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Acid-free microwave extraction of citrus pectins: Linking molecular structure to functional properties☆

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ABSTRACT

Exploring greener and more controlled processes remains a key challenge in food ingredient development. In this study, pectins from orange, lemon, and lime peels were extracted by acid-free hydrothermal microwave-assisted extraction (MAE) and characterized for their structural and functional properties. Competitive yields were achieved without external acid addition, reaching up to 26.38% at 160 °C for 5 min. Extraction conditions and citrus source significantly affected the degree of esterification, monosaccharide composition, and molar mass distribution. Orange pectins were classified as high-methoxyl pectins (HMP), whereas lemon and lime pectins remained HMP only below 180 °C for 10 min and 160 °C for 10 min, respectively. More severe conditions produced low-methoxyl pectins (LMP). Enzymatic de-esterification using pectin methylesterase (PME) further reduced the degree of methylation by 77.8–91.8%, yielding LMP with enhanced water-holding capacity, improved emulsion stability, and shear-thinning behavior. Compared with commercial pectins, MAE- and PME-derived pectins exhibited higher antioxidant activity, likely due to co-extracted phenolic compounds. FTIR and thermogravimetric analyses confirmed the structural modifications induced by MAE and PME. Overall, acid-free MAE combined with enzymatic de-esterification offers a sustainable strategy to produce citrus pectins with tunable structures and functional properties for diverse food applications.

1. Introduction

Pectin, or pectic substances, are complex dietary fibers present as key structural components in plant cell walls, contributing to porosity, surface charge, mechanical integrity, and rigidity, besides playing a role in the activation of plant defense (Voragen et al., 2009). In the food sector, pectin is notably recognized for its versatile applications as emulsifier, stabilizer, gelling agent, and fat replacer (Xiang et al., 2024). Moreover, due to its numerous human health benefits, such as immunomodulatory, antioxidant, cholesterol reduction, wound healing, and prebiotic activities, among others, pectin also presents applicability in the pharmaceutical and cosmetic sectors (Macias-Frotto et al., 2025; Soomro et al., 2024).

Structurally, pectin is a heteropolysaccharide composed mainly of D-galacturonic acid (GalA), with galactose (Gal) and arabinose (Ara) as the predominant neutral sugars, along with smaller amounts of other monosaccharides (Mao et al., 2019). These monosaccharides are distributed in different domains. The homogalacturonan (HG) domain, often referred to as the smooth region, typically represents the largest portion of the pectin structure, approximately 60 to 65% (Picot-Allain et al., 2022; Voragen et al., 2009). In contrast, the rhamnogalacturonan I (RG-I), rhamnogalacturonan II (RG-II), xylogalacturonan (XG), and apiogalacturonan (APG) domains form the so-called hairy regions (Jin et al., 2021). The proportion between the smooth and hairy fractions in the pectin structure is responsible for the different properties of pectin, including gelation, emulsification, and bioactivity (Kpodo et al., 2018; Lu et al., 2025; Zhang et al., 2025). Moreover, the physicochemical properties of pectins are also strongly influenced by their degree of esterification (DE), molecular weight, branching chain length, monosaccharide composition, charge density, glycosidic linkages, and spatial conformation (Einhorn-Stoll, 2018; Li et al., 2022).

Commercially, pectins are predominantly extracted from apple pomace, citrus peels, and sugar beet pulp, typically using harsh acidic conditions and prolonged extraction times (Mao et al., 2019). The conventional extraction process using acids has some limitations, including the need for treatment of acid waste and it can degrade the native pectin structure (Pattarapisitporn & Noma, 2025). Several efforts have been made to investigate the application of alternative green

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extraction approaches for pectin recovery, including ultrasound-assisted extraction (Panwar et al., 2023), pulsed electric field (Du et al., 2024), and subcritical water extraction (Benito-Román et al., 2024). Among these innovative methods, microwave-assisted extraction (MAE) has shown promising potential, particularly due to its reduced extraction time and lower solvent consumption compared with conventional maceration (Gomez et al., 2020). In MAE, non-ionizing electromagnetic radiation is applied in the frequency range of 300 MHz to 300 GHz, the energy penetrates the material, causing ionic conduction and dipole movement, the two predominant phenomena responsible for the conversion of microwave energy into heat (Ferrara et al., 2023). Both processes promote a rapid buildup of internal pressure caused by liquid vaporization within the cell, leading to cell swelling and subsequent rupture, which in turn facilitates the diffusion of target compounds (Flórez et al., 2015; Vinatoru et al., 2017).

Although microwave-assisted extraction (MAE) has been extensively investigated for the recovery of pectins from citrus peels, most reported studies rely on acidic conditions, typically involving the addition of citric acid or other mineral and organic acids combined with short extraction times (< 6 min) (Iñiguez-Moreno et al., 2025; Jurić et al., 2025; Rahmani et al., 2020; Rodsamran & Sothornvit, 2019; Wani & Patidar, 2025). More recently, Turan et al. (2024) applied MAE to orange peel pectin extraction, achieving high yields under relatively harsh conditions (90 °C, 60 min) and requiring the addition of a deep eutectic solvent as an extraction additive. In another study, Chen et al. (2016) investigated the application of acid-free MAE of tangerine peel pectins under mild conditions (704 W, 52.2 °C, 41.8 min), reaching a yield of 19.9% and isolating a purified pectic polysaccharide of 17.8 kDa with notable antioxidant activity. Nevertheless, their work did not address the techno-functional behavior of the recovered pectins and relied on a prolonged extraction time that limits the energy efficiency typically associated with MAE. Despite these advances, the potential of acid-free hydrothermal MAE as a green approach for pectin recovery remains poorly explored across major citrus sources such as orange, lemon, and lime peels. To the best of our knowledge, this is the first study to comparatively evaluate acid-free hydrothermal MAE across these three citrus peels, exposing behavior that single-source studies cannot capture and providing a rational basis for selecting the raw material and conditions according to the target pectin class, while linking the resulting structural features to the functional properties of the obtained pectins and to their controlled enzymatic modification.

In this context, the present study evaluates an acid-free hydrothermal MAE strategy for the extraction of pectins from different citrus byproducts (orange, lemon, and lime peels), eliminating the need for external acid or solvent additives, aiming to investigate the feasibility of this process and to understand its impact on the structure and functionality of the recovered pectins. Beyond extraction yield, this work provides a systematic evaluation of how MAE process variables influence pectin structural features and further elucidates the role of pectin methyl esterase treatment in tailoring pectin characteristics and functional properties. Overall, this combined approach offers new insights into a greener and more controlled route for producing functional citrus pectins.

2. Experimental

2.1. Materials

Citrus peels from orange (*Citrus sinensis*), lemon (*Citrus limon*) and lime (*Citrus aurantifolia*) were kindly provided by CEAMSA (Porrino, ES). In addition, commercial food-grade pectins produced from the same citrus sources using a conventional acid-based extraction method were provided for comparison purposes. The purity of commercial pectins was estimated as 65–74% based on the galacturonic acid content.

Citrus peels were ground and sieved (particle size below 500 μm) prior to use. Biomasses were stored in polyethylene bags at room temperature until characterization. The pH of the different citrus peels was measured after simple dispersion in water (1:10; w/v) under magnetic stirring for 3 h at 23 °C, resulting in the following values: orange peel (4.58 ± 0.24), lemon peel (4.15 ± 0.02), and lime peel (4.20 ± 0.04).

Ethanol 96% was purchased from VWR (Mölndal, SE). Sodium azide 1% solution was ordered from G-Biosciences (St. Louis, MO, USA). Pectin methyl esterase (Novoshape®) was acquired from Novozymes (Bagsværd, DK). Other chemicals used in the present study were purchased from Sigma-Aldrich (Schnelldorf, DE), unless otherwise stated.

2.2. Methods

2.2.1. Pectin extraction

Pectin extraction from orange, lemon, and lime was performed in an Ethos Lean microwave (Milestone, Bergamo, Italy). For this, tap water (pH 7.27 ± 0.05) and a solid:liquid ratio of 1:10 were used (final volume of 30 mL). The extraction variables included time (5, 10, and 15 min) and temperature (140, 160, and 180 °C). Pectin was precipitated with 96% ethanol in the proportion of 1:2 (v/v), kept overnight at approximately 4 °C, and then recovered by centrifugation. The recovered pellet was further subjected to vacuum evaporation using a MiVac system (Genevac, SP Industries, Ipswich, UK).

2.2.2. Effect of treatment with pectin methyl esterase (PME)

Extracted citrus pectins were subjected to enzymatic demethoxylation with pectin methyl esterase (PME) according to Zhao et al. (2015) with modifications. For this, pectins were resuspended at 1:10 (w/v) in Milli-Q water, followed by pH adjustment to 4.5 with 1 M NaOH solution. Commercial PME (Novoshape®) was added to the pectin solutions (500 μL/g of pectin) and the reaction was kept at 40 °C for 2 h. The reaction was performed twice in total and stopped by raising the temperature to 80 °C for 5 min, after which the samples were cooled down and recovered as described in Section 2.2.1.

2.2.3. Characterization of extracted citrus pectins

2.2.3.1. Extraction yield. Global extraction yield (Y) was gravimetrically determined as a function of the dry extract mass (We) in relation to the initial dry biomass weight (Wb).

2.2.3.2. Monosaccharide profile. The monosaccharide profile of the extracted pectins was evaluated in terms of neutral sugars and uronic acids using a two-step methanolysis followed by trifluoroacetic acid (TFA) hydrolysis in accordance with the method of Willför et al. (2009). Analyses were performed by High-Performance Anion-Exchange Chromatography coupled with Pulsed Amperometric Detection (HPAEC-PAD) using a Dionex ICS-6000 system (Thermo Fisher Sci., Waltham, USA). Separation was achieved on a CarboPac™ PA20 column (3 × 150 mm, Thermo Fisher Sci., Waltham, USA) at 0.4 mL/min under the gradient conditions described by Massironi et al. (2024). Quantification was conducted using calibration curves prepared with neutral sugars (fucose, arabinose, rhamnose, galactose, glucose, xylose, and mannose) and uronic acids (galacturonic and glucuronic acids) at different concentrations.

