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Comparative rheology and antioxidant potential of high-methoxyl sugar acid gels of unrefined powder and acid-extracted pectin from two hawthorn (*Crataegus pinnatifida*) fruit cultivars

Mengmeng Guo [a,b,1], Kang Xu [a,b,1], Josephine Yee [b], John R. Dutcher [b], Mario M. Martinez [b,c], Laura Roman [b,c,d,*]

[a] Key Laboratory of Food Nutrition and Healthy in Universities of Shandong, College of Food Science and Engineering, Shandong Agricultural University, Tai'an 271000, China
[b] Department of Physics, University of Guelph, 50 Stone Road East, Guelph, Ontario, N1G 2W1, Canada
[c] Center for Innovative Food, Department of Food Science, Aarhus University, AgroFood Park 48, Aarhus N, 9200, Denmark
[d] Food Technology Area, Department of Agricultural and Forestry Engineering, University of Valladolid, Av. Madrid 50, 34004, Palencia, Spain

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ABSTRACT

Hawthorn fruits (*Crataegus pinnatifida*) present high content of high-methoxyl pectin, able to gel under high-sugar acidic conditions. In this work, the proximate and phytochemical composition of two cultivars of hawthorn fruit and the gelling ability of their unrefined (not further processed) dried powders and their extracted pectins were evaluated and systematically compared with citrus pectins (CP1 and CP2). Mianqiu (MI), a less known cultivar, showed two-fold higher pectin content and titratable acidity than Dajinxing (DA), one of the most common cultivars. DA showed higher starch, insoluble dietary fiber, pasting viscosity and total and extractable (EPP) phenolic compounds. EPP content was almost two-fold higher in DA than MI, resulting in stronger antioxidant properties. All high-methoxyl sugar acid gels exhibited a predominantly elastic response. MI resulted in hawthorn-powder gels with higher elastic modulus (G′) after gel-making (initially stronger gels), and lower G′ increase during storage (hardening) than DA. Citrus pectins (CP2 > CP1) showed higher gel-strength and faster gelling ability than hawthorn pectin gels (DA > MI) based on the lower G' and lack of gel formation after 90 min of cooling in hawthorn pectin-based gels. The gelation results were closely linked to the starch-to-pectin ratio, purity, and degree of methyl esterification.

1. Introduction

Hawthorn (*Crataegus* spp.) belongs to a large genus of small trees from the Rosacea family, whose fruits are widely consumed either fresh or processed into canned fruits, jams, jellies, candies, and soft drinks. Hawthorn fruits are rich in phenolic compounds, mainly flavonoids and oligomeric procyanidins, as well as dietary fiber constituents, namely pectic polysaccharides (Li et al., 2020, 2022). Due to the presence of these compounds, hawthorn consumption has been associated with several health benefits, including improving lipid metabolism, lowering blood pressure, cardio-vascular protection, antioxidant, anti-inflammatory, or anti-bacterial potential, among others (Alirezalu et al., 2020; Li et al., 2022; Yang & Liu, 2012).

Hawthorn fruit is especially rich in high-methoxyl pectic polysaccharides (~10–16 % w/w, dry basis), with galacturonic acid (GalA) ranging from 68 to 90 %, and, hence, composed of pectins rich in the smooth homogalacturonan domains, formed by long linear α-1,4-linked D-GalA chains, rather than in the hairy rhamnogalacturonan-I region (Chen, Qi, Zhu, & Wang, 2019; Cuevas-Bernardino, Lobato-Calleros, Román-Guerrero, Alvarez-Ramirez, & Vernon-Carter, 2016; Guo, Du, Jiang, Goff, & Cui, 2019; Roman et al., 2021). The structural features of hawthorn pectins, including their monosaccharide composition, chain conformation, molecular weight (M_w), degree of methyl-esterification (DM) and branching are closely related to the extraction procedure,

* Corresponding author. Food Technology Area, Department of Agricultural and Forestry Engineering, University of Valladolid, Av. Madrid 50, 34004, Palencia, Spain.
E-mail address: laura.roman.rivas@uva.es (L. Roman).
[1] These authors contributed equally.

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fruit cultivar and maturity state, which, in turn, determine the presence of co-extracted non-pectin polymers and plant secondary metabolites, as well as their functional properties (Basak & Annapure, 2022; Guo et al., 2019; Li et al., 2021; Linares-García, Ramos-Ramírez, & Salazar-Montoya, 2015; Roman et al., 2021). The fine structure of hawthorn pectins extracted under different methods and their antioxidant, film-forming and emulsifying properties have been extensively explored (Chen et al., 2019; Fu et al., 2024; Jiang, Du, Zhang, & Li, 2018; Roman et al., 2021). In this regard, high-methoxyl hawthorn pectins from *Crataegus pubescens, Crataegus sanguinea* and other undisclosed species were reported to possess higher viscosity in solution, and greater gelling capacity (up to three-fold higher), emulsifying and stabilizing properties than commercial citrus pectins (Cuevas-Bernardino et al., 2016; Guo et al., 2019; Linares-García et al., 2015). Because of the above-mentioned properties, hawthorn fruits are commonly used as stabilizer, thickener, and/or gelling agents in the preparation of some traditional hawthorn jellies and candies (Zhou et al., 2021). However, to date, there is no studies that thoroughly evaluate the natural gelling ability of dried hawthorn fruit powders, without the need for further pectin purification, in order to promote the utilization of less refined ingredients for their application by the food industry.

