



# Valorization of apple pomace: Structural and rheological characterization of low-Methoxyl Pectins extracted with green agents of citric acid/sodium citrate

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## ABSTRACT

Traditional production of low-methoxyl pectin (LMP) is labor-intensive and environmentally harmful. This study explores using citric acid/sodium citrate as a green extractant for valorizing LMPs from apple pomace. Two types of pectin, AP-5 and AP-8, were extracted at pH 5.0 and 8.0, resulting in LMPs with methoxylation degrees of 42.33 % and 34.68 %, respectively. Monosaccharide composition and SEC-MALLS analysis revealed that APs are rich in arabinose side chains and contain heterogeneous fractions, while FTIR spectra confirmed their low-methoxyl structure. Compared to the commercial low-methoxyl pectin, APs exhibited higher critical pH but lower critical  $\text{Ca}^{2+}$  levels for acid- and  $\text{Ca}^{2+}$ -induced gels, where hydrogen bonding and electrostatic interactions were the dominant intermolecular forces. Notably, AP-8 and AP-5 demonstrated superior stability in acid- and  $\text{Ca}^{2+}$ -induced gels. Additionally, combined acidic and  $\text{Ca}^{2+}$  conditions enhanced the gel strength and stability of APs. These findings highlight the potential of APs in developing novel, particularly low-calorie, food products.

## 1. Introduction

The global apple industry generates approximately 4 million tons of apple pomace annually, a byproduct of juice extraction and cider making (Kausar et al., 2024). Despite being considered waste, apple pomace is rich in valuable compounds, particularly pectin, making it a prime candidate for valorization (Morales-Contreras et al., 2020). Pectin, a complex polysaccharide found in plant cell walls, is widely used in the food, pharmaceutical, and cosmetic industries due to its gelling, thickening, and stabilizing properties (Roman-Benn et al., 2023). Pectin consists of linear chains of  $\alpha$ -(1 → 4)-linked D-galacturonic acid units, some of which are methyl-esterified. This structural variability classifies pectin into high-methoxyl pectin (HMP) and low-methoxyl pectin (LMP) (Singh et al., 1997). Traditionally, HMP is mostly extracted from citrus peel or apple pomace using mineral acids (e.g. hydrochloric acid and nitric acid), while LMP is produced by the de-methoxylation of HMP, a process that is both labor-intensive and environmentally harmful (El-Nawawi & Heikal, 1995).

To address these challenges, there is growing interest in developing direct extraction methods for LMP, which could simplify its production and enhance sustainability. While green and eco-friendly methods for

pectin extraction have been widely reported, most approaches rely on mineral or organic acids to adjust the extraction pH, typically within a highly acidic range (pH 1–3) (Oliveira et al., 2016; Panwar et al., 2022; Su et al., 2019). In our previous study, we proposed a new pectin extraction method using Generally Recognized as Safe (GRAS) agents—citric acid and sodium citrate—as green extractants. This method combines citric acid and sodium citrate to create a broader, more neutral pH range for extraction. It not only addresses the limitations of highly acidic conditions in previous methods but also enables a one-step process to produce LMP, improving both extraction efficiency and reducing environmental impact compared to conventional processes (He et al., 2021). Despite the potential of citric acid/sodium citrate-based extraction methods for direct LMP extraction, comprehensive studies on the rheological properties of such LMP are still lacking.

LMP is particularly valuable in food formulations for its ability to form gels in the presence of calcium ions ( $\text{Ca}^{2+}$ ), unlike HMP, which requires low pH and high concentrations of co-solutes (Chan et al., 2017). This property makes LMP ideal for low-calorie products and applications requiring mild gelling conditions (Wan et al., 2021). The gelling capacity of LMP, however, is greatly influenced by its source and extraction method (Cui et al., 2021). While  $\text{Ca}^{2+}$ -induced gelation of

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LMP is well-documented, our previous studies suggest that LMP can also form gels at low pH (He et al., 2021). However, the acid-induced gelling capacity of LMP has not been widely studied and requires further exploration. Understanding the  $\text{Ca}^{2+}$ - and acid-induced gelling behavior of citric acid/sodium citrate-extracted LMP is essential for optimizing its use in various applications.

In this study, the citric acid/sodium citrate-based green extraction method was introduced for the valorization of pectin from apple pomace. The aim is to investigate the potential of extracting LMP from apple pomace using citric acid/sodium citrate as green extractants and to analyze the structural and rheological properties of the extracted LMP. By addressing these research objectives, this proof-of-concept study aims to advance sustainable apple pomace valorization and pectin production, meeting the evolving demands of the food industry for innovative and eco-friendly ingredients.

## 2. Materials and methods

### 2.1. Pectin extraction

Fuji apples were used in this study, with pomace collected post-juicing. Fuji apples were selected for this study based on their large-scale cultivation in China and their relatively high pectin content, making them an ideal choice for exploring the pectin extraction process (Zhou et al., 2023). This selection also ensures reproducibility and relevance for future research and industrial applications. Fresh apple pomace was used to ensure controlled conditions and minimize variability associated with industrial processing techniques. In this study, citric acid and sodium citrate were used as extracting agents due to their well-known “green” and eco-friendly properties. Citric acid is a naturally occurring organic acid, widely available from renewable sources and biodegradable, making it an environmentally sustainable option. Similarly, sodium citrate, as the sodium salt of citric acid, shares these properties and is also biodegradable and non-toxic. Both agents are affirmed as GRAS for use as food ingredients by the Food and Drug Administration (FDA) (He et al., 2021).

The apple pomace was air-dried overnight at 60 °C and then ground and passed through a 40-mesh sieve to obtain a fine powder for pectin extraction. The extraction followed the method detailed in our prior research (He et al., 2021), employing a citric acid/sodium citrate solution (CSS) as a green extractant. Briefly, apple pomace was mixed with 0.1 M CSS adjusted to pH 5.0 and 8.0, respectively, at a ratio of 1:25 (w/v). The extraction was conducted at 85 °C for 3 h. After extraction, the mixture was centrifuged at 8990g for 15 min, separating pectin extracts in the supernatant. Pectin in the extract was precipitated using anhydrous ethanol (v/v, 1:1.5), and subsequently separated via centrifugation at 8990g for 10 min, followed by washing with anhydrous ethanol and oven-drying at 60 °C overnight. The pectin extracted using CSS at pH 5.0 and 8.0 was denoted as AP-5 and AP-8, respectively. Additionally, commercial low-methoxyl apple pomace pectin (A001831, Andre), labeled as LAP, was included for comparative analysis in subsequent tests.

### 2.2. Physicochemical properties

The degree of methoxylation (DM) of pectins was determined using the titration method (Liu et al., 2010). The total sugar content was determined using the phenol-sulfuric method (Dubois et al., 1956), and protein content was determined by the Bradford method (Bradford, 1976). Additionally, the pH of pectin solutions (0.1 % w/v) was measured at 25 °C using a pH meter (PHS-3C, Leici, Shanghai, China).

### 2.3. Monosaccharide composition

The monosaccharide composition of pectins was determined according to a previously reported method (Zhu et al., 2021). Pectins were

hydrolyzed with 2 M trifluoroacetic acid at 121 °C for 2 h. The resulting monosaccharides were analyzed by high-performance anion-exchange chromatography (HPAEC, ICS5000, Thermo Fisher Scientific, USA) equipped with a pulsed amperometric detector (PAD, Dionex, USA) and a CarboPac PA-20 anion-exchange column (3 × 150 mm, Dionex, USA). The mobile phase consisted of solvent A (double-distilled water), solvent B (0.1 M NaOH) and solvent C (0.1 M NaOH and 0.2 M NaAc). The gradient program was as follows: the volume ratio of solvent A, B, and C was 95:5:0 at 0 min, 85:5:10 at 26 min, 85:5:10 at 42 min, 60:0:40 at 42.1 min, 60:40:0 at 52 min, 95:5:0 at 52.1 min, and 95:5:0 at 60 min. The flow rate was 0.5 mL/min.