2.2.3.3. Methyl esterification and acetylation degree. The esterification degree was evaluated in terms of methylation degree (DM) and acetylation degree (DAc) following the method of Voragen et al. (1986). In brief, pectin samples (30 mg) were subjected to saponification at 23 °C using 0.4 M sodium hydroxide prepared in an isopropanol:water mixture (1:1) for 2 h, followed by centrifugation and filtration through 0.22 μm nylon filters. The methanol and acetic acid released were quantified using an HPLC-RI system (LC4000, Jasco Inc., Tokyo, JPN) equipped with an Aminex® HPX-87H column (300 × 7.8 mm, Bio-Rad,

Hercules, USA) operated at 30 °C, using 5 mM H_2SO_4 as the mobile phase at 0.6 mL/min for 40 min, with the refractive index detector maintained at 40 °C.

2.2.3.4. Phenolic composition. The phenolic profile of the extracted and treated citrus pectins was investigated in terms of free phenolic compounds and bonded phenolic compounds. The free phenolic compounds profile was determined by resuspension of the samples (10 mg) with a 50:50 mixture of the 1% formic acid and methanol, followed by vertexing and filtration through 0.22 μm nylon filters. Meanwhile, for the bonded phenolic compounds, samples (10 mg) were subjected to saponification with 500 μL of a 2 M sodium hydroxide solution in an inert atmosphere at 30 °C overnight, followed by adjustment of the pH to 2 with HCl. A liquid-liquid partition with ethyl acetate in a proportion of 1:2 (sample:ethyl acetate; v/v) was performed in triplicate. Samples were left to evaporate to dryness under a nitrogen stream and resuspended in a 0.1% formic acid and methanol mixture (50:50; v/v). Identification and quantification were performed on a HPLC system (Vanquish™ Core, Thermo Fisher Sci., Waltham, USA) coupled to a diode array detector (Thermo Fisher Sci., Waltham, USA). For separation, a reverse-phase Luna 3 μm C18(2) column (150 × 4.6 mm; Phenomenex, Torrance, USA) was used at a flow rate of 0.7 mL/min, with a gradient separation using 0.1% formic acid as eluent A and methanol as eluent B applied as follow: 95% eluent A for 50 min, 50% eluent A for 15 min, and equilibration with 95% eluent B for 10 min. Quantification was performed against hydroxybenzoic acids (gallic acid, gentisic acid, and vanillic acid) hydroxycinnamic acids (chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid, sinapic acid, and cinnamic acid), and flavonoids (catechin, rutin, quercetin, and luteolin) at 280 nm.

2.2.3.5. Total phenolic content (TPC). TPC from the extracted and treated citrus pectins were determined using Folin-Ciocalteu (FC) reagent following the method described by Cicco et al. (2009). In short, samples solubilized in water and Folin-Ciocalteu reagent were added to a 96-well microplate at the proportion of 1:1, followed by the addition of sodium carbonate (5%; w/v) at 5 times the final volume of the sample: FC reagent. Microplates were incubated at 37 °C in the dark for 20 min and read at 760 nm using a FLUOstar Omega microplate reader (BMG Labtech, DE). Gallic acid was used as standard, and the results were expressed in mg GAE/g of dry sample.

2.2.3.6. Size exclusion chromatography with multi-angle laser light scattering (SEC-MALLS). The molar mass of the extracted and enzymatic treated pectins was investigated using in a high-performance size-exclusion chromatography system composed of a 1260 Infinity II HPLC (Agilent Technologies, California, USA) equipped with a TSKgel PW_{XL} guard column (12 μm, 6 × 40 mm, TOSOH Bioscience, Griesheim, DE) and a TSKgel $GMPW_{XL}$ main column (7.8 mm × 30 cm; TOSOH Bioscience, Griesheim, DE) main column for separation. The system was coupled to a DAWN HELEOS-II multiangle laser light scattering detector and an Optilab rEX refractive index detector. For elution, a mobile phase of 0.02% $NaNO_3$ and 0.02% NaN_3 was applied at a flow of 0.5 mL/min was applied. The weight-average molar mass (M_w), number-average molar mass (M_n), Mw/M_n, and polydispersity index (PDI) values were assessed using ASTRA 5.3.4 software (Wyatt Technology, Santa Barbara, CA, USA). A refractive index increment (dn/dc) of 0.146 mL/g was employed for analysis.

2.2.3.7. Fourier transform infrared (FT-IR) spectroscopy analysis. Characterization of the molecular profile of citrus pectins was carried out using a Spectrum 3 spectrometer (PerkinElmer, Waltham, USA), equipped with a Universal Attenuated Total Reflectance (ATR) device (GladiATR, Pike Technologies, Madison, USA) consisting of a diamond crystal. The measurements were performed by averaging 32 scans with a resolution of 4 cm^{-1} from 4000 cm^{-1} to 600 cm^{-1}.

2.2.3.8. Thermogravimetric analysis (TGA). The thermal stability of the pectins was investigated by thermogravimetry using a Q500 thermoanalyzer (TA Instruments, New Castle, USA). Approximately, 5 mg of each sample was weighed and heated from 25 °C to 700 °C at 10 °C/min under a nitrogen atmosphere.

2.2.3.9. Antioxidant activity. The antioxidant activity of citrus pectins was evaluated in terms the electron transfer (ET) methodologies of radical scavenging activity against the radicals DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2′2-azinobis (3-ethyl-benzothiazoline-6-sulphonate)), as described in Pereira and Jiménez-Quero (2025), in which the absorbance was recorded at 517 nm and 734 nm, respectively. The Ferric Reducing Power Assay was employed to evaluate the reducing capacity from the pectins following the method of Benzie and Strain (1996), in which the absorbance at 593 nm was measured. Complementarily, the Oxygen Radical Absorbance Capacity (ORAC) assay was used to evaluate the hydrogen atom transfer (HAT) mechanism, in accordance with the method described by Zulueta et al. (2009). The fluorescence was measured at excitation and emission wavelengths of 400 to 480 nm and 520 nm, respectively. All measurements were performed using a FLUOstar® Omega plate reader (BMG LABTECH, Ortenberg, Germany).

2.2.4. Techno-functionality

2.2.4.1. Water holding capacity (WHC) and oil holding capacity (OHC). The water and oil holding capacities of the pectin samples were determined using according to the method of Gan et al. (2020), with slight modifications. In summary, 200 mg of powdered pectin were mixed with 10 mL of distilled water or 5 mL of sunflower oil, and vortexed vigorously, and incubated at 25 °C at and 100 rpm for 24 h. The mixture was then centrifuged at 4800 rpm for 10 min. After removing the supernatant, the residue was weighed. Water and oil holding capacities were expressed as the amount of water or oil retained per gram of pectin. All measurements were carried out in triplicate.

2.2.4.2. Emulsifying properties. Oil-in-water emulsions were prepared by homogenizing 10 wt% rapeseed oil and 90 wt% aqueous pectin solution at different concentrations (3, 1.5, and 1%) at 23 °C using a high-speed homogenizer at 15.000 rpm (T18 digital Ultra-Turrax, IKA, Works Inc., USA) for 1 min. Fine emulsions were then obtained by sonicating the coarse emulsion using a sonicator (Sonifier 450, Branson Ultrasonics Co., USA) operated at 30% amplitude for 60 s (alternating 3 s on and 2 s off) in accordance to Zhu et al. (2025).

Emulsion stability of standing emulsions was evaluated as a function of standing time at 23 °C over 24 days. The emulsion layers height for the different assays was measured using a digital caliper, and the emulsion stability was expressed as a percentage and calculated as a function of serum layer height per total emulsion height in accordance with Gu et al. (2005).

The microstructure of the emulsions was evaluated at the beginning at the preparation day (day 0) and after 24 days after storage at room temperature, using an optical microscopy (Primostar 3, Carl Zeeis AG, Jena, DE). For this, an aliquot of each sample was diluted 1:10 with deionized water, and 10 μL of the diluted emulsion was placed over glass slides and covered. The analysis was performed at 100× magnification.

2.2.4.3. Rheology. Apparent viscosity was determined for different pectins at 3% (w/v), stirred overnight. Steady shear rate sweeps were carried out in a controlled-stress rheometer (DHR-3, TA Instruments, New Castle, USA) using a 40 mm stainless steel parallel plate geometry with a gap of approximately 0.8 mm. Temperature was maintained at 25 °C using a peltier plate. A shear sweep between 1 s^{-1} and 100 s^{-1} shear rates, first in ascending and, subsequently in descending order, with the latter being considered for analysis. The experiments were performed in

duplicate.

2.2.5. Statistics analysis

Statistical significance was investigated by One-way Analysis of Variance (ANOVA) when the interactive factors were not relevant for investigation; in contrast, Two-way ANOVA was applied when interactions were relevant. In both cases, a significance level of 5% was chosen. One-way ANOVA, when significant, was followed by Fisher's LSD post-hoc test. Meanwhile, when Two-way ANOVA was significant, a Tukey test was applied for mean comparison. The principal component analysis (PCA) was further applied to verify the effect of the variables on the emulsion stability. Statistical analyses were carried out using R Statistical Software (v4.5.0; R Core Team 2025).