Gel formation in high-methoxyl pectins (DM >50 %) occurs in the presence of an acidic environment and a co-solute containing sugar (Evageliou, Richardson, & Morris, 2000; Löfgren, Guillotin, Evenbratt, Schols, & Hermansson, 2005). The presence of the co-solute reduces water activity, fostering hydrophobic interactions between methyl-ester groups of pectin molecules, while the low pH favors hydrogen bonds between pectin chains by suppressing electrostatic repulsion, enabling the formation of the gel network (Löfgren et al., 2005). Following this gelation mechanism, sweetened roll films or candies are manufactured under high-sugar acidic conditions (sugar >55 %, pH = 2.0–3.5), taking advantage of the natural acidic pH of unrefined hawthorn powders from dried fruits, rich in organic acids (Li et al., 2021; Liu et al., 2010). Thus, hawthorn gels are generally prepared by boiling a mixture of hawthorn fruit, water and sucrose, followed by cooling and drying. During gelling, pectin content, its structural features and the composition of hawthorn powders are critical as they will determine the rate and extent of the gelation process, and, in turn, the hardness and elasticity of the obtained gels and their aging during storage. Therefore, variations in the gelling and rheological properties of unrefined hawthorn fruit powders would not only depend on their pectin attributes but also on the presence and interactions with other non-pectin components coexisting in the fruits. Despite the strong dependence of composition and structure on the genotypical differences of hawthorn fruits (Liu et al., 2010; Liu, Kallio, Lü, Zhou, & Yang, 2011; Wen et al., 2015), no mechanistic studies evaluate the gelling ability of unrefined hawthorn fruit powders and their extracted pectins, from various *Crataegus pinnatifida* cultivars, for the formation of high-sugar acidic gels. The mechanistic understanding of the gelling performance of different cultivars could be particularly relevant for guiding hawthorn berry processors and food manufacturers into better utilizing and selecting hawthorn fruits for oriented thickening and gelling applications.

In this work, one of the most common cultivars of *Crataegus pinnatifida* in China, Dajinxing cultivar, and, Mianqiu, a less used cultivar gaining popularity for its larger fruits of softer texture, were thoroughly characterized for their proximate composition, phenolic fractions, antioxidant capacity, viscosity behavior and gelling performance. The gelling ability of hawthorn fruit dried powders and their high-methoxyl extracted pectins was evaluated in high-sugar acid gels through dynamic oscillatory rheology at different storage times, and systematically compared with commercial citrus pectins. The present work aims to understand the potential of using non-refined hawthorn powders as gelling agents to save costs related to pectin purification, while enhancing bioactivity associated with their endogenous polyphenols.

2. Materials and methods

2.1. Materials

Fresh hawthorn (*Crataegus pinnatifida*) berries from Mianqiu (MI) and Dajinxing (DA) cultivars (Fig. 1), supplied by Yijia Food Co. (Linqu, Shandong, China), were manually collected at the 20th week after full bloom day in the central mountainous region of Shandong province (36°04′ N - 36°37′N, 118°14′E – 118°49′E). Citrus pectins, ESS 4400 (CP1) and RS 4700 (CP2), with 67.9 and 79.9 % DM, respectively (Roman et al., 2021) were gently provided by Ceamsa (Porriño, Spain).

2,2-diphenyl-1-picrylhydrazyl, (DPPH), Folin & Ciocalteu's phenol reagent, 3-phenylphenol and galacturonic acid were obtained from Sigma-Aldrich (St. Louis, USA). 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) 2,2-azobis-(2-methylpropionamidine)-dihydro chloride and phosphate buffered saline (PBS 10X), were purchased from Thermo Fisher Scientific (Massachusetts, USA). Megazyme® Integrated total dietary fiber and total starch HK kits (Megazyme, Wicklow, Ireland) were used for quantitative analysis of total dietary fiber and starch, respectively. Tris buffer salt and Celite® were also purchased from Megazyme. All other reagents were at least of analytical grade.

2.2. Methods

2.2.1. Preparation of hawthorn powders

Hawthorn fruits from MI and DA cultivars were cut into slices (1–2 mm thickness) after manually removing the seeds and dried at 40 °C until moisture was below 10 %. Subsequently, dried slices were ground to powder using an electric grinder and sieved under a 270 μm mesh screen to obtain hawthorn powder fractions (MIF and DAF). Hawthorn powders were stored in sealed polyethylene bags at −18 °C until analysis.

2.2.2. Isolation of hawthorn crude pectins

Crude pectins from MI and DA hawthorn cultivars (MIP and DAP) were prepared following an acidic extraction method (Fig. S1), reported in Roman et al. (2021), where the compositional and structural information of the extracted pectin-rich residues was also analyzed. Detailed compositional information of the pectins, including monosaccharide composition, GalA, DM and M_w, is reported in Table S1. DA and MI

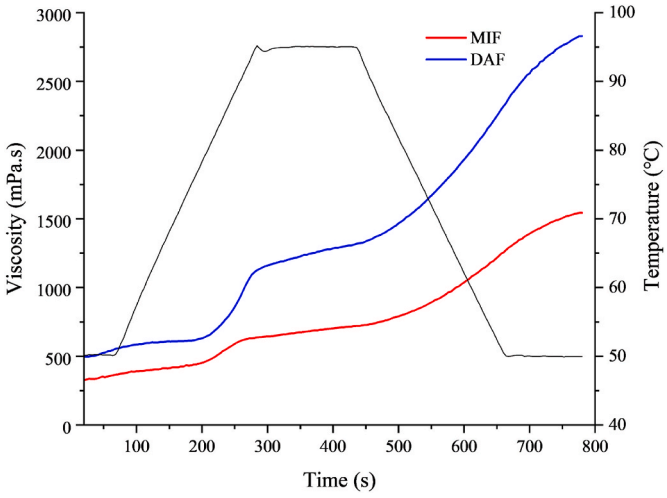

Fig. 1. Pasting profiles of two different hawthorn powders obtained from hawthorn fruit after a heating-cooling cycle. Temperature profile during the heating-cooling cycle is reported in black colored line. MIF: hawthorn powder obtained from Mianqiu (MI) fruit cultivar. DAF: hawthorn powder obtained from Dajinxing (DA) fruit cultivar.

cultivars exhibited similar pectin recovery (10.79–10.65 % w/w, respectively), while GalA varied in DAP (38.3 %) and MIP (61.2 %).