### 2.4. Molecular weight

The determination of molecular weight of pectins was conducted using size exclusion chromatography (SEC) coupled with multi-angle static light scattering (MALS) and a differential refractive index (RI) detector, following a previously reported method (Z. Zheng et al., 2019). Briefly, pectins were dissolved in a 0.1 M  $\text{NaNO}_3$  solution containing 0.02 %  $\text{NaN}_3$  to achieve a concentration of 1 mg/mL. The pectin solutions were then filtered through a 0.45  $\mu\text{m}$  membrane filter before injection into the SEC system, which consisted of a U3000 system (Thermo, USA), a DAWN HELEOS-II detector (Wyatt Technology Co., USA), and an Optilab T-rEX RI detector (Wyatt Technology Co., USA). Isocratic elution was performed with a mobile phase of 0.1 M  $\text{NaNO}_3$  solution at a flow rate of 0.3 mL/min on tandem columns (300 × 8 mm, Shodex OH-pak SB-805 and 803, Showa Denko K.K., Tokyo, Japan) maintained at 45 °C. The molar mass was calculated using a  $\text{dn}/\text{dc}$  value of 0.141 mL/g.

### 2.5. Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of pectins were recorded by a Vertex70 spectrometer (Bruker, Germany). Briefly, pectins were mixed with dehydrated KBr, ground, and pressed into pellets. The sample pellets were then scanned over the range of 4000–400  $\text{cm}^{-1}$  by the spectrometer.

### 2.6. Rheological properties

The rheological properties of pectins were evaluated by a rotational rheometer (DHR-1, TA Instruments) equipped with a 40 mm parallel plate, according to a previously reported method with minor modification (Li et al., 2023). Briefly, pectins were completely dissolved in distilled water to a concentration of 3 % (w/v). The prepared pectin solutions were then divided into four groups: (1) control groups: pectin solutions without any treatment; (2) acid-induced gelling groups: pectin solutions adjusted to pH 2.5–5.0, respectively; (3)  $\text{Ca}^{2+}$ -induced gelling groups: pectin solutions supplemented with calcium chloride ( $\text{CaCl}_2$ ) to achieve the final  $\text{Ca}^{2+}$  concentration of 0.62–16.67 mM, respectively; (4) acid- and  $\text{Ca}^{2+}$ -induced gelling group: pectin solutions adjusted to pH 4.0 and 5.0 in the presence of  $\text{Ca}^{2+}$  (4.67 mM), respectively. For each measurement, AP solutions/gels were loaded onto the parallel plate with a 1 mm gap. Silicone oil was used to seal the gap between the plate and the sample to minimize water evaporation during measurement. The rheological behavior of pectin solutions/gels under different conditions was investigated using the following tests.

#### 2.6.1. Flow sweep test

A flow sweep test was conducted over a shear speed of 0.1–100  $\text{s}^{-1}$  at 25 °C, with an equilibrium time of 300 s, to determine the apparent viscosity and shear stress during steady shear. Viscosity parameters including the consistency coefficient (K) and flow behavior index (n), were calculated using the Power-law model:

$$\sigma = K\dot{\gamma}^n$$

where  $\sigma$  represents the shear stress (mPa), and  $\dot{\gamma}$  represents the shear speed ( $s^{-1}$ ). In this model, K is an important indicator of the fluid's resistance to deformation, with a higher K value corresponding to a higher viscosity of the fluid, while n indicates whether the fluid is pseudoplastic ( $n < 1$ ), Newtonian ( $n = 1$ ), or dilatant ( $n > 1$ ).

### 2.6.2. Amplitude sweep test

An amplitude sweep test was performed over a strain range of 0.01–1000 % at an angular frequency of 1.0 Hz with an equilibrium time of 120 s at 25 °C, to identify the linear viscoelastic region. Changes in  $G'$  (storage modulus) and  $G''$  (loss modulus) were recorded during the measurement.

### 2.6.3. Temperature sweep

A temperature sweep was conducted over the range of 0–40 °C at a ramp rate of 2.0 °C/min at an angular frequency of 1.0 Hz with an equilibrium time of 300 s, to record changes in  $G'$  and  $G''$  during the measurement. The strain was determined based on the linear viscoelastic region identified via the amplitude sweep.

### 2.6.4. Frequency sweep

A frequency sweep was performed over the angular frequency range from 100 to 0.1 rad/s with an equilibrium time of 120 s at 25 °C, to record changes in  $G'$  and  $G''$  during the measurement.

## 2.7. Intermolecular interaction analysis of pectin gels

The intermolecular force of pectin gels was analyzed according to the method reported by Yan et al. (2021). Urea and sodium chloride were used to evaluate the role of hydrogen bonds (H-bonds) and electrostatic interactions in the pectin gelation. Briefly, 1 M urea and 1 M NaCl were respectively added during the preparation of the acid-induced and  $Ca^{2+}$ -induced AP-5, AP-8 and LAP gels, respectively, and the gel-form capacity is characterized by an inversion test.

## 2.8. Statistic analysis

All experiments were conducted in triplicate, and the results were expressed as means  $\pm$  standard deviations (SD). Statistical analysis was performed using the SPSS software (version 20.0, SPSS Inc., Chicago, USA), employing one-way analysis of variance (ANOVA) with Tukey's post hoc test to identify significant differences ( $p < 0.05$ ) among the samples.

## 3. Results and discussion

### 3.1. Physicochemical properties

In this study, two apple pomace pectins (APs), AP-5 and AP-8, were extracted using CSS at pH 5.0 and 8.0 from apple pomace. The extraction yields were 6.13 % for AP-5 and 11.73 % for AP-8, indicating that extraction pH significantly affects yield. This finding is consistent with previous studies showing that higher extraction pH leads to higher yields, likely due to enhanced extraction of RG-I pectic polysaccharides under alkaline conditions, whereas acidic conditions may cause degradation and removal of these polysaccharides (Yilmaz-Turan et al., 2023). Although the extraction yields for AP-5 (6.13 %) and AP-8 (11.73 %) are comparable to yields reported in similar studies (Wang & Lü, 2014; Zhou et al., 2023), further optimization of the extraction process, including extraction time, temperature, and solvent ratios, could potentially increase the yield for industrial applications. The physicochemical properties of APs are presented in Table 1. Both AP-5 and AP-8 had a low protein content (less than 1 %) and a high total sugar content (84–88 %), comparable to commercial low-methoxyl apple pomace pectin (LAP). AP-5 exhibited a higher protein content but a lower total sugar content than AP-8. The protein content of pectin reflects its purity, as proteins are often co-extracted during the extraction process (Wang & Lü, 2014). The lower protein content and higher total sugar content in AP-8 compared to LAP indicates that AP-8 is a purer form of pectin. This difference may be attributed to the more efficient extraction conditions used for AP-8, which reduced the co-extraction of proteins and other impurities. The degree of methoxylation (DM) was 41.43 % for AP-5 and 33.65 % for AP-8, classifying them as low-methoxyl pectins. When dissolved in water, AP-5 and AP-8 solutions displayed nearly neutral pH values of 5.70 and 6.81, respectively, compared to the more acidic pH of 3.79 for the LAP solution. This suggests that the carboxylic acid groups in AP-5 and AP-8 may be present in ionized forms rather than in the free form, thus increasing their pH (He et al., 2021).