3. Results & discussion

3.1. MAE performance to recovery citrus pectins

The effect of the MAE process on an acid-free hydrothermal approach for pectin recovery from citrus peels was assessed based on extraction yield (Table 1). Pectin yields ranged from 8.09 to 21.23% for orange peels, 5.09 to 25.67% for lemon peels, and 3.27 to 26.38% for lime peels, depending on extraction temperature and time. For all three citrus peels, 160 °C for 5 min gave the highest extraction yield, and 180 °C consistently lowered it through thermal degradation of the pectin. For orange pectin, only the main effects of temperature and time were significant ($p < 0.05$), where yield increased from 140 to 160 °C and then declined, and shorter times were more favorable, with 5 min as the best condition. For lemon and lime pectin, the temperature and time interaction was significant ($p < 0.05$), so yield depended on the specific combination. In both cases, the optimum was 160 °C for 5 min, where extraction was rapid and most efficient, while longer times at this temperature reduced the yield. The main difference was at 180 °C, where the yield remained low throughout and, for lime, collapsed as time increased, corroborating that excessive heat and prolonged exposure degrade the extracted pectin. This behavior is consistent with previous studies reporting reduced pectin recovery or degradation under excessive thermal conditions (Iñiguez-Moreno et al., 2025; Wani & Patidar, 2025). Comparable yields were reported by Hossain et al. (2024) for pomelo peel pectin using MAE (26.63%). Lower yields were reported under acidic conditions using hydrochloric acid for lime peel pectin (14.13 to 15.91%; Rodsamran & Sothornvit, 2019). Under similar pH conditions, Wani and Patidar (2025) obtained 20.80% for lemon peel pectin, which increased to 48.00% after adjusting the pH to 1. Similarly, Iñiguez-Moreno et al. (2025) reported 35.75% for orange peel using citric acid at pH 2.48. Conventional extraction yielded comparable or lower values: 8.79% for orange (Kute et al., 2020) and 16.12 to 23.59% for lime peel (Rodsamran & Sothornvit, 2019).

Although pH adjustment enhances pectin solubilization, the present results demonstrate that competitive recoveries can be achieved without acid addition by appropriately tuning MAE conditions. Avoiding pH adjustment reduces chemical consumption and minimizes downstream neutralization steps, offering a more sustainable alternative while achieving comparable or improved yields relative to traditional extraction. The initial pH of all citrus peels under prolonged exposure to water was approximately 4.3 before the MAE process and remained essentially stable across the extraction conditions, close to 3.0 for all citrus extracts. This result is likely due to the presence of citric acid naturally occurring in citrus peels, as well as other reactive species that may further contribute to the acidification of the extracts. Although some treatment combinations differed significantly ($p < 0.05$), the absolute variation in pH was very small and of negligible practical relevance, and its direction was not consistent among the sources.

The impact of MAE variables on pectin profiles from different citrus

Table 1
Extraction yield, methylation (DM) and acetylation (DAc) degrees, total phenolic content (TPC), and soluble protein content from the extracted citrus pectins obtained from microwave assisted extraction at different conditions.

Sample	Extraction condition (°C-min′)	Yield (%)	pH	DM (%)	DAc (%)	Soluble protein (mg·g DW^{-1})
Orange peel	140–5	12.76 ± 1.16^{bA}	$3.04 \pm 0.04^{b*}$	$53.65 \pm 2.78^{d*}$	$0.71 \pm 0.09^{g*}$	$1.64 \pm 0.31^{de*}$
	140–10	12.11 ± 2.33^{bAB}	$3.09 \pm 0.01^{ab*}$	$63.31 \pm 2.97^{bc*}$	$1.79 \pm 0.12^{fg*}$	$2.28 \pm 0.10^{cde*}$
	140–15	12.43 ± 0.00^{bB}	$3.07 \pm 0.01^{ab*}$	$65.36 \pm 0.44^{abc*}$	$1.86 \pm 0.08^{f*}$	$1.42 \pm 0.20^{e*}$
	160–5	21.13 ± 2.44^{aA}	$3.04 \pm 0.03^{b*}$	$65.22 \pm 1.19^{abc*}$	$2.02 \pm 0.07^{e*}$	$3.67 \pm 0.86^{cd*}$
	160–10	17.89 ± 3.50^{aAB}	$3.06 \pm 0.01^{b*}$	$63.04 \pm 1.19^{bc*}$	$2.21 \pm 0.17^{de*}$	$6.10 \pm 0.96^{ab*}$
	160–15	15.18 ± 1.46^{aB}	$3.08 \pm 0.02^{ab*}$	$61.73 \pm 0.90^{c*}$	$3.17 \pm 0.21^{cd*}$	$6.76 \pm 0.54^{a*}$
	180–5	10.08 ± 0.82^{cA}	$3.11 \pm 0.01^{a*}$	$68.33 \pm 1.34^{ab*}$	$3.51 \pm 0.21^{bc*}$	$3.21 \pm 0.47^{cde*}$
	180–10	9.83 ± 1.07^{cAB}	$3.12 \pm 0.03^{a*}$	$67.98 \pm 0.12^{ab*}$	$8.77 \pm 1.40^{a*}$	$4.16 \pm 0.99^{bc*}$
	180–15	8.10 ± 0.83^{cB}	$3.06 \pm 0.03^{ab*}$	$70.70 \pm 4.31^{a*}$	$3.94 \pm 0.20^{ab*}$	$6.00 \pm 1.31^{ab*}$
Lemon peel	140–5	$6.80 \pm 0.08^{d*}$	$3.13 \pm 0.04^{a*}$	$72.02 \pm 0.36^{bc*}$	$1.05 \pm 0.11^{e*}$	$3.40 \pm 0.48^{c*}$
	140–10	$19.40 \pm 2.59^{b*}$	$3.02 \pm 0.01^{d*}$	$74.63 \pm 1.08^{ab*}$	$1.29 \pm 0.14^{de*}$	$3.28 \pm 0.65^{c*}$
	140–15	$14.30 \pm 2.17^{bc*}$	$3.04 \pm 0.03^{cd*}$	$69.60 \pm 0.43^{cd*}$	$1.24 \pm 0.16^{de*}$	$4.71 \pm 1.00^{bc*}$
	160–5	$25.66 \pm 1.00^{a*}$	$3.13 \pm 0.01^{a*}$	$67.67 \pm 0.47^{de*}$	$2.66 \pm 0.09^{bc*}$	$7.14 \pm 0.20^{b*}$
	160–10	$19.40 \pm 2.59^{b*}$	$3.08 \pm 0.01^{abcd*}$	$71.92 \pm 0.47b^{c*}$	$2.31 \pm 0.41^{bc*}$	$11.66 \pm 1.02^{a*}$
	160–15	$15.30 \pm 3.22^{b*}$	$3.07 \pm 0.01^{abcd*}$	$72.30 \pm 3.47^{bc*}$	$1.61 \pm 0.10^{cd*}$	$10.33 \pm 2.42^{a*}$
	180–5	$8.91 \pm 1.62^{cd*}$	$3.06 \pm 0.01^{bcd*}$	$78.12 \pm 0.68^{a*}$	$3.85 \pm 0.13^{ab*}$	$6.54 \pm 0.25^{b*}$
	180–10	$5.09 \pm 0.35^{d*}$	$3.11 \pm 0.02^{ab*}$	$36.39 \pm 4.99^{e*}$	$17.39 \pm 4.90^{a*}$	$5.85 \pm 1.26^{bc*}$
	180–15	$8.11 \pm 1.36^{d*}$	$3.09 \pm 0.02^{abc*}$	$49.82 \pm 7.61^{de*}$	$19.45 \pm 2.36^{a*}$	$4.21 \pm 0.75^{bc*}$
Lime peel	140–5	$18.20 \pm 2.95^{d*}$	$3.14 \pm 0.03^{a*}$	$78.27 \pm 12.97^{a*}$	$2.77 \pm 0.08^{de*}$	$1.57 \pm 0.30^{b*}$
	140–10	$17.86 \pm 0.40^{b*}$	$3.14 \pm 0.01^{ab*}$	$63.74 \pm 0.69^{bc*}$	$3.13 \pm 0.02^{d*}$	$2.46 \pm 0.45^{b*}$
	140–15	$20.35 \pm 2.54^{bc*}$	$3.03 \pm 0.02^{e*}$	$69.33 \pm 1.13^{ab*}$	$2.64 \pm 0.02^{ef*}$	$1.17 \pm 0.15^{b*}$
	160–5	$26.38 \pm 2.83^{a*}$	$3.06 \pm 0.01^{cde*}$	$58.76 \pm 0.70^{cd*}$	$2.42 \pm 0.16^{f*}$	$6.16 \pm 0.35^{a*}$
	160–10	$18.55 \pm 0.70^{b*}$	$3.12 \pm 0.04^{abc*}$	$53.06 \pm 0.70^{de*}$	$3.03 \pm 0.21^{d*}$	$5.39 \pm 0.76^{a*}$
	160–15	$17.99 \pm 0.32^{b*}$	$3.07 \pm 0.02^{cde*}$	$15.86 \pm 1.55^{g*}$	$9.62 \pm 0.64^{a*}$	$5.54 \pm 1.00^{a*}$
	180–5	$13.06 \pm 2.18^{cd*}$	$3.05 \pm 0.01^{de*}$	$35.55 \pm 5.19^{f*}$	$4.45 \pm 0.55^{bc*}$	$5.69 \pm 0.51^{a*}$
	180–10	$4.25 \pm 1.05^{d*}$	$3.10 \pm 0.01^{abcd*}$	$36.86 \pm 2.07^{g*}$	$4.13 \pm 0.05^{c*}$	$5.53 \pm 0.15^{a*}$
	180–15	$3.26 \pm 0.36^{d*}$	$3.08 \pm 0.01^{bcde*}$	$14.13 \pm 3.71^{g*}$	$8.02 \pm 2.40^{ab*}$	$6.50 \pm 0.31^{a*}$

Results are presented as mean ± standard deviation.
Results followed by different lowercase letters in the column of means differ among temperatures; means followed by different Uppercase letters in the column of means differ among times (Tukey, $p < 0.05$).
Different letters followed by "*" indicate significant differences in the interaction of variables (Tukey, $p < 0.05$).
TPC are expressed in mg gallic acid equivalent/g of sample on dry weight basis.
Soluble proteins are expressed in mg BSA equivalent/g of sample on dry weight basis.