2.2.3. Compositional characterization of hawthorn powders

Moisture and total starch in hawthorn powders were determined following AACC 44–15.02 and 76–13.01 methods (AACC, 2015), respectively. Crude protein was determined following AACC 46–30.01 methods (AACC, 2015) using an automatic elemental analyzer (Leco, St. Joseph, USA) for nitrogen analysis, using a 6.25 factor to convert nitrogen into protein. Ash was analyzed according to AACC 08–01.01 method (AACC, 2015). Reducing sugars were extracted as in Pico et al. (2019) and measured as in Roman, Sahagun, Gomez, and Martinez (2019). Titratable acidity (TA) was determined by titration with 0.1 mol/L NaOH and results expressed as g of citric acid equivalents per 100 g (Li, Hu, & Xu, 2015). Pectin content was estimated using the *m*-hydroxydiphenyl method for GalA content (Kintner & Buren, 1982) using D-galacturonic acid as standard. Total dietary fiber (TDF), insoluble dietary fiber (IDF) and soluble dietary fiber precipitated with ethanol (SDFP) were measured according to AACC 32–45.01 and 32–50.01 methods (AACC, 2015). Proximal composition was expressed as weight percentage in dry basis (%, d.b.).

2.2.4. Pasting properties, water absorption capacity (WAC) and oil absorption capacity (OAC) of hawthorn powders

The viscosity profile of hawthorn fruit powders and their pasting properties (Table S2) were analyzed on a Rapid Visco Analyzer (RVA 4800, Perten Instruments, Sydney, Australia) using 3.5 g of hawthorn powder in 25 mL of distilled water following AACC method 61–02.01 (AACC, 2015). WAC and OAC were analyzed as follows; 10 mL distilled water or refined vegetable oil (Mazola, CPC International, USA) were added to 0.5 g of hawthorn powder, vortex-mixed and kept at 25 °C for 30 min. The resulting mixture was centrifuged at 2000×*g* for 10 min in a pre-weighed centrifuge tube and the supernatant was decanted. WAC and OAC were calculated as the weight difference between the initial sample weight and the weight after the removal of the non-bound water/oil. Results were expressed as g of water/oil bound per g of hawthorn powder (d.b.).

2.2.5. Visual evaluation of hawthorn powders and pectins

Hawthorn powders and pectins were imaged using a Canon EOS (1300D, Tokyo, Japan) to evaluate their macrostructure. Microstructure was observed using a NeoScope JCM-5000 scanning electron microscope (JEOL, Tokyo, Japan). Hawthorn powders and pectins were stuck to carbon tape on a 25 mm diameter specimen holder. Photomicrographs were taken in high-vacuum mode with a 5 kV accelerating voltage.

2.2.6. Phenolic fractions and antioxidant activity

Extraction and determination of extractable polyphenols (EPP), hydrolyzable non-extractable polyphenols (HPP) and non-extractable proanthocyanidins (NEPA) in hawthorn powders, as well as their DPPH and ABTS radical scavenging capacities (DPPH-RSC and ABTS-RSC, respectively), were performed following the procedure detailed in Pico et al. (2019). IC_{50} of EPP and HPP through both DPPH and ABTS activities is shown for comparison of the antioxidant ability of the phenolic fractions. However, only IC_{50} from DPPH is reported for NEPA because of its immiscibility with the aqueous PBS buffer of ABTS reagent (Pico et al., 2019). EPP and HPP fractions were expressed as mg of gallic acid equivalents per g of hawthorn powder (d.b.), while anthocyanins-NEPA fraction was expressed as mg of delphinidin equivalents per g of samples (d.b.). The antioxidant capacity, IC_{50}, indicates the phenolic concentration (mg of extract) needed to inhibit 50 % of the DPPH or ABTS activity. EPP fraction was also evaluated in the obtained high-sugar acidic gels and discussed in supplementary material (Table S3).

2.2.7. Preparation of high-sugar acid gels

High-sugar acidic gels were prepared at least in triplicate using the two cultivars of hawthorn powders (MIF and DAF) as well as their extracted pectins (MIP and DAP). Additional gels using CP1 and CP2 were also obtained for comparison. For the preparation of the gels from MIF and DAF hawthorn powders, 3.5 g of powders and 25 mL of distilled water were mixed for 30 min at 95 °C under continuous stirring in a hot plate (IKA Inc., Canada) equipped with a heating accessory for 50 mL tubes. Then, 15.0 g of sucrose were incorporated, followed by continuous stirring for 60 min. The hot pastes were quickly poured into plastic circular molds (12 disks of 20 mm diameter and 3.5 mm height), spread out evenly to fill the molds and left to cool for 90 min at 20 °C for gelling evaluation.

For the preparation of pectin-based gels from hawthorn and citrus pectins, gels were made following the method of Chen et al. (2019) and Löfgren, Walkenström, and Hermansson (2002) with some modifications. Briefly, 0.15 g of dry crude pectin were dissolved in 10.0 mL of 0.1 mol/L citrate buffer (pH = 2.0) and stirred for 30 min at 95 °C. Then, 6.0 g sucrose was added and stirred as indicated above for hawthorn powder gels, following the same procedures for molding and storage of the obtained gels. The pectin-rich gels were prepared in citrate buffer to ensure the acidic pH, needed for pectin gelation. The large amount of acid in unrefined hawthorn powders, showed by the high total acidity (Table 1), could satisfy entirely the acidic requirement for gelling, as the formation of hawthorn powder gels did not depend on citric acid incorporation.

2.2.8. Small amplitude oscillatory shear (SAOS) testing of high-sugar acid gels

Dynamic oscillatory rheological experiments of hawthorn gels were carried out immediately after the 90 min cooling period and denoted as day 0. The remaining gels were kept in the plastic circular molds described above, capped with a bolted plastic cover to avoid drying and kept in an incubator at 20 °C. After 24 h (denoted as day 1) and 7 days (denoted as day 7) of storage, the gels were subjected to the same rheological characterization. For each storage time and gel type, gel disks were analyzed on a controlled stress rheometer (DH3 Rheometer, TA Instruments, New Castle, DE, USA) controlled by Trios software (TA Instruments, New Castle, USA) equipped with a Peltier system to control the temperature. 100 grit medium sandpaper was attached to the bottom plate and to the 20 mm parallel plate geometry to avoid sample slippage. The gap between plates was set to 2 mm and vaseline oil was used to avoid drying of the surface between plates. Before each rheological experiment, gel disks were left to equilibrate in the measurement position for 10 min.

