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Structure and properties of citrus pectin as influencing factors of biomarkers of metabolic syndrome in rats fed with a high-fat diet

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ABSTRACT

Pectin, widely used as a food and pharmaceutical ingredient, has garnered attention in recent years due to its bioactive properties. We conducted an *in vivo* study to evaluate the effects of citrus pectin on biomarkers of metabolic syndrome (MtS), including lipid profile, hypertension, and adipose tissue. Supplementing a high-fat diet (60% energy from fat) with 20% pectin for 4 weeks significantly reduced body weight and fat accumulation, improved insulin resistance, and decreased circulating leptin levels, demonstrating a beneficial effect on MtS. Pectin exhibited excellent viscosity, emulsifying properties, and water-holding capacity, forming a viscous gel in the gastrointestinal tract. This gel delays gastric emptying, enhances satiety, and reduces food and calorie intake, leading to lower weight gain in rats fed pectin. Its viscosity also interferes with lipase activity, lipid hydrolysis, and absorption, while its oil-holding capacity may help prevent lipid absorption. The presence of galactose in pectin's structure showed potential for improving insulin resistance. Furthermore, both degree of esterification (DE) and pH influence pectin's functionality. At acidic pH levels, such as those found in the stomach and duodenum, high methoxyl pectin (HMP) retains fats and bile salts more effectively, contributing to better cholesterol regulation. These effects, combined with the antioxidant properties of pectin, helped reverse arterial hypertension associated with MtS. Overall, our findings highlight the potential of citrus pectin as a natural bioactive ingredient for combating obesity-related disorders, complementing pharmacological treatments and promoting health through innovative dietary approaches.

1. Introduction

Metabolic syndrome (MtS) is associated with excessive visceral adiposity, hypertension, hyperglycemia, insulin resistance, and dyslipidemia (Ravaut et al., 2021). Chronic consumption of fats, comprising 45–60% of total energy intake, enhances weight gain, obesity, and MtS (Qin et al., 2018). However, consuming 14 g of fiber per 1000 kcal is associated with a decrease in cholesterol and other lipids in the blood, a lower glycemic index, and improvements in gut microbiota (Cronin et al., 2021).

Fiber, present in significant amounts in by-products of the agri-food industry, is important not only from an environmental perspective but also for its applications in managing certain diseases, particularly those related to metabolic disorders (Gerschenson et al., 2021). Fiber is

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primarily composed of bioactive carbohydrates, with pectin being one of the most important components. The primary sources of pectin include by-products from orange and apple pomace (Nadar et al., 2022). This heteropolysaccharide mainly consists of a linear chain of galacturonic acid (GalA), known as homogalacturonan (HG), which alternates with α-1,2-linked rhamnose units (rhamnogalacturonan-I, RG-I). RG-I features various side chains composed mainly of arabinose and galactose (Muñoz-Almagro et al., 2021a).

Pectin is classified according to its degree of methyl esterification (DE) into high methoxyl pectin (HMP) (>50% carboxyl groups esterified) and low methoxyl pectin (LMP) (<50% carboxyl groups esterified). Due to its structure and techno-functional characteristics, pectin can be used as a gelling agent, emulsifier, thickener, and stabilizer in various industrial food products, including beverages, fermented dairy-based drinks, and meat products (Muñoz-Almagro et al., 2021b).

Pectin is highly resistant to digestive processes and can be fermented in the colon by the microbiota, producing short-chain fatty acids (SCFAs) (Ferreira-Lazarte et al., 2021). Consequently, pectin is considered a potential prebiotic, contributing to gut microbiota homeostasis (Muñoz-Almagro et al., 2021b; Méndez-Albiñana et al., 2022).

According to the European Food Safety Authority (EFSA), a cause-and-effect relationship has been established between pectin consumption and a reduction in various cardiometabolic disturbances. In this context, pectin effectively adsorbs glucose both *in vitro* and *in vivo* (Djaoud et al., 2022; Sánchez et al., 2008). It also helps maintain normal blood cholesterol concentrations while reducing low-density lipoprotein (LDL) levels (Scientific Opinion on the substantiation, 2010). Additionally, various studies have reported improvements in blood pressure levels associated with hypertension following pectin consumption (Cabral et al., 2021; Agarkova et al., 2019).