### 3.2. Monosaccharide composition

The monosaccharide composition of APs is summarized in Table 1. APs were found to contain six monosaccharides: D-galacturonic acid, arabinose, rhamnose, glucose, galactose, xylose, and fucose (Fig. 1A), consistent with those found in APs reported in other studies (Calvete-Torre et al., 2021; Chen & Lahaye, 2021; Wang & Lü, 2014). However, variations in monosaccharide composition were observed among different APs. For AP-5 and AP-8, D-galacturonic acid was the predominant monosaccharide, constituting 72.74 % and 46.12 % of the total composition, respectively, followed by arabinose at 12.51 % and 27.22 %, respectively. Notably, the arabinose content in AP-5 and AP-8

**Table 1**  
Physicochemical properties and chemical composition of pectins.

Sample	Yield (%)	Protein content (%)	Total sugar content (%)	DM (%)	pH	Monosaccharide composition (%)						HG (%)	RG-I (%)	Mn (kDa)	Mw (kDa)	PDI	
						Rha	Ara	Gal	Glc	Fuc	Xyl						GalA
AP-5	6.13 $\pm$ 0.16 <sup>a</sup>	0.77 $\pm$ 0.02 <sup>b</sup>	84.00 $\pm$ 0.11 <sup>c</sup>	42.33 $\pm$ 0.91 <sup>a</sup>	5.70 $\pm$ 0.03 <sup>b</sup>	2.44	12.51	3.42	7.39	0.59	0.91	72.74	70.3	20.81	52.55	207.93	1.86
AP-8	11.73 $\pm$ 0.32 <sup>b</sup>	0.56 $\pm$ 0.01 <sup>c</sup>	88.39 $\pm$ 0.18 <sup>a</sup>	34.68 $\pm$ 1.03 <sup>c</sup>	6.81 $\pm$ 0.10 <sup>a</sup>	6.01	27.22	8.00	9.67	0.62	2.37	46.12	40.11	47.24	24.73	218.90	3.96
LAP	–	0.88 $\pm$ 0.01 <sup>a</sup>	86.55 $\pm$ 0.24 <sup>b</sup>	39.23 $\pm$ 1.41 <sup>b</sup>	3.79 $\pm$ 0.04 <sup>c</sup>	4.17	3.37	5.24	2.60	0.45	3.42	80.75	76.58	16.95	174.60	320.63	8.85

Means in each column with different lowercase letters are significantly different ( $p < 0.05$ ). “–” indicates not applicable. Abbreviations: Rha (rhamnose), Ara (arabinose), Gal (galactose), Glc (glucose), Fuc (fucose), Xyl (xylose), and GalA (D-galacturonic acid). The molar percentage of HG and RG-I was calculated as the following formulas: HG (%) = GalA (mol %) – Rha (mol %) and RG-I (%) = 2Rha (mol %) + Ara (mol %) + Gal (mol %) (He et al., 2021).

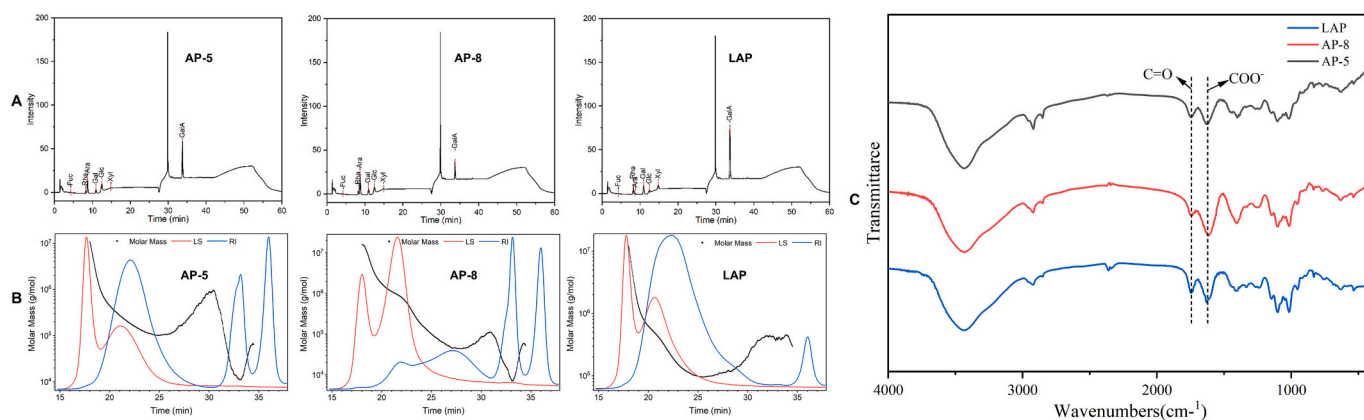


Fig. 1. The monosaccharide composition (A), molecular weight distribution (B) and FTIR spectra (C) of pectins.

was higher than reported in other studies, suggesting that these two extracted APs may have arabinan and/or arabinogalactan-rich side chains (Calvete-Torre et al., 2021; Wang & Lü, 2014). Additionally, they exhibited relatively higher glucose content at 7.39 % and 9.67 %, respectively, possibly due to residual free sugars from the apple pomace or co-extracted non-pectic polysaccharides (e.g., hemicelluloses and cellulose) during extraction (He et al., 2021). Other monosaccharides in AP-5 constituted less than 5 %, while AP-8 displayed relatively higher contents of galactose (8.00 %) and rhamnose (6.01 %). In comparison, LAP exhibited the highest content of D-galacturonic acid at 80.75 %, with neutral monosaccharides constituting less than 5 % (except for galactose at 5.24 %). This suggests that LAP had fewer branches in the pectin molecule, as neutral monosaccharides are mainly present in the pectin branches. The relative content of homogalacturonan (HG) and rhamnogalacturonan-I (RG-I) further supported this observation. AP-5 and AP-8 showed higher RG-I content than LAP, with AP-8 having the highest RG-I content at 47.24 %, suggesting that AP-8 could be highly branched (Zhang et al., 2018). The preservation of side chains in APs is due to the citric acid/sodium citrate used in their extraction, which has a lower dissociation constant compared to the mineral acids used in the extraction of LAP. This lower hydrolyzing capacity leads to less depolymerization and better preservation of the pectin side chains (RG-I and RG-II regions) (Marić et al., 2018).

### 3.3. SEC-MALLS analysis

The molecular weight distribution of APs is illustrated in Fig. 1B. AP-5 and AP-8 exhibited a multimodal distribution pattern, suggesting the presence of heterogeneous polysaccharide fractions with varying molecular weights. This pattern is consistent with the molecular weight distribution of citrus peel pectin extracted using CSS in a prior study (He et al., 2021). In contrast, LAP displayed a unimodal distribution pattern, indicating the presence of a uniform polysaccharide fraction. The calculated number average molecular weight ( $M_n$ ), weight average molecular weight ( $M_w$ ), and polydispersity index (PDI,  $M_w/M_n$ ) are listed in Table 1. LAP demonstrated the highest  $M_n$  and  $M_w$  at 174.60 kDa and 320.63 kDa, respectively. AP-5 and AP-8 showed similar  $M_w$  values at approximately 210 kDa, but AP-5 exhibited a higher  $M_n$  (174.60 kDa) compared to AP-8 (24.73 kDa). The PDI, which measures the broadness of the molecular weight distribution, further elucidated differences in weight distribution among the samples (Liang et al., 2022). LAP exhibited the lowest PDI at 1.86, indicating a relatively narrow weight distribution. In contrast, AP-5 and AP-8 displayed significantly higher PDIs at 3.96 and 8.85, respectively, suggesting broader weight distributions and the presence of heterogeneous fractions. This broader distribution in AP-5 and AP-8 is likely due to the absence of further purification processes, such as column chromatography, in this study.