A frequency sweep was performed at 25 °C from 0.1 to 10 Hz at 0.5 % constant strain, followed by a temperature sweep from 25 to 90 °C at

Table 1
Proximate composition (g/100 g, in dry basis, d. b.), acidity and water (WAC) and oil (OAC) absorption capacities of the two hawthorn fruit powders (MIF and DAF) from Mianqiu (MI) and Dajinxing (DA) cultivars.

Composition	MIF	DAF
Crude protein (%, d. b.)	3.0 ± 0.0b	3.6 ± 0.0a
Total starch (%, d. b.)	10.9 ± 0.4b	14.7 ± 0.2a
Reducing sugars (%, d. b.) [a]	14.7 ± 1.1a	16.3 ± 0.9a
Pectin (%, d. b.) [b]	14.2 ± 0.4a	7.6 ± 0.4b
IDF (%, d. b.)	25.5 ± 2.1b	39.2 ± 0.9a
SDFP (%, d. b.)	14.5 ± 1.9a	9.4 ± 0.1b
TDF (%, d. b.)	40.0 ± 0.1b	48.6 ± 1.0a
Ash (%, d. b.)	2.6 ± 0.1a	2.8 ± 0.1a
Titratable acidity (%, d. b.) [c]	14.5 ± 0.1a	11.0 ± 0.0b
WAC (g/g)	4.1 ± 0.0b	4.5 ± 0.1a
OAC (g/g)	1.0 ± 0.0b	1.1 ± 0.0a

Mean values ± standard deviations within the same row followed by different letters are significantly different ($p < 0.05$). [a] Glucose Equivalents. [b] Galacturonic acid Equivalents. [c] Citric acid Equivalents. IDF, insoluble dietary fiber; SDFP, soluble dietary fiber precipitated with ethanol; TDF, total dietary fiber.

5 °C/min, 0.5 % strain and 1 Hz. The applied strain for these tests was selected based on a previously performed strain sweep from 0.1 to 25% to guarantee the existence of a linear viscoelastic response (see **supplementary information 1**). For each testing day, G′ at 1 Hz was obtained from the frequency sweep at 25 °C. ΔG′ refers to the G′ increase at 1 Hz from day 0 to day 1 or from day 1 to day 7, respectively, from the frequency sweep at 25 °C. Relative G′ drop was calculated from the temperature sweep as the G′ difference at 25 °C and 90 °C divided by the G' value at 25 °C, expressed as a percentage. Coefficient of variation among triplicates was less than 15 %.

2.2.9. Statistical analysis

All analyses were conducted at least in triplicate. Statistical analysis was performed using SPSS Statistics 22 software (IBM, USA) and results expressed as mean value ± standard deviation. Significant differences were compared by Fisher's Least Significant Difference (LSD) test ($p < 0.05$).

3. Results and discussion

3.1. Proximate composition of hawthorn powders

The proximate composition of the two cultivars of hawthorn powders is reported in Table 1. Significant differences were found in the compositional attributes of MI and DA cultivars grown under the same environmental conditions. Crude protein content (d.b.) was low, but significantly higher for DAF (3.6 %) compared to MIF cultivar (3.0 %). Meanwhile, reducing sugars (14.7–16.3 %) and ash content (2.6–2.8 %) were not significantly different between cultivars. Results for protein and ash were in the range of those reported by Özcan, Hacıseferoğulları, Marakoğlu, and Arslan (2005), with protein and ash values in wild hawthorn fruits of 2.5 and 2.3 %, respectively. Reducing sugar content in MIF and DAF cultivars were also consistent with Liu et al. (2010), who showed that fructose and glucose were the main reducing sugars in hawthorn fruits and their combined content ranged from 10.9 to 34.6 % for different cultivars. In addition, starch was abundant in both DA and MI cultivars, with relatively higher content in DAF (14.7 %) than in MIF (10.9 %). These differences should be attributed to differences in starch biosynthesis rather than maturity stage as cultivars were harvested at the same maturity stage (Li et al., 2015). Furthermore, MIF denoted a significantly higher pectin content (GalA equivalents) and SDFP, while DAF not only showed higher total starch content, but also higher IDF and TDF contents. Most of the dietary fiber in hawthorn powders was insoluble (IDF), with values of 25.5 % and 39.2 % for MI and DA, respectively. Interestingly, the amount of SDFP was in close agreement with the results for pectin content, especially for the MI variety, suggesting that most of the soluble dietary fiber fraction consisted of pectic polysaccharides. Thus, hawthorn pectin, characterized as high-methoxyl pectin (Roman et al., 2021, Table S1), represented 14.2 % and 7.6 % of the dry matter content in MIF and DAF cultivars, accounting for 98.1 % and 81.0 % of SDFP, and 35.7 % and 15.7 % of the TDF, respectively. In this work, MIF showed a 2-fold greater pectin yield (in terms of GalA) than DAF, being recommended as an excellent cultivar for pectin extraction (Roman et al., 2021).

In addition, the titratable acidity (citric acid eq.) results indicated that MIF presented higher presence of acidic components than DAF (14.6 and 11.0 %, respectively), with both cultivars being highly acidic, and, therefore, able to form gels in high sucrose media, without the need of adding acids. Wide variations in the titratable acidity have also been reported in literature, where citric acid was the most abundant acid (Liu et al., 2010).

3.2. Pasting properties, WAC and OAC of hawthorn powders

The viscosity profile and WAC and OAC of hawthorn powders are reported in Fig. 1 and Table 1, respectively. The pasting profile of hawthorn powders was used to investigate the contribution of starch swelling to the apparent viscosity observed upon heating. DAF displayed higher viscosity than MIF during the whole pasting profile, including higher initial viscosity at 50 °C, before starch gelatinization, and maximum and final viscosities upon heating and cooling. However, no significant differences were observed for the pasting time and temperature (Table S2) between MIF and DAF, indicative of similar start of swelling upon heating. The higher viscosity of DAF, especially after the peak viscosity was reached, should be associated with its higher starch content (see Table 1). We note that gelatinized and dispersed starch molecules could reassociate upon cooling and significantly contribute to the increased setback and final viscosity and could also interfere with the gelling behavior of the system (see 3.5 section). In fact, viscosity differences between hawthorn powders were greater after the pasting temperature was reached (~82 °C) and upon cooling.