Previous research has demonstrated the benefits of a fiber-rich diet on various MtS biomarkers. Thus, Sánchez et al. (2008) studied the effects of apple pectin supplementation on different MtS biomarkers without considering the relationship between pectin's structure and properties with the changes in disease-related biomarkers. Additionally, the animal model used by these authors was a genetically obese model that exhibited congenital alterations, such as dyslipidemia and disruptions in glucose metabolism. Conversely, Llévenes et al. (2020) studied the effect of the fiber present in a nutraceutical in an animal model fed a high-fat diet to induce MtS. However, this study focused on the impact of the treatment on the systemic and vascular alterations characteristic of MtS and explored the molecular mechanisms involved, particularly the roles of neuronal nitric oxide synthase (nNOS) and protein kinase A (PKA), without delving deeper into the relationship between the physicochemical and structural properties of the fiber and its function in the organism. Lastly, Hu et al. (2022) reported metabolic improvements following pectin supplementation, but noted the need for further studies to link pectin's structure and properties with its effects on the organism, as well as to better understand the synergy between its physicochemical and bioactive properties. Although these studies examine the effects of fiber on different biomarkers, they lack a deeper investigation into how the structural and physicochemical properties of fiber, including pectin, influence the improvements in the various biomarkers studied.

2. Materials and methods

2.1. Characterisation of pectin

Samples of citrus pectin (4400) were kindly provided by CEAMSA (Porriño, Spain). The pH was measured using a pH meter (Mettler Toledo GmbH, Schwerzenbach, Switzerland). The dry weight was gravimetrically determined at 102 °C over 72 h. Protein determination was performed using the Bradford method ((Bradford, 1976)).

2.2. Structural evaluation of pectin

Estimation of relative Mw of pectin was conducted by Size Exclusion Chromatography (SEC) according to Muñoz-Almagro et al. (2021a), using an Agilent Technologies 1220 Infinity LC System (Agilent Technologies, Germany), equipped with TSK-GEL columns (G5000 PWXL, 7.8 × 300 mm, particle size 10 μm; G2500 PWXL, 7.8 × 300 mm, particle size 6 μm) (Tosoh Bioscience, Stuttgart, Germany). Samples (20 μL) were eluted with 0.01 M ammonium acetate at a flow rate of 0.5 mL/min for 50 min at 30 °C, which was evaporated using an ELSD 1260 Infinity (Böblingen, Germany) at 30 °C. Pullulans with Mw of 805-0.3 kDa were used as standards. Standard curves of pullulans for Mw estimation were obtained considering the logarithm of Mw versus the corresponding elution volume.

The determination of neutral sugars and galacturonic acid (GalA) was performed by GC-FID using a 7890A gas chromatograph (Agilent Technologies, Wilmington, Delaware, USA) equipped with a flame ionization detector (FID). Sample preparation and chromatographic analysis were carried out as reported by Muñoz-Almagro et al. (2021a).

The degree of esterification (DE) of pectin was determined at the Research InterDepartmental Service (SIDI) of the Autonomous University of Madrid. To determine the degree of methylation (DM), the pectins were combined with potassium bromide and analysed using a Bruker IFS66v spectrometer (Bruker, US). The FT-IR spectra were recorded in the mid-infrared region, covering a frequency range of 400–4000 cm−1 with a resolution of 4 cm−1 and 250 co-added scans. The DM was calculated as the ratio of the peak area at 1734 cm−1 (COO-R) to the total peak area of 1734 cm−1 (COO-R) and 1612 cm−1 (COO−).' According as previous described by Muñoz-Almagro et al. (2020).

$$DE = (A1760 / A1760 + A1630)\ x\ 100$$

The DE is represented by the ratio of the area of the band at 1760 cm^{-1} to the sum of the areas of the bands at 1760 cm^{-1} and 1630 cm^{-1}

2.3. Rheological properties and zeta potential

Pectin was dissolved in deionized water (2% w/v). The apparent viscosity was determined using a rotational rheometer (Haake MARS; Thermo Fisher Scientific Inc., Waltham, MA, USA), equipped with a temperature-controlled circulating bath set at 25 °C. The rheometer used a double cone-plate system with a 60 mm diameter, a cone angle of 2°, and a gap of 0.084 mm (DC60/2°; Haake, Vreden, Germany), along with a solvent trap to prevent evaporation. The procedure was programmed using Rheo Win 4 Job Manager (Thermo Fisher Scientific Inc.). The viscosity curve was measured over a shear rate range of 1–1000 s^{-1}, with ascending and descending ramps of 5 min each, and a holding time of 1 min at 25 °C.