peels was investigated in terms of monosaccharide composition and chain profile (Fig. 1). No significant differences ($p > 0.05$) were observed among MAE parameters for the carbohydrates content of the extracted samples. Regardless of pectin source, galacturonic acid was the main monosaccharide, followed by arabinose or galactose depending on extraction temperature. Although the final carbohydrates content was the same, the population of HG and RG-I tended to be different as a function of extraction severity. At 180 °C, galactose content increased while arabinose decreased, suggesting that galactose from galactan side chains is more resistant to hydrolysis than arabinans. This is consistent with trends in molar ratios MR_1 (pectin linearity) and MR_2 (side-chain branch length) (Hosseini et al., 2019; Rahmani et al., 2020). A similar structural behavior was obtained across the three citrus pectins, which showed less linear and more branched structures with increasing severity, differing in form and degree. Orange lost HG gradually and only through a temperature effect ($p < 0.05$), retained the most HG at 180 °C, showed a more modest RG-I enrichment (up to about 57%), and kept an essentially stable branch length (MR_2 around 8), making it the most robust of the three. Lemon and lime instead lost HG sharply through a temperature and time interaction ($p < 0.05$), concentrated at 180 °C for 10 and 15 min, and reached a higher RG-I content (about 69%); lemon stood out for the longest side chains (MR_2 about 12 under moderate conditions) and the lowest linearity ceiling, whereas lime spanned the widest range and yielded the most linear pectin of the set (MR_1 up to 1.75 at 160 °C, 15 min). Overall, the degradation pattern was common to all three, but orange was the most stable, lemon the most branched, and lime the most variable, showing that the raw material governs both the extent and the statistical form of the structural changes induced by MAE. MR_1 values ranged from 0.77 to 1.61 for orange, 0.54 to 1.28 for lemon, and 0.74 to 1.75 for lime peel pectin, lower than those reported for mandarin peel pectin obtained by electromagnetic heating ($MR_1 = 2.98$) (Yang et al., 2024) and for sour orange peel pectin ($MR_1 = 1.91$) obtained by ultrasound-assisted extraction (Hosseini et al., 2019). This comparison indicates that acid-free hydrothermal MAE leads to the extraction of less linear pectins, attributed to a higher proportion of the hairy region. Rahmani et al. (2020) reported a MR_2 value of 7.81 for sweet lemon peel pectin using MAE, consistent with values obtained here at 140–160 °C for orange and lime pectins, suggesting longer branch sides under moderate extraction conditions. Herein for lemon, the MR_2 (about 12 at 140 to 160 °C) suggests even longer branch lengths.

The processing impact on the methylation degree of the pectins was evaluated, and the results are shown in Table 1. The degree of esterification of pectins, which are generally described in terms of methylation degree (DM) and acetylation degree (DAc), is crucial to the structure and properties of pectin. Across the different pectin sources, DM and DAc undergo significant changes as a function of the extraction conditions. The DM of all citrus pectins was relatively high (DM > 50%) under most extraction conditions, characterizing them as high-methoxyl pectins (HMP), which require acidic pH and co-solutes for gelation (Ditta et al., 2020). For orange peel, DM remained consistently high (54 to 71%) across all conditions, generally increasing with temperature, with the highest values ($p < 0.05$) at 180 °C and the lowest at the mildest condition (140 °C, 5 min), suggesting selective extraction of highly methylated regions or limited thermal demethylation. Lemon peel pectin showed higher DM than orange pectin under mild and moderate conditions (above 70%), with a drastic decrease to 36 to 50% at 180 °C for 10 and 15 min, indicating extensive demethylation with increasing extraction severity (Wani & Patidar, 2025). Lime peel pectin had high DM under mild conditions (around 63 to 70%), but its DM decreased with both temperature and time, collapsing to about 14 to 16% at 15 min combined with 160 and 180 °C, which converted it into low-methoxyl pectin (LMP) and mirrored the yield reduction observed under severe conditions, possibly due to degradation of the extracted pectins.

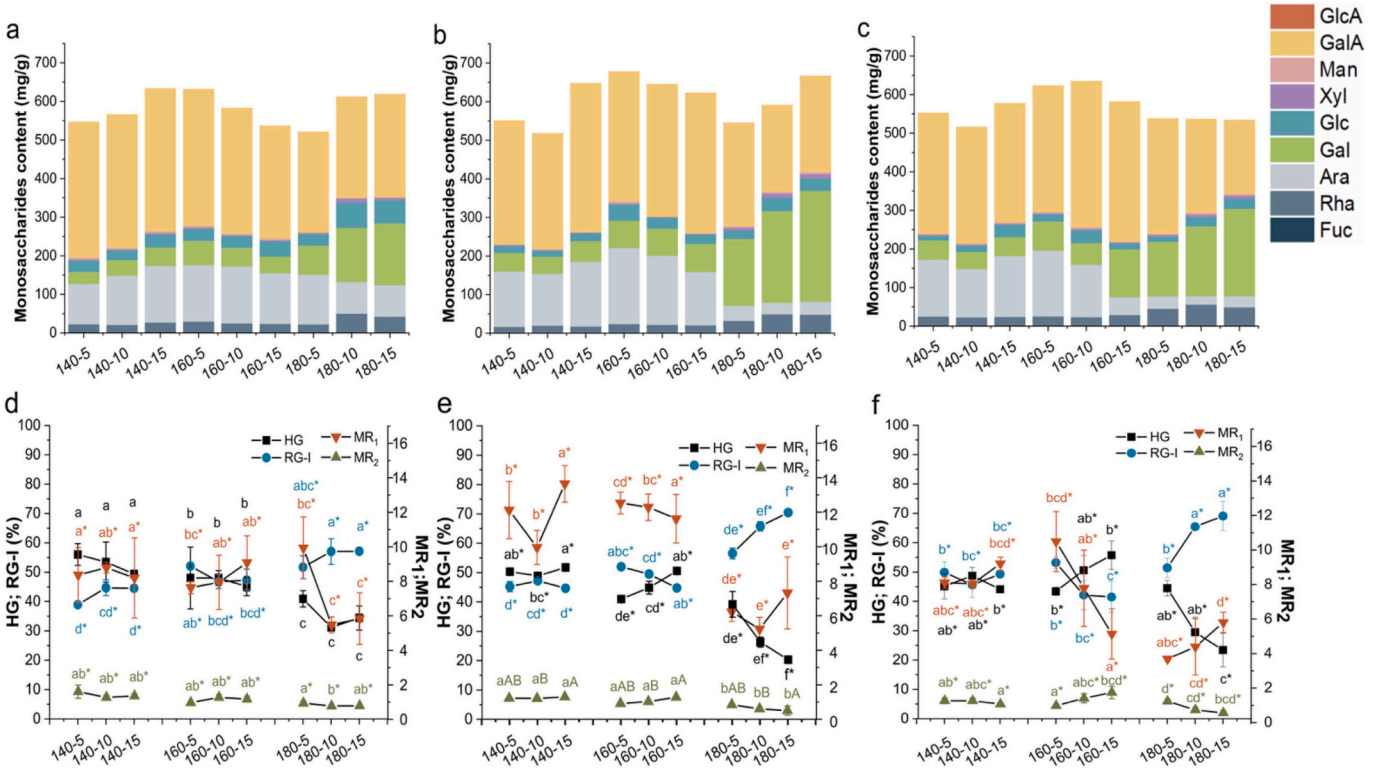

Fig. 1. Monosaccharide profile of the different citrus pectins including (a, d) orange, (b, e) lemon, and (c, f) lime peels expressed as total carbohydrate content (mg/g) and chain profile in terms of HG (%), RG-I (%), MR_1 (Ara + Gal/Rha) and MR_2 (GalA/(Fuc + Ara + Rha + Gal + Xyl)) calculated from the molar percentual and obtained by microwave assisted extraction at 140°, 160° and 180 °C for 5, 10, and 15 min. Different letters in the same colors shows significative differences (Tukey, $p < 0.05$).

Regarding DAc, orange peel pectin showed low values at 140 °C, which increased progressively with extraction severity. A significant increase ($p < 0.05$) was observed at 180 °C, 10 min (8.77 ± 1.40%), followed by a decrease at 180 °C, 15 min, suggesting preferential release of acetylated domains at high temperature with partial deacetylation upon prolonged exposure. Lemon peel pectin showed a dramatic DAc increase under severe conditions, reaching the highest values among all samples (17.39 to 19.45%; $p < 0.05$). The inverse relationship between DM and DAc at high temperatures suggests that methyl esters are more thermolabile in lemon pectin, while acetylated regions, possibly enriched through selective solubilization linked to increased RG-I domains, are more resistant. Evidence of acetylation on RG-I has been previously reported (Perrone et al., 2002), although further studies are needed to clarify the impact of MAE on the acetylation pattern of citrus pectins. In lime peel pectin, DAc increased markedly under severe conditions, reaching 9.56% at 160 °C, 15 min and 6.69% at 180 °C, 15 min, alongside a strong DM decrease, indicating high susceptibility to hydrothermal treatment. These differences highlight the influence of pectin source on esterification patterns and demonstrate that extraction parameters can be tuned to target specific DM and DAc balances, critical for tailoring functional properties such as gelation, emulsification, and ion interaction (Macias-Frotto et al., 2025; Renard & Jarvis, 1999).

Complementarily, the purity of the extracted citrus pectins was investigated in relation to their content of soluble protein, and the results are presented in Table 1. Soluble protein depended strongly on the extraction conditions and the pectin origin, with a significant temperature and time interaction ($p < 0.05$) in all three sources. In orange peel pectin, protein solubilization increased with temperature and reached its highest values at 160 °C, particularly at 15 min (6.76 ± 0.54 mg.g^{-1} DW), with no further gain at 180 °C, while it remained low at 140 °C (1.4 to 2.3 mg.g^{-1} DW). Lemon peel pectin showed the most pronounced response, with the highest soluble protein at 160 °C for 10 and 15 min (11.66 ± 1.02 and 10.33 mg.g^{-1} DW), followed by a significant decline at 180 °C (4.2 to 6.5 mg.g^{-1} DW, decreasing with time), consistent with protein denaturation under the most severe conditions. At 140 °C the soluble protein remained intermediate (3.3 to 4.7 mg.g^{-1} DW), and the value reached at 15 min (4.71 mg.g^{-1} DW) was statistically similar to those obtained at 180 °C, indicating that mild conditions with longer extraction times can solubilize protein amounts comparable to the most severe treatments. Lime peel pectin displayed a distinct, stepwise behavior: protein was low at 140 °C (1.17 to 2.46 mg.g^{-1} DW) and increased sharply from 160 °C onward, remaining high and statistically similar across all times at 160 and 180 °C (about 5.4 to 6.5 mg.g^{-1} DW). For all three pectins, the soluble protein contents were lower than those reported in the literature (12 to 47 mg.g^{-1}) (Hosseini et al., 2019; Liu et al., 2022; Rahmani et al., 2020), suggesting that the citrus origin and the extraction type may have great influence on the coextraction of proteins during the recovery of pectin.