Other components may also affect the viscosity and swelling development of the matrix. For example, pectic polysaccharides have been reported to cover the starch granules promoting their swelling restriction in water, thereby directly decreasing the peak viscosity (Sharma, Oberoi, Sogi, & Gill, 2009) while others have indicated that water-binding polysaccharides could contribute to deplete the free water, synergistically increasing the viscosity of starch-based systems (Matia-Merino et al., 2019). Regarding WAC and OAC (Table 1), although minimal differences were observed, DAF presented higher values for WAC (4.5 g/g) and OAC (1.1 g/g) than MIF (WAC: 4.1 g/g and OAC: 1.0 g/g), which could be related to their different composition, especially to their protein and carbohydrate fractions.

3.3. Visual evaluation of hawthorn powders and pectins

Fig. 2 shows the visual appearance of MI and DA hawthorn fruits, their ground unrefined powders and extracted pectins. It is clearly observed that MIF exhibited a stronger brownish color than DAF, which should be related to the varietal differences of the fruits (first-row images), where MI fruits presented a more reddish color than its DA counterpart. However, no visual differences were observed for the color of the obtained pectins (third-row, Fig. 2), suggesting that the pigments responsible for the reddish color of the fruit were successfully removed during the first steps of pectin extraction with organic solvents. Thus, crude hawthorn pectins showed a whitish cotton-like appearance, similar to the pectins obtained from fermented and steeped hawthorn wine pomace (Jiang et al., 2018). The microstructure of hawthorn powders and pectins is shown in the fourth and fifth row of Fig. 2, respectively. Both MIF and DAF were arranged in compact aggregated particles with no clear differences in the particle size and morphology between varieties, which agrees with the sieving process to which they were subjected after milling. Meanwhile, the two hawthorn pectin samples denoted similar microstructure which consisted of lamellate particles with irregular-shaped structures, although DAP presented a relatively more homogeneous surface with thinner fibrils.

3.4. Phenolic fractions and antioxidant capacities of hawthorn powders

The total content in phenolic compounds (TP), the different extractable and bound phenolic fractions, including EPP, HPP and NEPA fractions, and their relative antioxidant capacities, measured as DPPH and ABTS radical scavenging abilities, are summarized in Table 2. Remarkably, DAF (100.7 mg/g) showed a higher TP content than MIF (60.4 mg/g). Similarly, Lou et al. (2020) observed that total phenolic contents in frozen and freeze-dried hawthorn berries from *C. pinnatifida* were 66.7 mg/g and 67.2 mg/g, respectively. High variations in TP content (21.2 mg/g to 69.1 mg/g) among 15 different hawthorn fruit species were observed in another study (Alirezalu et al., 2020). Thus, the total phenolic content and the phenolic composition varies within hawthorn species and genotypes, as well as with growth conditions, maturity and processing of the hawthorn fruit (Li et al., 2015; Liu et al.,