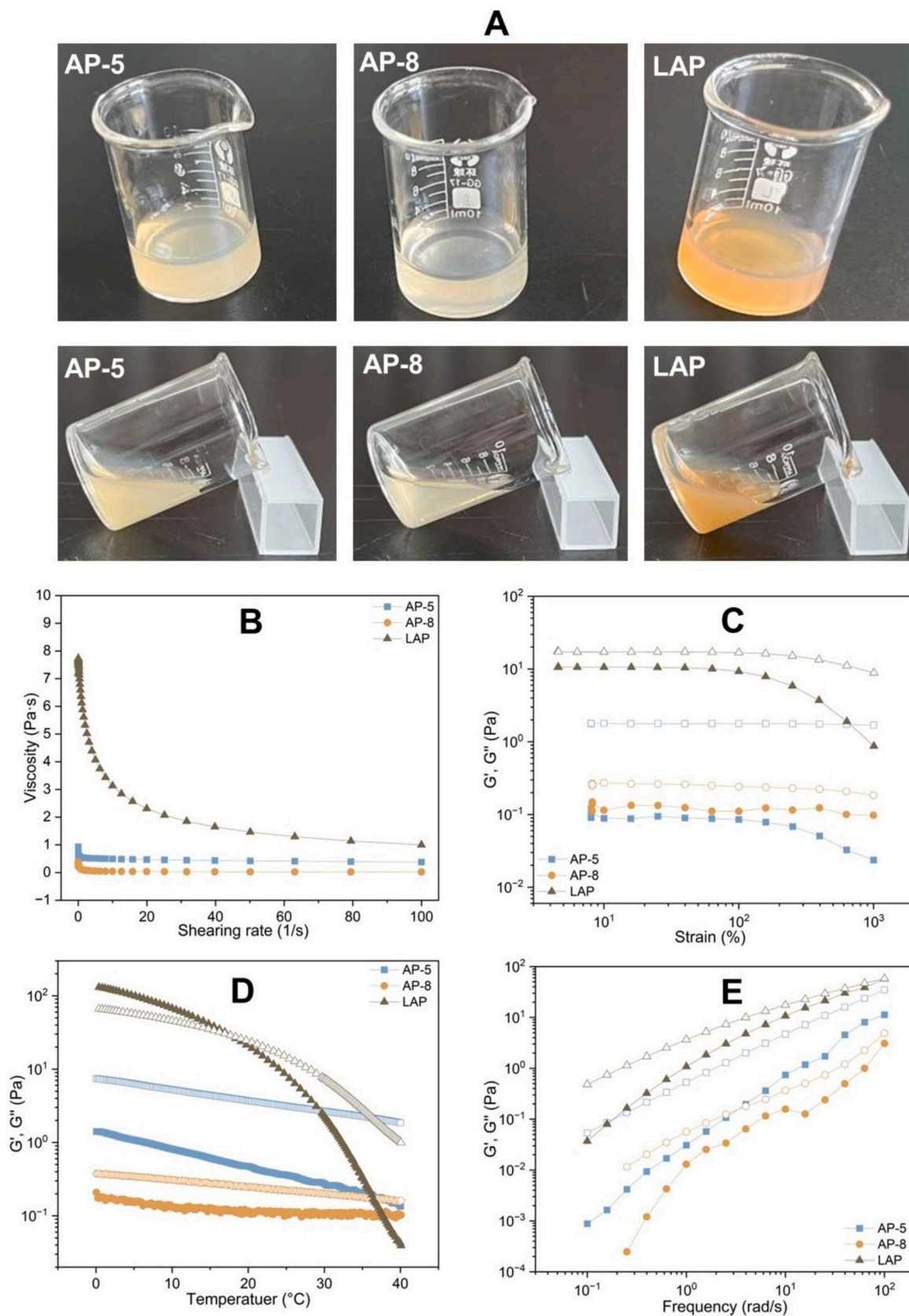
### 3.4. FTIR

The FTIR spectra of APs are shown in Fig. 1C. All pectin samples showed characteristic absorption peaks at  $1630\text{ cm}^{-1}$  and  $1750\text{ cm}^{-1}$ , corresponding to the stretching vibrations of ionic carboxylic groups ( $\text{COO}^-$ ) and methyl-esterified carboxyl groups ( $\text{C=O}$ ), respectively. The peak area for  $\text{C=O}$  was lower than that for  $\text{COO}^-$  in both APs and LAP, particularly in AP-8, indicating that the carboxylic groups in APs and LAP are less methyl-esterified, confirming their low degree of methoxylation (Oliveira et al., 2016). This observation is consistent with their DM values determined by the titration method. Additionally, APs exhibited variability in the absorption band at  $1300\text{--}800\text{ cm}^{-1}$ , attributed to pyranose cycle vibrations of monosaccharides, which is considered the fingerprint region of pectin (Wang & Lü, 2014). This variability corresponds to differences in their monosaccharide composition.

### 3.5. Rheological properties of APs

To evaluate the thickening and gelling capacities of APs, the rheological behavior of pectin solutions (3 %, w/v) is characterized and the result is shown in Fig. 2. At this concentration, all pectin solutions were unable to form gels at room temperature, manifesting instead as viscous solutions (Fig. 2A). In the flow sweep test (Fig. 2B), as the shear rate increased, the apparent viscosity of pectin solutions significantly decreased, exhibiting shear-thinning behavior. The flow curves of shear stress vs. shear rate were fitted by a Power-law model, which provides a consistency coefficient ( $K$ ) and flow behavior index ( $n$ ) that determines the viscosity behavior of the hydrocolloid. Based on the  $K$  value, the viscosity of the AP solutions followed the order:  $\text{LAP} > \text{AP-5} > \text{AP-8}$  (data not shown), indicating that AP solutions had lower viscosity than LAP solutions. The difference in apparent viscosity may be attributed to their molecular weight, as pectin with a higher molecular weight generally exhibits higher apparent viscosity than those with a lower molecular weight (Cui et al., 2020; Wang et al., 2014).

In the amplitude sweep test (Fig. 2C), the loss modulus ( $G''$ ) of pectin solutions consistently exceeded the storage modulus ( $G'$ ), indicating that pectin solutions exhibited viscoelastic liquid characteristics. Similarly, during the frequency sweep test (Fig. 2D), as the frequency increased, both  $G''$  and  $G'$  of pectin solutions gradually increased, with  $G''$  consistently surpassing  $G'$ , further indicating viscoelastic liquid behavior of the pectin solutions. In the temperature sweep test (Fig. 2E), with increasing temperature, both  $G''$  and  $G'$  of pectin solutions gradually decreased, with  $G''$  consistently surpassing  $G'$ . However, LAP exhibited an exception. When the temperature was below  $17\text{ }^\circ\text{C}$ ,  $G'$  exceeded  $G''$ , exhibiting characteristics of elastic solids. But when the temperature exceeded  $17\text{ }^\circ\text{C}$ ,  $G''$  exceeded  $G'$ , indicating a phase transition of the sample from gel to liquid, consistent with its liquid state at room temperature ( $25\text{ }^\circ\text{C}$ ).



**Fig. 2.** Appearance (A), flow sweep test (B), amplitude sweep test (C), frequency sweep test and temperature sweep test (D) of pectin solutions (3 %, w/v). Symbols: solid symbols represent  $G'$  (storage modulus), and open symbols represent  $G''$  (loss modulus).

This suggests that LAP solution exhibited certain thermal reversibility.

Overall, the pectin solutions did not spontaneously form gels. Our previous study showed that low-methoxyl citrus peel pectin extracted by CSS exhibited excellent acid and  $\text{Ca}^{2+}$ -induced gelling capacity (He et al., 2021). Therefore, to further evaluate the processing potential of the extracted APs, the rheological properties of APs under acidic and  $\text{Ca}^{2+}$ -supplemented conditions were characterized.