The phenolic content was evaluated in the recovered pectins in terms of free and bonded phenolic content (Fig. 2). Overall, all pectins presented a higher content of free phenolic compounds, possibly due to co-extraction during pectin precipitation in an adsorbed form or as electrostatic complexes (Liu et al., 2020). The two-way ANOVA showed a significant temperature × time interaction for the three pectins, indicating that the effect of extraction time on the phenolic content depended on the temperature applied.

Orange pectin did not follow a consistent severity trend: the highest ($p < 0.05$) phenolic contents were obtained at 140–15′, 180–15′ and 160–5′, which were statistically similar to one another, whereas the lowest value was found at 160–15′. The time effect reversed with temperature, increasing at 180 °C but decreasing at 160 °C, which explains the significant interaction. Lemon peel pectin, in contrast, showed a clear decline in phenolic content as temperature increased. The highest values occurred at 140 °C regardless of time, dropping sharply at 180 °C for 10 and 15 min, which evidences thermal degradation of phenolics under the most severe conditions. Lime peel pectin displayed the opposite behavior, with a temperature-driven increase: the highest phenolic contents were reached at 180 °C and were statistically equivalent across all times; 160 °C gave intermediate and time-independent values, and 140 °C was the most variable, ranging from the lowest content of the whole set at 5 min to a comparatively high value at 10 min. This suggests that, in lime, higher temperatures favored the release of phenolics, possibly through hydrolysis from the bonded form attached to the pectin structure to the free form during the MAE process, an effect that prevailed over degradation and was already maximized at 180 °C independently of time. The phenolic profile (Fig. S1) revealed that in the free fraction the main phenolics are composed of two possible flavonoid derivatives (quantified here as quercetin equivalents), possibly attributed to hesperidin and naringin, the main flavonoids in citrus peels (AlZahabi & Mamdouh, 2025). The same trend was not observed for the free phenolic compounds of lemon and lime peel pectins, where sinapic acid was the secondary phenolic compound. Nasso et al. (2026) reported

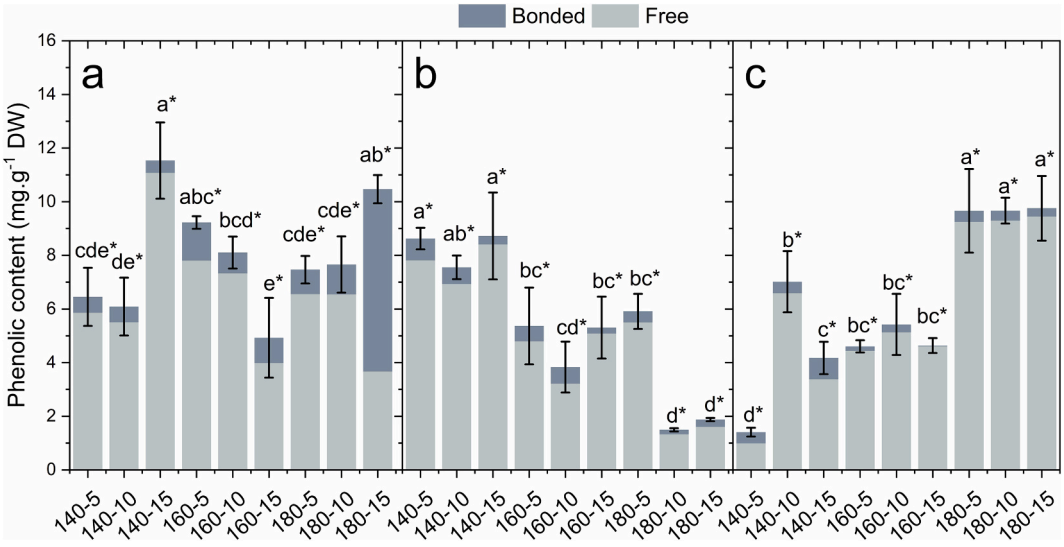

Fig. 2. Phenolic content determined by HPLC-DAD, expressed as bonded and free phenolic fractions, for pectins extracted from (a) orange peel, (b) lemon peel, and (c) lime peel. Standard deviation is shown for total phenolic content (bonded + free phenolics), and different letters indicate statistically significant differences among means according to Tukey's test.

hesperidin as the main flavonoid obtained in lemon peel extracts. Regarding the bonded phenolic profiles, in orange pectin, ferulic and sinapic acid are the main phenolic compounds found at low-severity extraction conditions, which tended to decrease drastically, giving rise to flavonoids. For lemon peel pectin, the main phenolic compounds in the bonded form were myricetin and *p*-coumaric acid. Meanwhile, in lime pectin, *p*-coumaric acid was found as the main bonded phenolic compound.

MAE at 160 °C for 5 min was selected for all three pectins for showing the highest extraction yield at shorter times to keep the energy input low, in line with the acid-free hydrothermal approach goal. The yields for orange, lemon, and lime peel (21.13, 25.66, and 26.38%) under a single comparable condition. At this extraction condition, the pectins were high-methoxyl (DM 59 to 68%) and lightly acetylated (DAc 2.0 to 2.7%), thereby preserving the native structure lost at higher severity. Regarding soluble proteins, the selected condition supported a favorable moderate co-extraction for orange and lemon (3.67 and 7.14 mg.g DW^{-1}), which increased significantly when the treatment was prolonged at 160 °C (up to 6.76 and 11.66 mg.g DW^{-1} at 10 to 15 min), while the yield declined. For lime, protein co-extraction was already at its highest level at 160 °C for 5 min (6.16 mg.g DW^{-1}), statistically comparable to the more severe treatments and significantly above the mildest ones. Phenolic content remained substantial, among the highest for orange and intermediate for lemon and lime, since maximizing it would require harsher, source-specific conditions (140 °C for lemon, 180 °C for lime) incompatible with a single condition and with yield and structural preservation. A similar condition was also optimized in the study of Iñiguez-Moreno et (2025) for an acidic MAE process for extraction of pectin from orange peels.

3.2. Influence of de-esterification on pectin physicochemical properties

The structural features described above, particularly the degree of esterification, molar mass distribution, and the balance between HG and RG-I domains, are expected to directly govern the functional behavior of the extracted pectins in food systems. To evaluate this structure-function relationship, the techno-functional properties of commercial, MAE, and PME-treated pectins were assessed in terms of water and oil holding capacities, emulsifying performance, and rheological behavior, and were compared with their commercial form (obtained chemically). Enzymatic treatment of MAE-obtained pectins with pectin methyl esterases (PME) allows the release of methoxyl groups, thereby modify the functional properties of the resulting de-esterified pectins (Wu et al., 2024; Yoo et al., 2009). FTIR spectra of commercial, MAE, and PME-treated pectins were compared as shown in Fig. 3a.

Although characteristic absorption bands are observed around 3300 cm^{-1} (–OH) and 2930 cm^{-1} (–CH), the main differences among commercial, MAE-, and PME-treated pectins appear at 1740 cm^{-1} and 1630 cm^{-1}, which are attributed to the stretching vibrations of ester carbonyl (C=O) and free carboxylate (COO^-) groups, respectively. These differences are mainly related to variations in the C=O/COO^- ratio, which follows a similar trend regardless of the citrus source. Commercial pectins, with a degree of methylation (DM) ranging from 52.65% to 58.27%, exhibit a higher C=O/COO^- ratio, consistent with their esterification level. In contrast, pectins obtained by MAE, with DM values between 58.76% and 67.67%, show a slight increase in C=O band