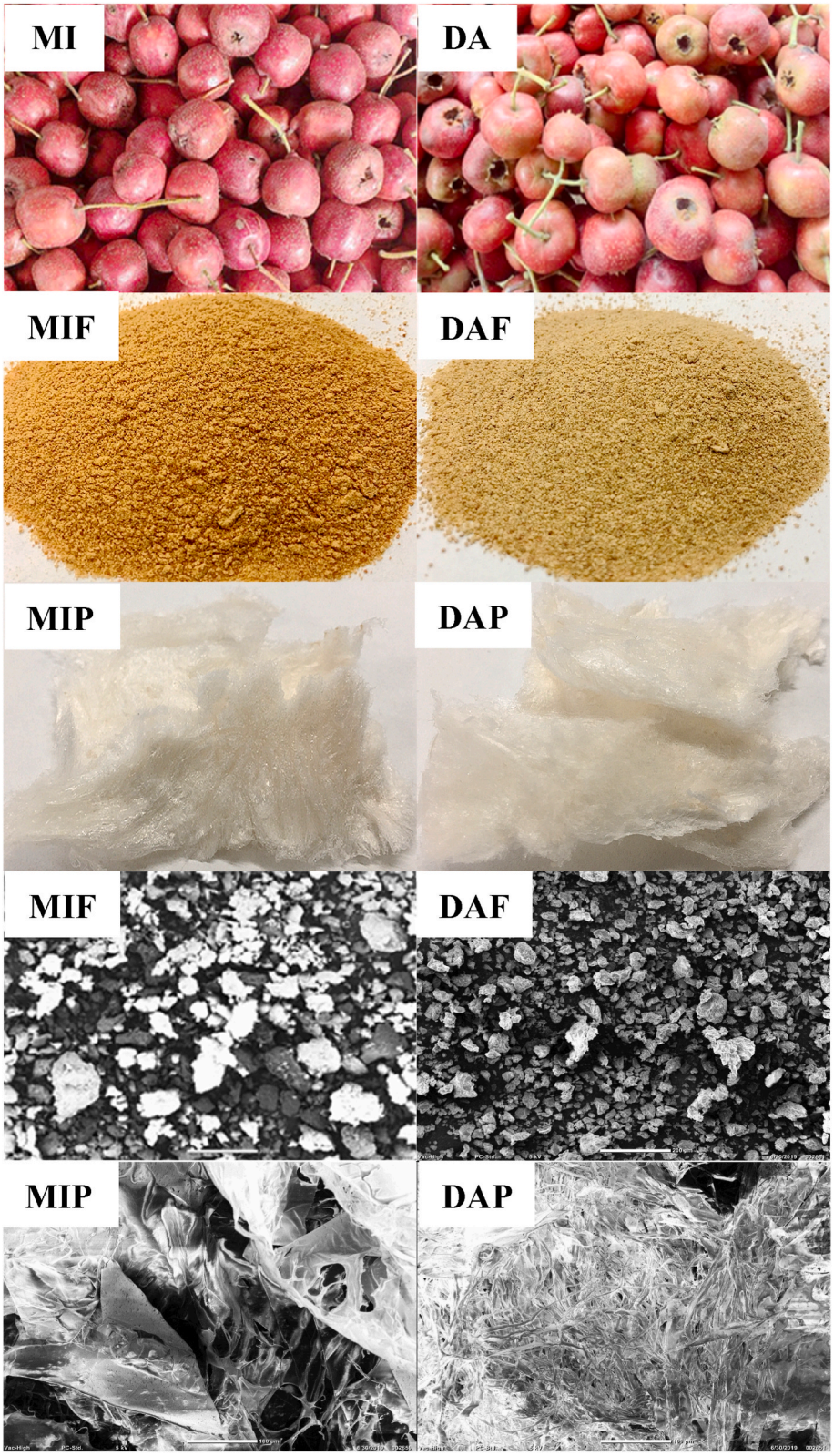

Fig. 2. Visual appearance of hawthorn fresh fruits (first row), dried fruit powders (second row) and extracted pectins (third row) and microstructure of hawthorn powders (fourth row) and pectins (fifth row). The scale bar in the scanning microscopy images indicates a magnification of 200 μm (hawthorn powders) and 100 μm (hawthorn pectins). MI: hawthorn fruits of Mianqiu cultivar, DA: hawthorn fruits of Dajinxing cultivar; MIF: hawthorn powder of MI, DAF: hawthorn powder of DA; MIP: pectin extracted from MIF; DAP: pectin extracted from DAF.

Table 2
Phenolic fractions and their antioxidant capacities for the two different hawthorn fruit powders.

		MIF	DAF
EPP (mg/g)		40.9 ± 0.7d	77.7 ± 0.4b
HPP (mg/g)		18.1 ± 1.2b	21.3 ± 0.9a
NEPA (anthocyanins, mg/g)		1.4 ± 0.0b	1.7 ± 0.0a
TP (mg/g)		60.2 ± 0.4b	100.6 ± 0.5a
ABTS-RSC	EPP (IC_{50}, mg)	0.025 ± 0.001a	0.011 ± 0.000c
	HPP (IC_{50}, mg)	0.184 ± 0.002a	0.116 ± 0.007b
DPPH-RSC	EPP (IC_{50}, mg)	2.122 ± 0.030a	1.179 ± 0.018c
	HPP (IC_{50}, mg)	0.857 ± 0.000a	0.744 ± 0.012b
	NEPA (IC_{50}, mg)	0.124 ± 0.001a	0.113 ± 0.002b

Mean values ± standard deviations within the same row followed by different letters are significantly different ($p < 0.05$). EPP, extractable phenolic compounds; HPP, hydrolyzable non-extractable phenolic compounds; NEPA, non-extractable proanthocyanidins; TP, total phenolics (EPP + HPP + NEPA). EPP and HPP are expressed as mg of Gallic Acid Equivalents, and NEPA-anthocyanins is expressed as delphinidin equivalents. Antioxidant capacity is given as IC50 values (mg extract) of DPPH-RSC, DPPH radical scavenging capacity; and ABTS-RSC, ABTS radical scavenging capacity. MIF, DAF, fruit powders prepared with MI and DA hawthorn cultivars, respectively.

2011). For both MIF and DAF, EPP was the most abundant phenolic fraction, indicating that most of the phenolics in hawthorn fruit were present in the free form, which agrees with Wen et al. (2015). DAF exhibited almost two-fold higher EPP content (77.7 mg/g) than MIF (40.9 mg/g), a trend also visible in their high-sugar acidic gels (see discussion in Table S3). The main phenolic compounds present in the free extracts of hawthorn fruits have been reported to include oligomeric procyanidins, epicatechin, hyperoside, isoquercitrin, and chlorogenic acid (Li et al., 2020; Liu et al., 2011; Wen et al., 2015).

Regarding the bound phenolics, HPP was also higher in DAF than MIF (21.3 and 18.1 mg/g, respectively), with values higher than those reported by Wen et al. (2015), which should be ascribed to the different extraction methods of the bound phenolics, processing of the fruits and/or varietal differences in the bioactive compounds. The non-extractable proanthocyanidins fraction (NEPA) was the least abundant phenolic fraction with values of 1.4–1.8 mg/g. Several bound phenolics have been identified in hawthorn fruit, including procyanidin B2, hyperoside, isoquercitrin and flavonoid glycosides such as 3-*O*-galactoside and 3-*O*-glucoside (Li et al., 2020).

In this study, the DPPH-RSC and ABTS-RSC were used to evaluate the radical scavenging capacity of the different phenolic fractions (Table 2). Whether free or bound, the phenolic extracts from DAF exhibited stronger antioxidant capacity than those of MIF, as the lower values of IC_{50} indicated, which agrees with its higher phenolic content, suggesting a straight correlation between phenolic content and antioxidant capacity (Chen et al., 2019). EPP obtained from DAF exhibited stronger ABTS-RSC than DPPH-RSC due to higher ABTS sensitivity (Pico et al., 2019), as detailed in methods section. It was also observed that ABTS-RSC was stronger in EPP than HPP, which agrees with the commonly reported higher antioxidant activity of the free phenolic extracts (Wen et al., 2015).