### 3.5.1. Acid-induced rheological properties of APs

To characterize the acid-induced gelling capacity of APs, an acid-induced gelation test was conducted within the pH range of 2.5 to 5.5. As shown in Fig. 3A, when the pH of AP and LAP solutions was above 2.9 and 2.5, respectively, none of the pectin samples formed gels. However, when the pH decreased to 2.9 for AP-5 and AP-8 and to 2.5 for LAP, all pectin solutions form gels. Therefore, the critical acid-gelling pH for AP-5 and AP-8 was determined to be 2.9, whereas for LAP, it was 2.5. This finding aligns with previous studies, which reported that lowering the pH below 3 leads to the formation of strong gels in LMP, as the acid-induced gelation of LMPs, mediated by hydrogen bonding, only occurs at  $\text{pH} \leq 3$  (Lootens et al., 2003; Yuliarti et al., 2017).

Above the critical acid-gelling pH (3.0–5.5), a significant thickening effect was observed in the pectin solutions, indicated by the increased consistency coefficient (K) with pH reduction in the flow sweep test. As shown in Table 2, when the pH decreased from 5.5 to 3.0, the K value of AP-5 and AP-8 solutions increased from 0.21 and 0.25 to 5.06 and 5.61, respectively, while for LAP, it increased from 0.10 to 8.11, indicating a higher acid-induced viscosity than AP solutions. The flow behavior index (n) of all pectin solutions remained less than 1, indicating retained pseudoplasticity.

Within the critical acid-gelling pH range (2.5–2.9), pectin solutions started to gel but exhibited different behaviors. In the frequency sweep test (Fig. 3B), for AP-5, at pH 2.9, the solution formed a gel as indicated by  $G'$  exceeding  $G''$ . However, at high frequencies,  $G''$  gradually surpassed  $G'$ , indicating a potential collapse of its gel network structure, transitioning to viscoelastic fluid characteristics. For AP-8,  $G'$  consistently exceeded  $G''$  even at high frequencies, indicating a more stable gel state compared to AP-5. For LAP, at pH 2.7 and 2.9,  $G''$  exceeded  $G'$ , preventing gel formation. Only at pH 2.5 did  $G'$  surpass  $G''$ , confirming a lower critical acid-gelling pH, consistent with the acid-induced gelation test.

The thermal stability of acid-induced gels varied among the pectins. In the temperature sweep test (Fig. 3C), the AP-8 acid-induced gel demonstrated the strongest thermal stability, with minimal changes in  $G'$  and  $G''$  upon temperature change and  $G'$  consistently exceeding  $G''$ . In contrast, AP-5 and LAP acid-induced gels were susceptible to temperature changes, with significant decreases and phase transitions observed in  $G'$  and  $G''$  during heating. For AP-5 acid-induced gels, at pH 2.7 and pH 2.9,  $G'$  exceeded  $G''$  below 74.6 °C and 34.1 °C, respectively, but  $G''$  surpassed  $G'$  at higher temperatures, indicating thermal reversibility. Similarly, at pH 2.7 and pH 2.9, LAP acid-induced gels exhibited thermal reversibility with much lower temperatures of 12.5 °C and 18.4 °C, respectively, remaining liquid at room temperature (25 °C). The acid-induced gelation of low-methoxy pectin has been shown to be thermally reversible, with this phase transition primarily governed by the conformational changes in the pectin chains during heating and cooling cycles (Gilsenan et al., 2000).

A gelation test using a hydrogen bonding inhibitor (urea) and electrostatic interaction inhibitor (NaCl) further demonstrates the intermolecular interaction in acid-induced AP gels. As shown in Fig. 3D, at pH 2.5, AP solutions failed to form gels with the presence of 1 M urea but maintained their gel state with 1 M NaCl. This indicates that hydrogen bonding, rather than electrostatic interactions, is the dominant intermolecular force in acid-induced AP gels, consistent with the previous study (Yuliarti et al., 2017). Conversely, the gelation of LAP was significantly inhibited by both urea and NaCl, suggesting that both hydrogen bonding and electrostatic interactions play critical roles in

acid-induced LAP gels. Additionally, this result suggests that the gel stability of acid-induced AP gels is higher than that of LAP, as it is not affected by the electrostatic interaction inhibitor.

Compared to HMP, the rheological properties of LMP are more sensitive to acidic environments, resulting in an increase in viscosity or even gel formation at low pH (Fang et al., 2008; Mierczyńska et al., 2017). This heightened sensitivity is due to the high density of non-methyl-esterified carboxylic acid groups in LMP, which provide more sites for hydrogen bonding (Vincent et al., 2013). Although LMP can also exhibit acid-induced gelation, it requires more stringent conditions; specifically, the pH of low-methoxyl pectin solutions must be lower than the pKa values of uronic acid residues in pectin (approximately pH 2.9) (Ralet et al., 2001). The acid-induced rheological changes in LMP are attributed to the protonation of carboxylic acid groups in pectin. Under acidic conditions, the protonation of these groups increases intermolecular hydrogen bonding, leading to more associations and entanglement, and ultimately, gel formation (He et al., 2021). Additionally, the neutral side chains in pectin can offer more attachment points for intermolecular entanglement, which is more favorable than electrostatic repulsion between galacturonic acid chains (Gawkowska et al., 2018). However, gels formed from pectin with fewer branch chains tend to have decreased gel stability due to a less-entangled gel network with fewer inter-chain connections (Kaczmarek et al., 2024). This explains why APs with higher branch chains (RG-I region) exhibit overall better acid-induced gelation and gel stability compared to LAP.

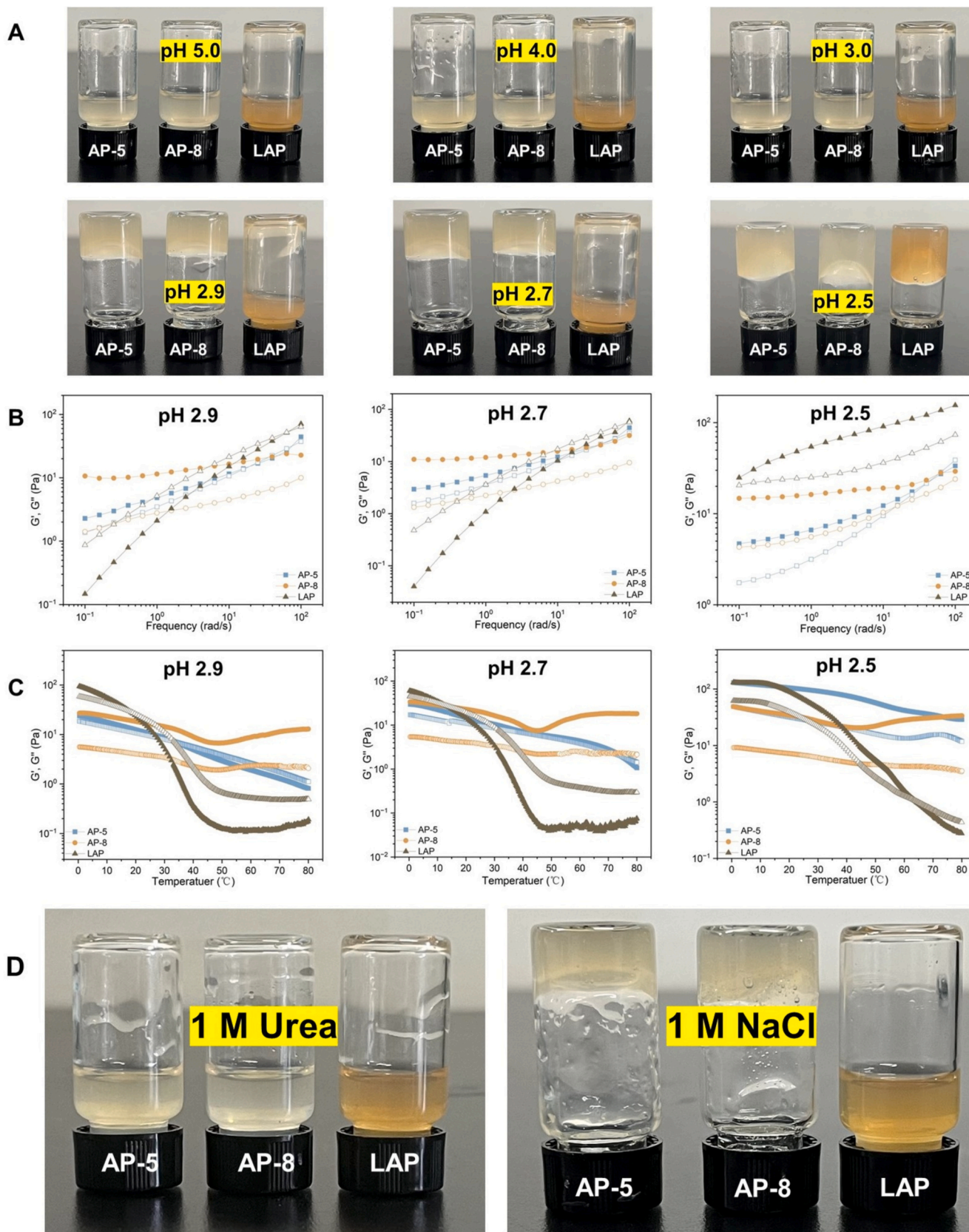
### 3.5.2. $\text{Ca}^{2+}$ -induced rheological properties of APs