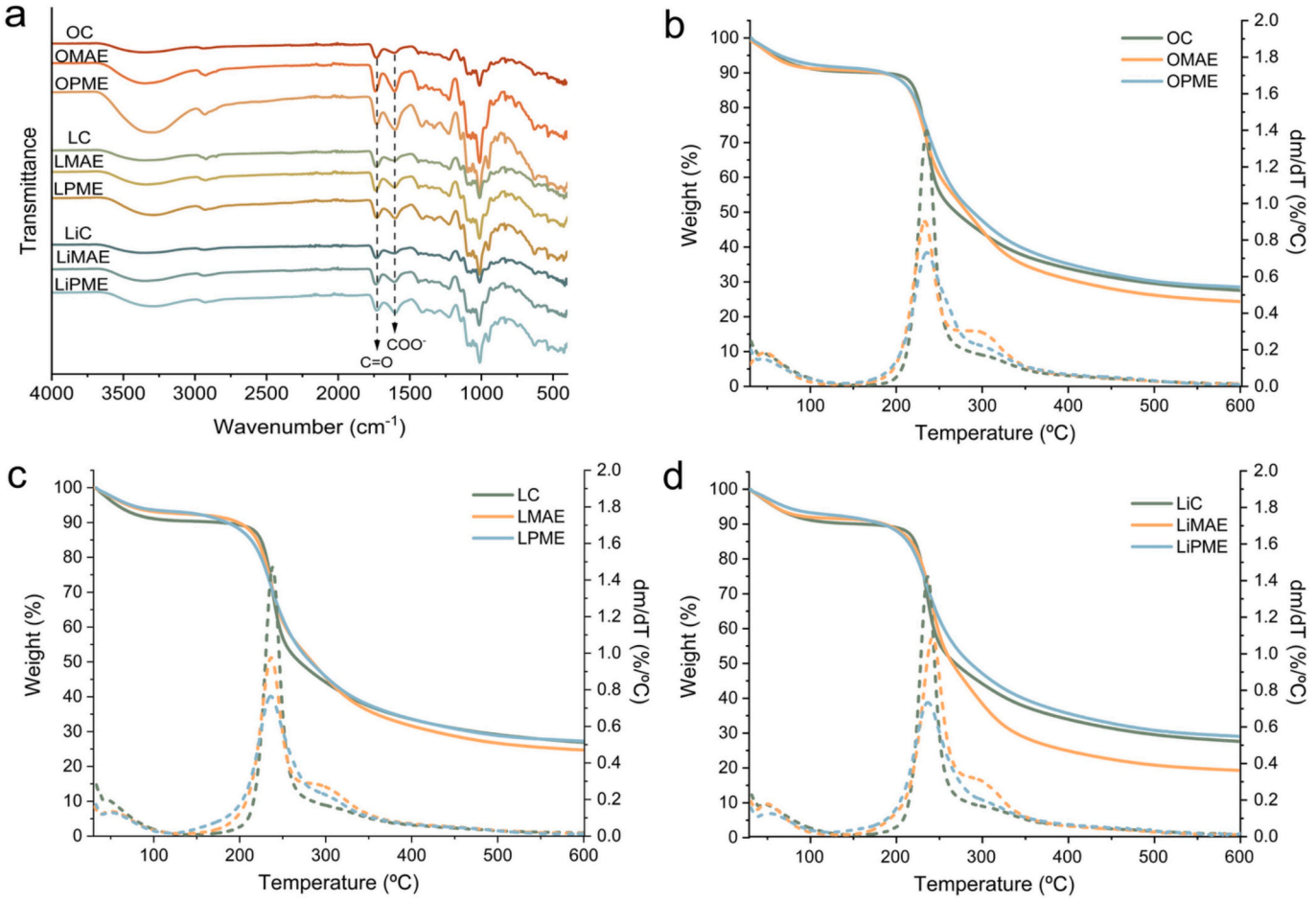

Fig. 3. (a) FTIR spectra and thermograms from the different citrus pectins including (b) orange-O, (c) lemon-L, and (d) lime-Li peels obtained by microwave assisted extraction (MAE), treated with pectin methyl esterase (PME) and compared with commercial pectins (C).

intensity relative to the COO^- band, reflecting their higher degree of esterification. As expected, PME-treated samples display a pronounced decrease in the C═O band relative to COO^- compared with MAE samples, in agreement with the reduced methylation degree (Table 2) observed for these samples, which decreased by 77.8%, 91.8%, and 89.1%, for orange (OPME), lemon (LPME), and lime (LiPME) pectins, indicating the presence of more free carboxyl groups after the enzymatic treatment. These results are in accordance with the trends observed by Yang et al. (2025) on the de-esterification of passion fruit pectins.

The thermal stability of the different pectins is shown in Fig. 3b, c, and d. All pectins presented a similar profile of thermal degradation with three main events. An initial event was observed up to 100 °C, attributed to the evaporation of water and other volatile compounds, followed by the main degradation step at T_{onset} in the range of 213–233 °C (T_{max} = 215–235 °C), 211–224 °C (T_{max} = 236–238 °C), and 212–224 °C (T_{max} = 236–242 °C), for orange, lemon, and lime pectins, respectively, attributed to the depolymerization of polysaccharide chains and galacturonic acid rings. A small third degradation step is evident in all pectins, the exception of commercial lemon and lime pectins, potentially associated to either degradation of polysaccharides chains or breakdown of charred residue or organic structure into volatile gases (Iqbal et al., 2025). The de-esterification of PME-pectins contributed to a decrease in thermal stability in comparison with MAE-pectins, as observed from T_{max} values, a phenomenon also reported by Liang et al. (2022). Commercial pectins presented slightly better thermal stability than MAE and PME-treated pectins. It is also worth noting that the thermal degradation rate is higher in commercial pectins, indicating that, although degradation occurs at a later stage, it proceeds more rapidly compared with MAE- and PME-pectins. This behavior might be attributed to structural and conformational differences, such as variables in linearity and/or molar mass populations resulting from the distinct processing methods used to obtain the pectins (Liang et al., 2022), which may facilitate their thermal decomposition, such as differences in linearity and/or molar mass populations. For MAE pectins, the lower thermal stability can be attributed to the compounds co-extracted with the pectins, such as phenolics and proteins, whereas for PME-treated pectins, the decrease in the esterification degree seems to lower the thermal stability. In fact, according to Einhorn-Stoll and Kunzek (2009), free carboxyl groups facilitate thermal degradation reactions, favored by hydrogen bonds, reducing the energy barrier needed to trigger the reaction.

Table 2
Comparison among degree of methylation (DM), degree of acetylation (DA), number-averaged molar mass (M_n), Weigh-averaged molar mass (M_w), and molar mass polydispersity index (PDI) for: commercial (C), microwave-assisted extracted (MAE), and enzymatically de-esterified (PME) pectins from orange (O), lemon (L), and lime peels (Li).

Sample	DM (%)	DA (%)	M_n (kDa)	M_w (kDa)	PDI (M_n/M_w)
OC	58.12 ± 0.60^{d}	7.87 ± 0.18^{a}	89.20 ± 10.04^{a}	128.75 ± 13.93^{c}	1.44 ± 0.06^{e}
OMAE	65.22 ± 1.19^{b}	2.02 ± 0.07^{de}	38.33 ± 3.30^{de}	197.07 ± 13.67^{bc}	5.15 ± 0.34^{a}
OPME	14.51 ± 0.93^{f}	1.54 ± 0.50^{ef}	63.70 ± 0.28^{b}	304.60 ± 0.85^{a}	4.78 ± 0.01^{ab}
LC	59.92 ± 0.43^{c}	8.06 ± 0.07^{a}	99.00 ± 15.84^{a}	149.30 ± 22.91^{cd}	1.51 ± 0.01^{e}
LMAE	67.67 ± 0.47^{a}	2.66 ± 0.09^{c}	53.76 ± 6.58^{bc}	209.00 ± 37.79^{b}	3.93 ± 0.99^{bc}
LPME	5.58 ± 0.34^{g}	1.05 ± 0.12^{g}	56.60 ± 7.35^{bc}	170.75 ± 61.87^{bcd}	2.97 ± 0.71^{d}
LiC	52.65 ± 0.25^{e}	7.02 ± 0.60^{b}	90.3 ± 4.53^{a}	123.20 ± 7.42^{d}	1.36 ± 0.01^{e}
LiMAE	58.36 ± 0.70^{d}	2.41 ± 0.15^{cd}	28.45 ± 4.31^{e}	156.05 ± 50.28^{bcd}	5.42 ± 0.95^{a}
LiPME	6.35 ± 0.15^{g}	1.25 ± 0.12^{fg}	50.05 ± 1.63^{cd}	152.10 ± 7.21^{cd}	3.04 ± 0.05^{cd}

Results expressed in mean ± standard deviation.
Different letters along with the results shows significative differences (Fisher, $p < 0.05$).

The molar mass results, expressed as number-average (Mn) and weight-average (Mw) molar mass and as the polydispersity index (PDI), are shown in Table 2. Compared with commercial pectins, all MAE pectins had lower Mn but higher Mw ($p < 0.05$); since Mn is more sensitive to smaller molecules, this indicates that MAE degrades pectin only mildly relative to the conventional acid hydrolysis used commercially. Their higher PDI ($p < 0.05$) further shows that MAE is a heterogeneous recovery process regardless of the source.

After enzymatic treatment, and despite the close compositional similarity of the three pectins, only orange pectin showed a significant Mw increase (OMAE 197.07 to OPME 304.60 kDa, $p < 0.05$), whereas lemon and lime remained statistically unchanged. This rise is consistent with aggregation of the low-methoxyl pectin, as reported by Yoo et al. (2003), who attributed a comparable increase to incomplete solvation and persistent pectin-to-pectin interactions. The effect cannot be explained by the number of free carboxyl groups alone, since lemon and lime were more de-esterified (DM 5.58 and 6.35%) than orange (14.51%) yet did not aggregate. Following Yoo et al. (2006), the difference reflects the intramolecular distribution of methyl esters: pectins of similar global DE and Mw can develop different charge patterns upon de-esterification, and a blockwise arrangement of free carboxyl groups favors the junction zones that drive aggregation. The native methyl-ester pattern of orange pectin thus appears more prone to aggregation after PME treatment, reinforced by chain stretching from increased carboxyl repulsion (Alba et al., 2018), while the patterns in lemon and lime were less favorable, leaving their molar mass essentially unchanged.