3.5. Rheological characterization of the high-methoxyl sugar acid gels

Frequency, temperature and strain sweeps for the high-methoxyl sugar acid gels made with hawthorn powders and hawthorn and citrus pectins at different storage days are summarized in Table 3, Fig. 3 and Fig. S2. Samples showed a greater G′ compared to G″ (see Fig. S3), indicating a predominantly elastic response in all sugar acid gels. For hawthorn powder-based gels, G′ values at day 0 were higher for GMIF than GDAF, in agreement with its 2-fold higher pectin content (Table 1). Rheological experiments after 90 min (day 0) of gel-making could not be performed on the gels made with hawthorn pectins (MIP and DAP) as solid gels were not yet formed. The high presence of other non-pectic components in the crude hawthorn pectins may interfere with pectin-pectin associations for the formation of a structured network, delaying network formation, which could be consistent with the longer time needed for their gelation. Conversely, gels were set after 90 min for citrus counterparts, suggesting a more rapid-gelling ability, which could be related to the higher pectin purity and DM in the commercial citrus pectins (Roman et al., 2021). Interestingly, citrus pectin GCP2, with higher DM and M_w than GCP1, denoted the highest G′ value among all pectin-based gels (Table 3). This could highlight the importance of having both a higher M_w and DM for extended hydrophobic interactions in the formation of a stiffer network (Linares-García et al., 2015; O'Donoghue & Somerfield, 2008). In this sense, da Silva and Gonçalves (1994) indicated that weaker pectin networks (lower G′ values) were formed under thermal conditions unfavorable for hydrophobic interactions. For the pectin-based gels stored for 1 and 7 days, GMIP and GDAP showed lower G′ values than citrus pectin gels, especially GCP2. Regarding the differences between hawthorn pectin-based gels, the higher G′ of GDAP could be explained by its lower M_w and higher co-extracted starch content in its crude pectin, reported in Roman et al. (2021) and Table S1. Thakur et al. (1997) indicated that the presence of side branches, like those in starch, could significantly affect the gelling properties of pectins by preventing their aggregation and formation of inter-molecular junction zones. The mechanical spectra also revealed a G′ and G″ dependence with frequency in both hawthorn powder-based and pectin-based gels (Fig. 3 and Fig. S3). For the gels made with hawthorn powders, GMIF denoted higher initial G′ (stiffer gel) than GDAF, which could be related to the two-fold higher pectin content in the MI cultivar (see Table 1), and with no differences in their critical strain (see Fig. S2 and Table S4). Crude pectin gels presented significantly higher critical strains (extended LVR) and lower G′ modulus at the

Table 3
Rheological characterization of high-methoxyl sugar acidic gels made with hawthorn fruit powders (GMIF and GDAF) and pectins (GMIP and GDAP) compared to two commercial citrus pectins (GCP1 and GCP2) analyzed after 90 min (day 0), 24 h (day 1) and 7 days (day 7).

Test	Samples	GMIF	GDAF	GMIP	GDAP	GCP1	GCP2
Frequency Sweep (25 °C)	G′ day 0 (Pa)	3440 ± 83bC	2604 ± 244cC	–	–	3231 ± 149bB	5847 ± 386aB
	G′ day 1 (Pa)	13879 ± 1653bB	19245 ± 1526aB	955 ± 69eB	2026 ± 183eB	4796 ± 147dA	7919 ± 230cA
	G′ day 7 (Pa)	32872 ± 910aA	36333 ± 4727aA	1219 ± 157dA	2345 ± 163cdA	5120 ± 279cA	8066 ± 242bA
	ΔG′ increase day 1 (Pa)	10439	16640	–	–	1565	2072
	ΔG′ increase day 7 (Pa)	18992	17088	263	319	324	147
Temperature sweep	Relative G′ drop day 0 (%)	32.8 ± 1.2cB	44.3 ± 4.6bC	–	–	77.1 ± 4.8aA	45.8 ± 2.9bB
	Relative G′ drop day 1 (%)	47.9 ± 1.2dA	54.5 ± 0.9cA	69.6 ± 6.1bA	65.9 ± 1.5bB	82.2 ± 4.1aA	51.1 ± 2.6cdA
	Relative G′ drop day 7 (%)	47.1 ± 0.0dA	49.7 ± 1.3cdB	73.5 ± 4.4bA	70.2 ± 3.2bA	80.6 ± 2.7aA	53.3 ± 2.2cA

Mean values ± standard deviations followed by different lowercase or uppercase letters are significantly different ($p < 0.05$). G′ for the temperature sweep experiments was obtained at 1 Hz frequency and 25 °C. ΔG′ refers to the increase in G′ (25 °C) from day 0 to day 1 or from day 1 to day 7, respectively. '–': data was not obtained as gels were not formed at day 0. Lowercase letters indicate statistical differences between gel samples for each storage day. Uppercase letters indicate statistical differences between storage days (day 0, 1 and 7) for each gel sample.