To characterize the  $\text{Ca}^{2+}$ -induced gelling capacity of APs, a  $\text{Ca}^{2+}$ -induced gelation test was conducted within the  $\text{Ca}^{2+}$  concentration range of 0.62 to 16.67 mM. As shown in Fig. 4A, none of the pectin samples formed gels when the  $\text{Ca}^{2+}$  concentration of AP-5, AP-8, and LAP solutions was 0.62, 2.44, and 4.76 mM, respectively. However, when the  $\text{Ca}^{2+}$  concentration increased to 1.23, 4.76 and 9.09 mM for AP-5, AP-8 and LAP, respectively, all pectin solutions formed gels. Therefore, the critical  $\text{Ca}^{2+}$ -induced gelling concentration was determined to be in the ranges of 0.62–1.23 mM for AP-5, 2.44–4.76 mM for AP-8, and 4.76–9.09 mM for LAP.

At low  $\text{Ca}^{2+}$  concentration (0.62–2.44 mM), a significant thickening effect was observed in the pectin solutions, indicated by the increased consistency coefficient (K) with increasing  $\text{Ca}^{2+}$  concentration in the flow sweep test. As shown in Table 2, when the  $\text{Ca}^{2+}$  concentration increased from 0.62 mM to 2.44 mM, the K value of AP-8 and LAP solutions increased from 0.06 and 7.02 to 0.11 and 10.41, respectively, while for AP-5, it dramatically increased from 0.36 to 39.54, indicating a higher  $\text{Ca}^{2+}$ -induced viscosity than AP-8 and LAP at low  $\text{Ca}^{2+}$  concentration. The flow behavior index (n) of all pectin solutions remained less than 1, indicating retained pseudoplasticity.

At high  $\text{Ca}^{2+}$  concentrations (4.76–16.67 mM), pectin solutions started to gel but exhibited different behaviors. In the frequency sweep test (Fig. 4B), with the addition of  $\text{Ca}^{2+}$  at 4.76 mM, AP-5 and AP-8 solutions formed gels as indicated by  $G'$  exceeding  $G''$ . However, For LAP,  $G''$  surpassed  $G'$ , indicating the prevention of gel formation, although  $G'$  was higher than  $G''$  at high frequency. When the concentration of  $\text{Ca}^{2+}$  increased to 9.09 mM, the  $G'$  and  $G''$  of all pectin gels significantly increased with  $G'$  consistently exceeding  $G''$ , indicating enhanced gel strength. In particular, the  $G'$  and  $G''$  of LAP dramatically increased and were even higher than those of AP-5 and AP-8, indicating the gel formation of LAP was quite susceptible to  $\text{Ca}^{2+}$  concentration. When the concentration of  $\text{Ca}^{2+}$  further increased to 16.67 mM, the  $G'$  and  $G''$  of all pectin gels further increased, and both  $G'$  and  $G''$  showed minimal changes with increasing frequency, indicating a stronger gel strength and gel stability at such high concentration of  $\text{Ca}^{2+}$ . The order of  $G'$  and  $G''$  values followed the sequence LAP > AP-5 > AP-8, suggesting LAP's superior gelation capacity at high concentrations of  $\text{Ca}^{2+}$ .

The thermal stability of  $\text{Ca}^{2+}$ -induced gels varied among the pectins. In the temperature sweep test (Fig. 4C), the AP-5  $\text{Ca}^{2+}$ -induced gel



**Fig. 3.** Gelation test (A), frequency sweep test (B), temperature sweep test (C) and intermolecular force analysis (D) of acid-induced gelling properties of pectins. Symbols: solid symbols represent  $G'$  (storage modulus), and open symbols represent  $G''$  (loss modulus).

**Table 2**  
Rheological parameters of pectins under different conditions.

Condition	Sample	K (Pa·s <sup>n</sup> )	n
pH 3.0	AP-5	5.06 ± 0.27 <sup>c</sup>	0.49 ± 0.01 <sup>a</sup>
	AP-8	5.61 ± 0.85 <sup>b</sup>	0.18 ± 0.02 <sup>b</sup>
	LAP	8.11 ± 0.31 <sup>a</sup>	0.50 ± 0.01 <sup>a</sup>
pH 3.5	AP-5	4.25 ± 0.05 <sup>b</sup>	0.55 ± 0.00 <sup>b</sup>
	AP-8	3.25 ± 0.35 <sup>c</sup>	0.18 ± 0.02 <sup>c</sup>
	LAP	5.85 ± 0.21 <sup>a</sup>	0.58 ± 0.01 <sup>a</sup>
pH 4.0	AP-5	0.57 ± 0.01 <sup>a</sup>	0.82 ± 0.00 <sup>b</sup>
	AP-8	0.30 ± 0.04 <sup>b</sup>	0.62 ± 0.03 <sup>c</sup>
	LAP	0.11 ± 0.00 <sup>c</sup>	0.97 ± 0.00 <sup>a</sup>
pH 5.0	AP-5	0.25 ± 0.00 <sup>b</sup>	0.93 ± 0.00 <sup>b</sup>
	AP-8	0.26 ± 0.00 <sup>b</sup>	0.93 ± 0.00 <sup>b</sup>
	LAP	0.10 ± 0.00 <sup>a</sup>	0.99 ± 0.00 <sup>a</sup>
c(Ca <sup>2+</sup> ) = 2.44 mM	AP-5	39.54 ± 4.89 <sup>a</sup>	0.24 ± 0.02 <sup>c</sup>
	AP-8	0.11 ± 0.01 <sup>c</sup>	0.62 ± 0.01 <sup>a</sup>
	LAP	10.41 ± 0.32 <sup>b</sup>	0.51 ± 0.01 <sup>b</sup>
c(Ca <sup>2+</sup> ) = 1.64 mM	AP-5	8.09 ± 0.41 <sup>a</sup>	0.41 ± 0.01 <sup>c</sup>
	AP-8	0.08 ± 0.00 <sup>b</sup>	0.66 ± 0.01 <sup>a</sup>
	LAP	7.85 ± 0.28 <sup>a</sup>	0.55 ± 0.01 <sup>b</sup>
c(Ca <sup>2+</sup> ) = 1.23 mM	AP-5	2.79 ± 0.10 <sup>b</sup>	0.58 ± 0.01 <sup>b</sup>
	AP-8	0.07 ± 0.00 <sup>c</sup>	0.67 ± 0.01 <sup>a</sup>
	LAP	7.31 ± 0.28 <sup>a</sup>	0.55 ± 0.01 <sup>c</sup>
c(Ca <sup>2+</sup> ) = 0.62 mM	AP-5	0.36 ± 0.01 <sup>b</sup>	0.89 ± 0.00 <sup>a</sup>
	AP-8	0.06 ± 0.00 <sup>c</sup>	0.70 ± 0.00 <sup>b</sup>
	LAP	7.02 ± 0.25 <sup>a</sup>	0.56 ± 0.01 <sup>c</sup>