Antioxidant activity for the different pectins is shown in Fig. 4. Among the different antioxidant activity methods, the pectins presented a similar trend, in which commercial pectin showed the lowest antioxidant activity ($p < 0.05$) in comparison with the pectins obtained in the present study. The MAE pectins had the highest values of antioxidant activity for all the different methods of characterization used, followed by a significant reduction in the PME-treated pectins. The exception was observed for the DPPH assay in orange pectin, in which OMAE pectin did not differ statistically ($p < 0.05$) from the commercial and PME-treated samples. A similar trend of ABTS values being higher than DPPH values was previously reported in citrus pectins (Iñiguez-Moreno et al., 2025), which suggests a limitation of the DPPH assay in determining the antioxidant activity of pectins, in particular due to the use of an apolar solvent to solubilize the radical, which leads to pectin precipitation and a potential steric barrier that may affect the antioxidant mechanisms. Overall, the high antioxidant activities of MAE pectins may be attributed to the presence of a large fraction of free phenolic compounds co-extracted during pectin precipitation, as shown in Fig. 2. The high antioxidant activity of the PME-treated pectins relative to the commercial pectins, may be related to the extensive de-esterification promoted by the enzyme, which lowered the DM from 52 to 60% to below 15% in the PME-treated ones (Table 2). Removing methyl esters may expose free carboxyl groups and unmasks neighboring hydroxyl groups, increasing the dissociable, electron-donating and hydrogen-donating sites of the polymer. Since ABTS and FRAP rely on single electron transfer and ORAC on hydrogen atom transfer, these groups raise the capacity to scavenge radicals and reduce ferric ions, with the carboxyl groups also favoring chelation in FRAP, consistent with the degree of esterification dependence reported by Naqash et al. (2021). The absence of this enhancement in DPPH agrees with the same explanation, since there the de-esterified pectins precipitate and their groups become less accessible. Nonetheless, as the effect of the phenolic compounds co-extract was not completely isolated from the intrinsic antioxidant activity from PME-treated pectins, they may further contribute for the results observed (Fernandes & Coimbra, 2023). Overall, both MAE and enzymatic de-esterification may contribute to the antioxidant activity of pectin used as a dietary fiber, either by enabling the co-extraction of phenolic compounds or by exposing carboxyl and hydroxyl groups able

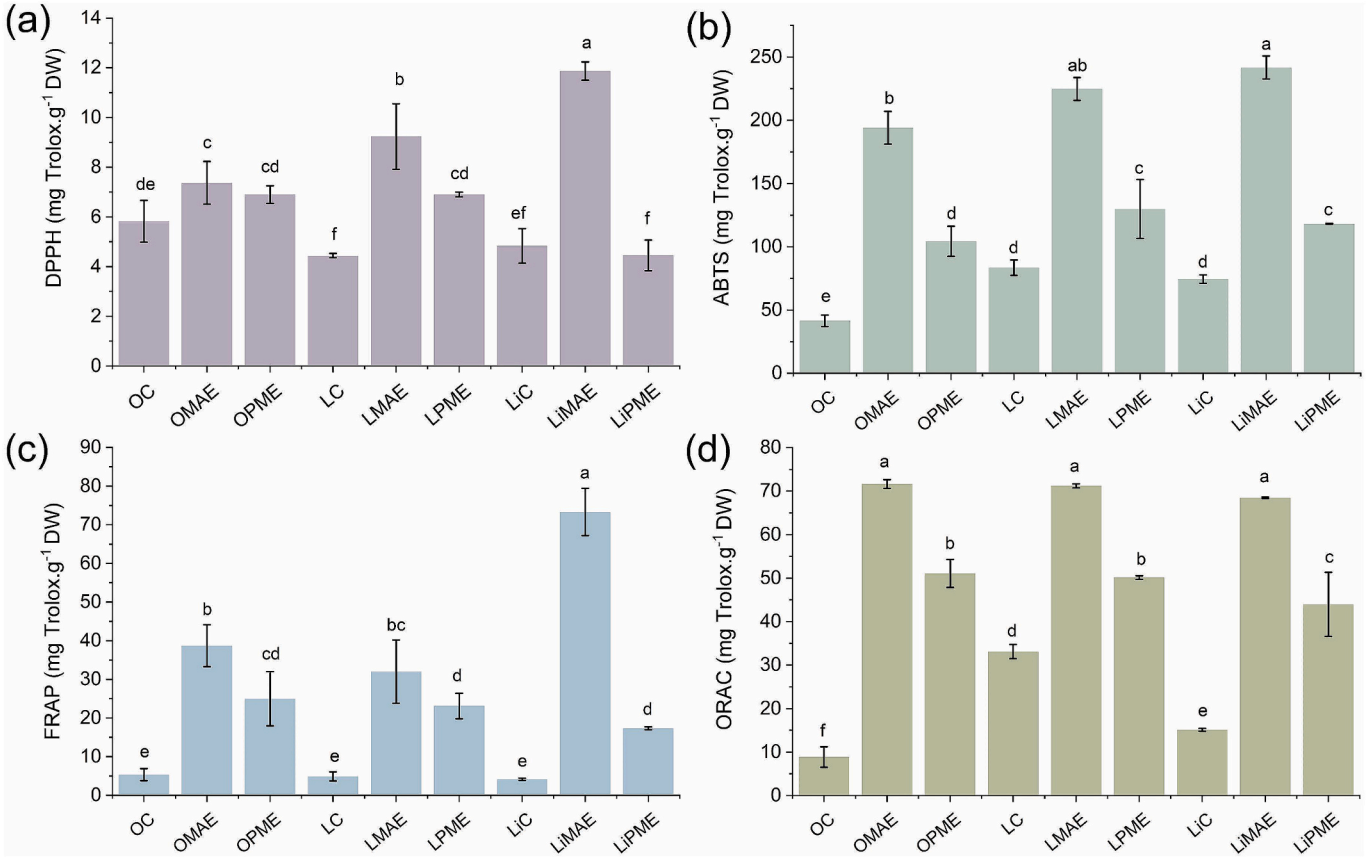

Fig. 4. Comparative of the antioxidant activity from the different citrus pectins including orange (O), lemon (L), and lime (Li) peels obtained by MAE-microwave assisted extraction, PME-treated with pectin methyl esterase, and compared with C-commercial pectins determined by different methods including DPPH (a), ABTS (b), FRAP (c), and ORAC (d). Different letters represent statistically significant means (Fisher, $p < 0.05$).

to donate electrons and hydrogen atoms to radicals (Wang et al., 2025).

3.3. Functionality of citrus pectin

In dietary fibers, water holding capacity (WHC) and oil holding capacity (OHC) are important physicochemical properties that influence technological and physiological properties. WHC reflects the ability of the pectin to withhold water under stress, preventing foodstuffs dehydration and promoting intestinal motility (He et al., 2022). OHC reflects its ability to retain oil or fat in the intestinal lumen, contributing to reduced serum cholesterol (Cui et al., 2019). Regardless of the source, MAE-pectins showed the lowest WHC ($p < 0.05$) compared with commercial pectins, while PME de-esterification significantly increased it (Fig. 5), indicating that the degree of esterification strongly influenced the WHC likely due to the free carboxyl groups, available for hydrogen bonding (Table 2). WHC values for the PME-treated pectins (7.57 ±

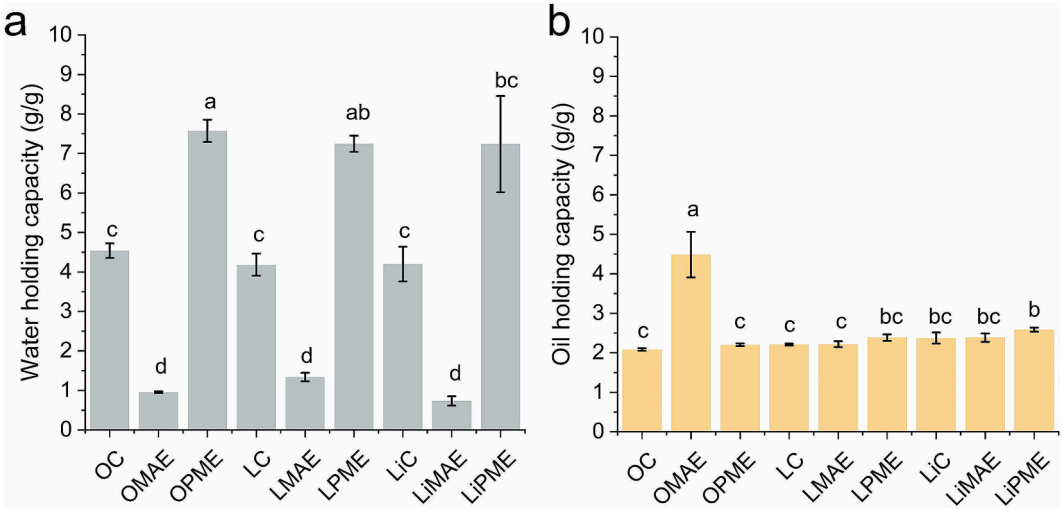

Fig. 5. (a) Water holding capacity (WHC), and (b) oil holding capacity (OHC) for commercial (C), microwave-assisted extraction (MAE), and enzymatically de-esterified (PME) pectins obtained from orange (O), lemon (L), and lime (Li) peels.

0.28, 7.24 ± 0.20, and 7.23 ± 1.20 g/g for OPME, LPME, and LiPME) were close to that reported for acidic MAE lemon peel pectin Wani and Patidar (2025). OHC was statistically similar results across the sources and processes, except for OMAE, which presented the highest value (4.49 ± 0.59 g/g; $p < 0.05$). Since no significant chemical structural feature of OMAE differentiates its behavior from that of other MAE-pectins, the main difference observed in OHC may be plausibly attributed to physical properties such as material porosity, which facilitates mechanical entrapment of oil in capillaries. These OHC values agree with reported values from lemon pectin (Wani & Patidar, 2025) and sweet lemon pectin (Sharma et al., 2023), and other factors such as bulk density, surface characteristics, hydrophobicity, and the microstructure of pectin may contribute to OHC values (Cui et al., 2019).

The performance of the different MAE and PME-treated pectins on the emulsifying properties was compared with the correspondent commercial pectin in function of the creaming index, as indirect measurement of emulsion stability (Fig. 6). Concentration was crucial factor, in which raising it from 1 to 3% favored the emulsifying properties of commercial and PME pectins, which behaved similarly, with no evident phase separation or creaming over 24 days of storage (Fig. 6a, b, c). The MAE-pectins, showed a short-term stability, with coalescence after 24 days, evidenced by larger oil droplets and agglomeration (Fig. 6).