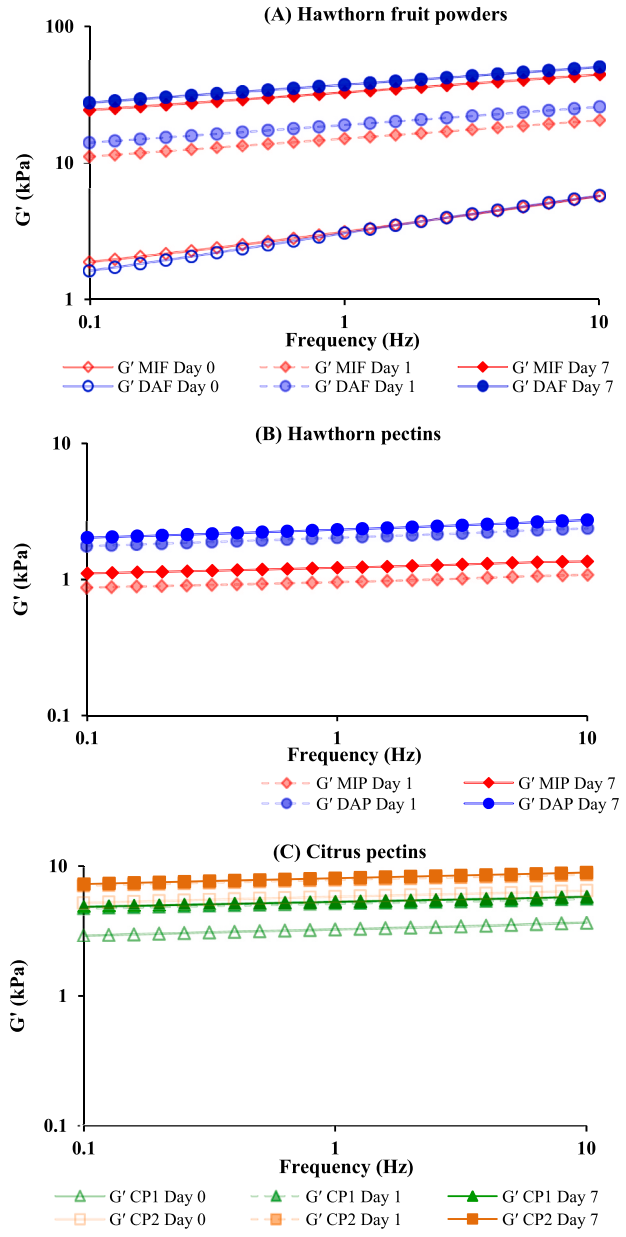

Fig. 3. Elastic modulus (G′) as a function of frequency for high-methoxyl sugar acid gels made with hawthorn fruit powders (MIF, DAF), hawthorn pectins (MIP, DAP) and citrus pectins (CP1, CP2) at 25 °C at three different storage times. Empty, light and dark colored symbols indicate G′ values for day 0, 1 and 7, respectively. Note the difference in the scale of the y-axis for figure A.

end of LVR than their powder counterparts, indicating stronger (i.e., higher critical strain) but less stiff networks. These differences should be related to the different composition of the gels, although in powder-based gels, the pectin content [1.1–2.0 % (w/w, pectin/water) for DAF and MIF] was kept similar to the crude pectin content in pectin-rich gels (1.5 %).

Regarding gel aging with the course of time (ΔG′ increase), both GMIF and GDAF showed a fast increase in G′ from both day 0 to day 1 and day 1 to day 7, indicating remarkable aging of the powder-based gels, together with an increase in the critical strain (i.e., extended LVR) as seen in Fig. 3 and supplementary material (Fig. S2 and Table S4). However, GMIF displayed a much more rapid stiffening during the first 24 h, which should be attributed to the role of starch retrogradation (Martinez et al., 2018), as DAF presented higher starch content and final viscosity during pasting than MIF counterpart (Table 1 and Fig. 1). This explanation is further supported by the almost lack of aging seen in the starch-depleted crude pectin samples (Table 3), also shown and further discussed in the textural maps of Fig. S2. From day 1 to day 7, minimum aging was observed in GMIP and GDAP, with a small but significant increase in G′, and no changes in their critical strain (Fig. S2B), indicating excellent stability of the network formed in hawthorn pectin gels, and similar to citrus pectins.

The thermal melting of pectin gels is critical for their quality evaluation, as it gives information about the network stability to heating conditions conducted during further processing. Furthermore, the loss of G′ modulus (G′ drop) upon heating was also evaluated to investigate possible factors responsible for gel properties (Table 3). GCP1 displayed the highest relative G′ drop, followed by GMIP, GDAP and GCP2, which was inversely correlated with the DM of the studied pectins measured by titration (Roman et al., 2021, Table S1), suggesting that methyl esters of pectins were heavily involved in hydrophobic intermolecular interactions that form the gel structure, as mentioned above. In this regard, previous research has reported that hydrophobic interactions are strengthened when increasing the temperature, while hydrogen bonds and electrostatic interactions weaken at higher temperatures (Alba, Bingham, Gunning, Wilde, & Kontogiorgos, 2018; da Silva & Gonçalves, 1994).

For hawthorn powder-based acid gels, GDAF showed a higher G′ drop than GMIF for day 0 and day 1, with G′ drop values increasing with storage time (i.e., extended intermolecular interactions), especially for GDAF. GDAF presented a higher starch-to-pectin ratio (2-fold higher vs 0.8-fold higher for DAF than MIF) in its gel composition, and, therefore, could indicate an extended melting of hydrogen bonds from starch chains with increasing temperature. All in all, our results highlight that not only pectin but also starch contributes to the gelling ability of hawthorn fruits, although its contribution should be further studied.

4. Conclusions

In this work, the proximate and phytochemical composition of two hawthorn fruit cultivars (MI and DA) was evaluated, together with the gelling ability of the unrefined fruit powders and their extracted pectins under high-sugar acidic conditions. Hawthorn powder from MI cultivar presented higher acidity and a 2-fold higher pectin content, making this cultivar more suitable for high-methoxyl sugar acidic gels. Meanwhile, DA cultivar was richer in starch, insoluble dietary fiber and free phenolics with stronger antioxidant capacity. These compositional differences could serve as a proxy for selecting more nutritional genotypes that help diversify hawthorn fruits' utilization.

Regarding high-sugar acidic gels, MI resulted in hawthorn powder-based gels with a higher G′ after gel-making than DA counterpart (i.e., more rapid gel formation), although DA gels became harder during aging, presumably associated with its higher starch content. For pectin-rich gels, citrus pectins exhibited higher strength and faster hardening than hawthorn pectin gels (based on G' values and the lack of gel formation after 90 min cooling for hawthorn pectin-based gels). Both hawthorn and citrus pectins resulted in more stable gels (i.e., lower aging) than hawthorn powder-based gels, being more attractive for their use as gelling agents if hardening phenomena is to be avoided. We demonstrated that the gelling performance, including gel strength and aging rate, of unrefined hawthorn fruit powders not only depended on the gelling ability of their high-methoxyl pectins, but was also affected by the presence of other coexisting components, with starch likely contributing to the network formation. However, further studies are needed to exploit the varietal differences of hawthorn fruit cultivars and fully understand the influence of their compositional traits on the processability and functionality of less-refined hawthorn fruit ingredients, for enhancing their utilization as sustainable thickening/gelling agents in the food industry.

CRediT authorship contribution statement

Mengmeng Guo: Writing – original draft, Methodology, Investigation. **Kang Xu:** Methodology, Investigation. **Josephine Yee:** Investigation. **John R. Dutcher:** Writing – review & editing, Methodology. **Mario M. Martinez:** Writing – review & editing, Visualization, Funding acquisition, Conceptualization. **Laura Roman:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare no conflicts of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2024.116331.

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