Means in each column under the same conditions with different lowercase letters are significantly different ( $p < 0.05$ ).

demonstrated the strongest thermal stability, with minimal changes in  $G'$  and  $G''$  upon temperature change and  $G'$  consistently exceeding  $G''$  at different  $\text{Ca}^{2+}$  concentration. In contrast, AP-8 and LAP  $\text{Ca}^{2+}$ -induced gels were susceptible to temperature changes, with significant decreases observed in  $G'$  and  $G''$  during heating with  $\text{Ca}^{2+}$  concentrations at 4.76 mM and 9.09 mM. Additionally, AP-8 and LAP  $\text{Ca}^{2+}$ -induced gel exhibited thermal reversibility at temperatures of 24.94 °C and 78.55 °C at  $\text{Ca}^{2+}$  concentrations of 4.76 mM and 16.67 mM, respectively. It is suggested that the pectin–calcium networks may introduce steric constraints on the conformational changes during the thermal phase transition. However, structural factors such as a high DM and branching may contribute to more flexible junction zones, facilitating thermally induced conformational changes, which could explain the differing thermal behavior among the various APs (Cardoso et al., 2003).

The dominated intermolecular force in  $\text{Ca}^{2+}$ -induced AP gels, as indicated by the gelatin test shown in Fig. 4D, was electrostatic interactions. This is evidenced by the fact that acid-induced gel formation for APs was significantly inhibited by 1 M NaCl rather than by 1 M urea. The  $\text{Ca}^{2+}$ -induced gelation is a typical characteristic of LMP, attributed to the interaction between  $\text{Ca}^{2+}$  and galacturonic acid in the HG region, leading to the formation of a cross-linking network due to the ionic bridge, as described by the typical “egg-box” model (Grant et al., 1973). Generally, the RG-I region did not support the egg-box formation even though it contains non-methoxylated galacturonic acid residue (Zheng et al., 2020). However, our study indicated that APs with a higher proportion of RG-I region exhibited higher  $\text{Ca}^{2+}$ -induced gelling capacity and gel stability than LAP. This could be attributed to the arabinose side chains in the RG-I region, which act as substitution preventing the efficient packing of the chains. They also form entanglements through hydroxyl groups, thereby strengthening the junction zones

during gel formation (Zheng et al., 2020). This is also confirmed by the observed decrease in gel strength of  $\text{Ca}^{2+}$ -induced AP gels in the presence of urea in this study (data not shown).

### 3.5.3. $\text{Ca}^{2+}$ -induced rheological properties of APs under acidic conditions

APs have shown significant acid- and  $\text{Ca}^{2+}$ -induced rheological change in the above results. However, in real food matrices, acidic and  $\text{Ca}^{2+}$  conditions may occur simultaneously. Therefore, a further study on the rheological properties of AP-5 and AP-8 under the combined influences of an acidic environment (pH 4.0 and 5.0) and  $\text{Ca}^{2+}$  (4.76 mM) was conducted. As shown in Fig. 5A, with  $\text{Ca}^{2+}$  addition at 4.76 mM, AP-5 and AP-8 can form stable gels at pH 4.0 and 5.0, in contrast to the observation that both AP-5 and AP-8 were unable to form gels at these pH levels without  $\text{Ca}^{2+}$  (Fig. 3A). This suggests that the addition of  $\text{Ca}^{2+}$ , even at a low concentration (4.76 mM), can greatly improve their gelation properties.

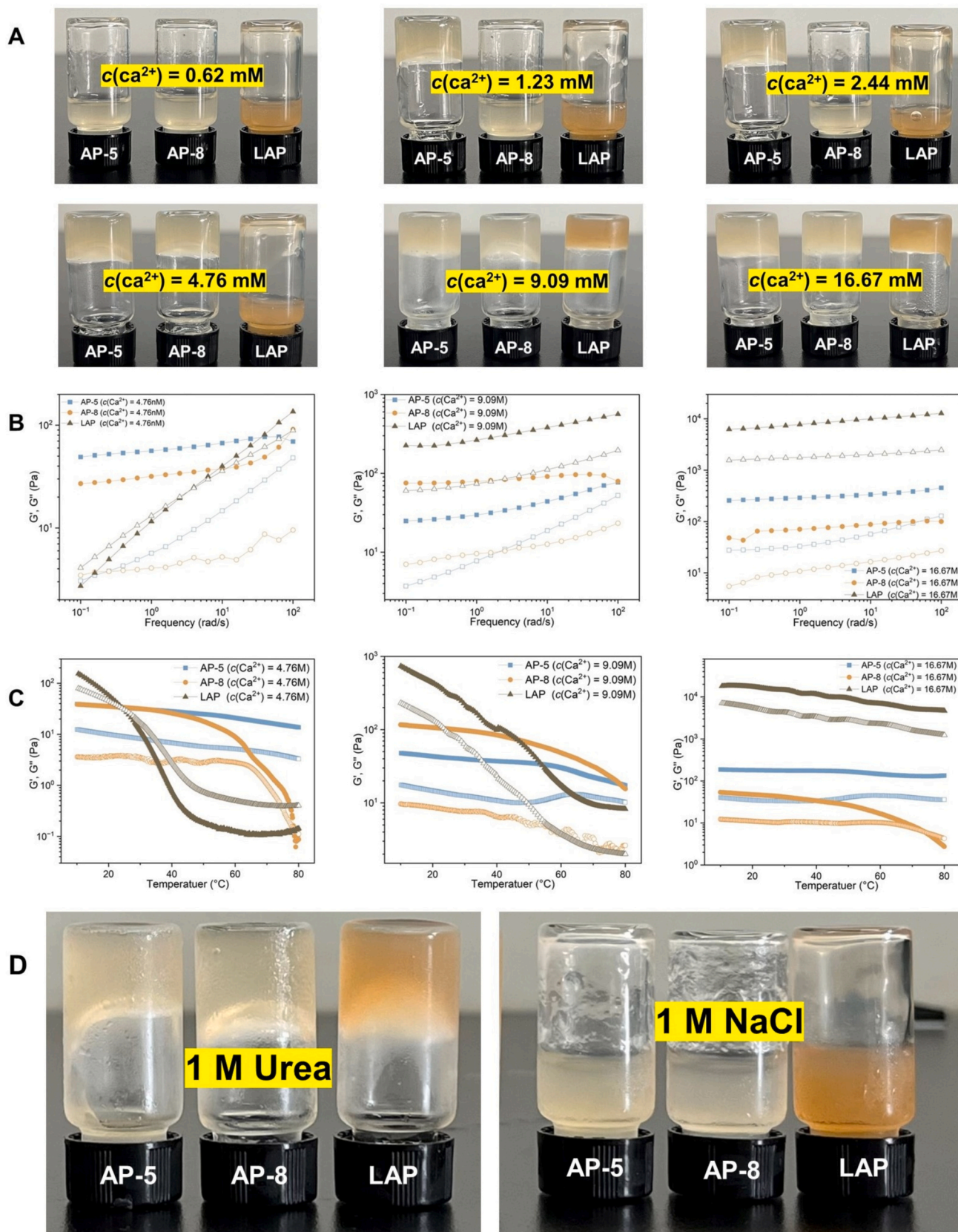
The frequency sweep test (Fig. 5B) indicated that the acidic conditions at pH 4.0 and 5.0 also improved the rheological properties of the  $\text{Ca}^{2+}$ -induced AP gels, resulting in higher  $G'$  and  $G''$  values compared to  $\text{Ca}^{2+}$ -induced AP gels without pH adjustment. Furthermore,  $\text{Ca}^{2+}$ -induced AP gels showed higher  $G'$  and  $G''$  values at pH 4.0 than at pH 5.0, indicating enhanced gel strength in a more acidic environment. Similarly, enhanced gel strength was also indicated in the temperature sweep test (Fig. 5C). Overall, AP-8 gels exhibited higher  $G'$  and  $G''$  values than AP-5 gels under the same conditions, suggesting a greater gelling capacity under the combined influences of acidic environment and  $\text{Ca}^{2+}$ . This synergy between acidic conditions and  $\text{Ca}^{2+}$  in strengthening the gel network aligns with findings from other studies (Wan et al., 2019; Yuliarti et al., 2017), highlighting the potential for optimizing pectin gel properties through controlled adjustment of pH and  $\text{Ca}^{2+}$  concentrations.