The structure-stability relationship was summarized by PCA (Fig. S2), whose first two components explained 85% of the variance (PC1 53%, PC2 32%) and separated the samples by process type. The PME pectins grouped on the positive side of PC1, aligned with the emulsion stability vector, HG, and MR1, indicating that their extensive de-esterification and high density of free carboxyl groups improved stability, most plausibly through electrostatic repulsion that limits droplet coalescence (Wan et al., 2019). The commercial pectins, although highly esterified (DM 52 to 60%), were equally stable but separated on a different axis linked to DAc and Mn, suggesting that they stabilize through acetylation rather than charge, as also reported for apple pectin (Naqash et al., 2021). In contrast, the MAE pectins occupied the opposite side, their instability associated with RG-I, branching (MR_2), and lower Mn; consistent with this, Yang et al. (2018) reported that highly branched RG-I domains create steric hindrance that hinders acetyl group interaction with oil droplets. DM did not align with stability and pointed toward both groups, showing that stability cannot be attributed to DM alone. Overall, these results highlight that PME-treated pectins exhibit a more stable emulsifying performance compared to MAE-treated pectins, with concentration and structural characteristics playing a key role in emulsion stability.

Complementarily, as the viscosity of pectin in solution can also influence emulsion stability and the performance of the polymer as a thickening agent, the apparent viscosity of the different pectins was compared at 3% (Fig. 7) and the flow curves were described with the power law and the Herschel-Bulkley models (Table S1).

The three pectin types exhibited distinct flow behavior. The commercial pectins were highly viscous and only weakly shear-thinning, with flow indices close to unity ($n = 0.88$ to 0.91) and no measurable yield stress, making the power law suitable for describing their flow. In contrast, the MAE pectins showed the lowest viscosity and consistency index ($K = 0.002$ to 0.008 Pa·s^n) and a flow index near one ($n = 0.93$ to 1.01), indicating essentially Newtonian behavior. This differs from the shear-thinning behavior reported for acidic MAE pectins by Yu et al. (2024) and Hosseini et al. (2019). PME pectins behaved markedly differently, exhibiting pronounced shear-thinning ($n = 0.49$ to 0.68) and

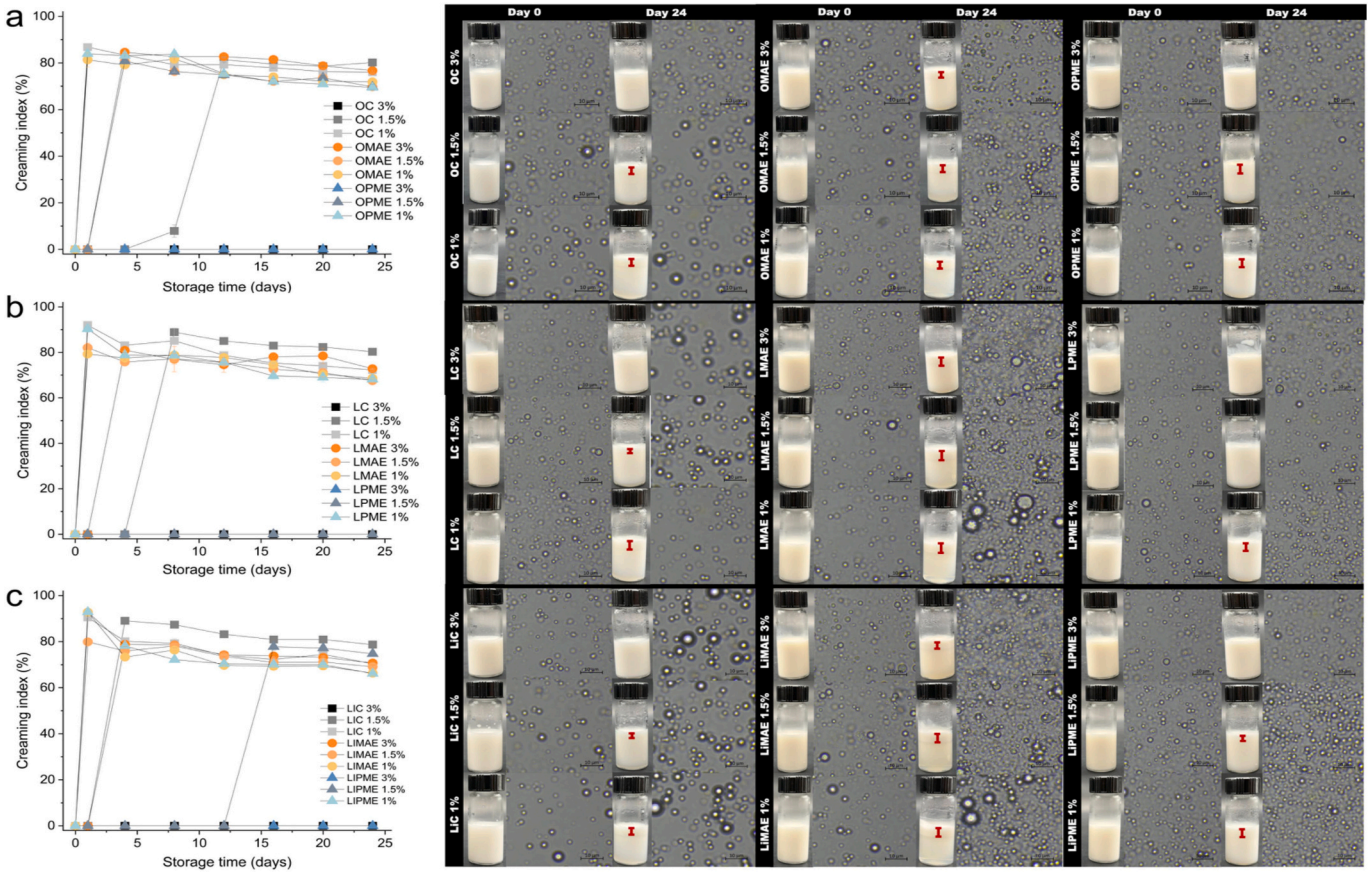

Fig. 6. Creaming index over 24 days, visual evaluation of emulsion stability for commercial (C), microwave-assisted extraction (MAE), and enzymatically de-esterified (PME) pectins obtained from (a) orange (O), (b) lemon (L), and (c) lime (Li) peels over time (day 0 and 24th) and their respective optical microscopy images. Bars in red represent the cream layer formation.

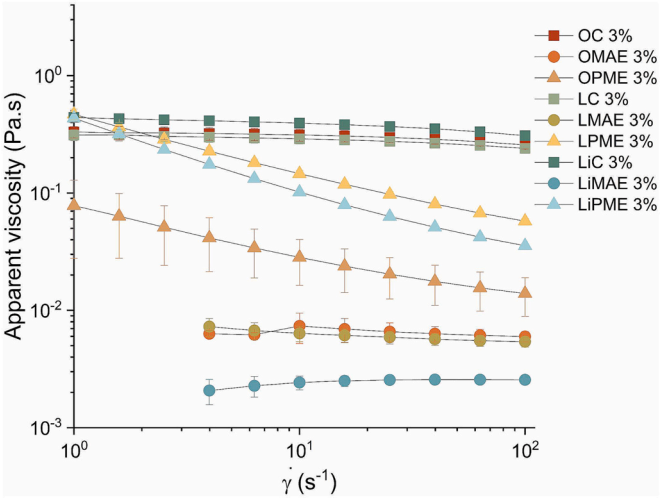

Fig. 7. Apparent viscosity for commercial (C), microwave-assisted extraction (MAE), and enzymatically de-esterified (PME) pectins obtained from orange (O), lemon (L), and lime (Li) peels.

being the only samples with a measurable yield stress ($\tau 0 = 0.04$, 0.16, and 0.29 Pa for OPME, LPME, and LiPME), suggesting the presence of a weakly structured network.

As PME pectins were obtained by enzymatic de-esterification of MEA pectins, a treatment that reduces methyl ester content while largely preserving molecular weight, the observed differences can be attributed to the degree of esterification (DE). Lowering the DE increased the consistency index and transformed a nearly Newtonian fluid into a shear-thinning material with a weak yield stress. This agrees with the inverse relationship between viscosity and DE reported for citrus pectins by Morris et al. (2000) and with the established role of DE governing pectin solution behavior (Löfgren et al., 2002). The low viscosity and flexible, coiled conformation of the MAE pectins, which may limit the accessibility of hydrophobic groups (Schmidt et al., 2015), are consistent with their poor emulsifying performance. Conversely, the more structured rheological behavior of PME pectins likely contributes to their improved emulsion stability. Overall, these results demonstrate that reducing DE enhances the thickening and structuring capacity of acid-free MAE pectins, making de-esterification essential for their effective use as thickening agents.

4. Conclusion

This study demonstrates that acid-free hydrothermal microwave-assisted extraction (MAE) is a practical and sustainable approach for recovering pectins from citrus byproducts while achieving competitive yields without the need for external acids. Among the conditions tested, 160 °C for 5 min was chosen for orange, lemon, and lime peels, based on the highest extraction yields and shorter extraction time, besides a satisfactory property such as methylation and acetylation degree, and phenolic compounds co-extraction. The citrus source played a key role in determining pectin characteristics. Even under the same extraction conditions, pectins from orange, lemon, and lime exhibit distinct differences in esterification patterns, carbohydrate profile, and temperature sensitivity. In general, the extracted pectins behaved as high-methoxyl polysaccharides with broad molar mass distributions and higher average molar mass than commercial pectins, suggesting that the acid-free method contributes to the preservation of structural integrity compared to commercial pectins. Subsequent enzymatic de-esterification using pectin methylesterase (PME) proved effective in further tailoring pectins functionality. The treatment significantly reduced the degree of methylation, enhanced water holding capacity, and improved emulsion stability compared to MAE derived pectins. FTIR analysis confirmed the observed esterification trends, while thermogravimetric analysis indicated slightly lower thermal stability for PME-treated pectins relative to commercial ones. In addition, PME treatment induced a transition in the flow behavior from nearly Newtonian to shear-thinning, which is desirable for thickening and emulsifying applications in food systems while preserves a satisfactory antioxidant property. Overall, the combination of acid-free hydrothermal MAE and enzymatic de-esterification provides a flexible and environmentally friendly strategy for producing citrus pectins with tunable properties.

CRediT authorship contribution statement

Pamela F.M. Pereira: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Amparo Jiménez-Quero:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors declare the use of Grammarly to improve linguistic quality and readability of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.carbpol.2026.125653.

Data availability

Data will be made available on request.

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