Considering the different intermolecular forces dominating the acid- and  $\text{Ca}^{2+}$ -induced gelling properties, synergistic effects on the rheological changes of AP gels have been confirmed in this study. Similar synergistic gelling properties were observed in enzymatically deesterified pectin, where pectin gels are supported by hydrophilic and hydrophobic interactions at low pH. However, as the pH increases to 4.0, these interactions become minor, and the pectin gel formation transitions to  $\text{Ca}^{2+}$ -induced complexation (Hua et al., 2018). In this study, at pH 4.0 and 5.0, the synergistic AP gels were constructed by the  $\text{Ca}^{2+}$ -induced cross-linking network, which is further “compressed” and strengthened under acidic conditions. This “synergistic” pectin gel allows for more tolerant conditions for gel formation, such as relatively low  $\text{Ca}^{2+}$  addition levels and higher pH, without requiring high levels of co-solutes as needed in HMP-based foods. This approach has potential applications in designing novel foods, such as low-calorie products.

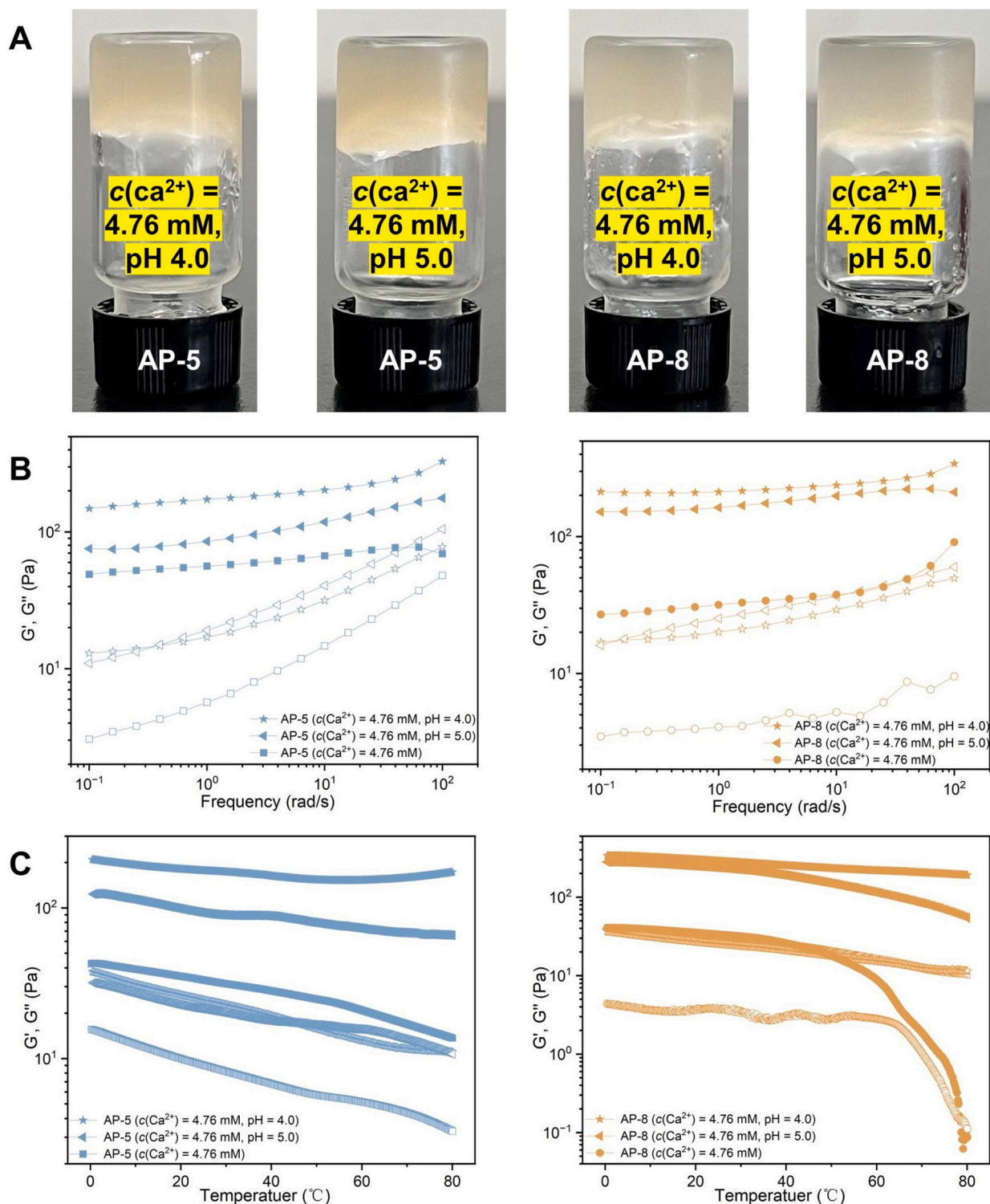
## 4. Conclusion

This study successfully extracted two low-methoxyl pectins, AP-5 and AP-8, from apple pomace using citric acid/sodium citrate as green extractants. The structural differences in monosaccharide composition, molecular weight distribution, and FTIR spectra of the APs were characterized. Specially, the rheological properties demonstrated that APs had significant thickening and gelling capacities influenced by both acidic and  $\text{Ca}^{2+}$  environments. Compared to LAP, APs exhibited superior gelation properties at lower  $\text{Ca}^{2+}$  concentrations and higher acidic pH, likely due to their higher proportion of neutral side chains. Additionally, the combination of acidic conditions and  $\text{Ca}^{2+}$  further enhanced the gel strength and stability, indicating synergistic effects. These findings highlight the potential of APs as valuable food ingredients with tailored rheological properties, providing opportunities for the development of novel food products, particularly low-calorie options. Future studies should explore the application of these APs in various food matrices to further understand their functional properties and optimize their usage in the food industry.





**Fig. 4.** Gelation test (A), frequency sweep test (B), temperature sweep test (C) and intermolecular force analysis (D) of  $Ca^{2+}$ -induced gelling properties of pectins. Symbols: solid symbols represent  $G'$  (storage modulus), and open symbols represent  $G''$  (loss modulus).



**Fig. 5.** Gelation test (A), frequency sweep test (B), temperature sweep test (C) of  $\text{Ca}^{2+}$ -induced gelling properties of APs at pH 4.0 and 5.0. Symbols: solid symbols represent  $G'$  (storage modulus), and open symbols represent  $G''$  (loss modulus).

#### CRediT authorship contribution statement

**Rui Guo:** Writing – original draft, Project administration. **Rong Fan:** Methodology, Formal analysis, Data curation. **Jiaxuan Hu:** Validation, Investigation. **Xinyue Zhang:** Visualization, Software. **Lin Han:** Supervision, Resources. **Min Wang:** Writing – review & editing, Resources. **Caian He:** Writing – review & editing, Funding acquisition.

#### 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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## Data availability

Data will be made available on request.

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