Zeta potential was analysed following the method proposed by Pacheco et al. (2019) using a Malvern Zetasizer NanoZS instrument (Malvern Instruments Ltd., Worcestershire, UK).

2.4. Techno-functional properties

For water holding capacity (WHC) a 2 % (w/v) pectin solution was shaken for 5 s every 15 min for 1 h. The solution was then centrifuged at 1000×*g* for 20 min. After centrifugation, the supernatant was removed, and the tube was allowed to drain on filter paper for 30 min. The WHC was expressed as grams of absorbed water per gram of pectin (Bayar et al., 2018).

Oil holding capacity (OHC) was determined using the same procedure, but using corn oil instead of water.

Pectin solutions at concentrations of 1%, 2%, and 4% (w/v) were prepared to evaluate emulsifying capacity (EC) and emulsifying stability (ES) (Bayar et al., 2018). 10 mL were mixed with 5 mL of corn oil and homogenized for 3 min. The emulsions were centrifuged at 1000 *g* for 5 min. EC was calculated using the equation:

$$EC\ (\%) = Vf\ /Vi \times 100$$

Were V_f is the volume of the emulsion and V_i is the total volume of the mixture.

For stability, the emulsions were incubated for 30 min at 80 °C and centrifuged at 1000 *g* for 5 min. The ES was calculated as following:

$$ES\ (\%) = V_t\ /Vf \times 100$$

Were V_t is the total volume of the emulsion after incubation and centrifugation and V_f is the volume of the emulsion.

2.5. Antioxidant activit and total phenolics content (TPC)

The antioxidant activity of pectin was evaluated by DPPH method (Wang et al., 2016). A DPPH solution at a ratio of 1:15 (2 mM DPPH: methanol) was used. Samples at concentrations of 2, 1.5, and 1 mg/mL were prepared and centrifuged at 1800×*g* for 5 min. Then, 150 μL of each sample was mixed with 150 μL of the DPPH solution and allowed to react for 30 min in the dark. Absorbance was measured at 517 nm. Three solutions of Trolox at concentrations of 2, 1.5, and 1 mg/mL were prepared, and their antioxidant activity was measured in the same way as the pectin samples. Results were expressed as the percentage of DPPH inhibition relative to the percentage inhibition of Trolox, using the following formula:

$$\%\ \text{DPPH inhibitory activity} = (C - CB) - (S - SB)/C - CB$$

Where C is the control (working solution), CB is the control blank (methanol), S is the sample, and SB is the sample blank (150 μL of the sample + 150 μL of methanol).

Total phenolic content (TPC) determination was performed using the Folin-Ciocalteu method (Singleton and Rossi, 1965).

2.6. Assays with animals

Four-week-old male Wistar rats (n = 24; initial weight: 173.9 ± 17 g) obtained from Charles River Laboratories (Spain) were raised and housed under controlled conditions (20–24 °C, 55% relative humidity, 12-h light-dark cycle) in the Animal Facility of the Autonomous University of Madrid (Registration number ES-28079-0000097). All procedures were approved by the Ethical Committee of the Autonomous University of Madrid and the Comunidad de Madrid (PROEX 341.4/21), and were conducted in compliance with NIH guidelines, ARRIVE guidelines (Animal Research: Reporting In Vivo Experiments) for reporting animal experiments, and the European Parliament Directive 2010/63/EU.

Animals were randomly divided into three groups: (1) Rats fed a standard diet (5.3% energy from fat, 3.950 kcal/g, LASQCdiet® Rod18-H, Altromin International, Germany) as the control group (Ctrl; n = 8); (2) rats with metabolic syndrome (MtS; n = 8) fed a high-fat diet (HFD, 60% energy from fat, 5.229 kcal/g, C 1090–60, Altromin International, Germany) for 11 weeks; and (3) rats with MtS fed the HFD for 11 weeks, supplemented with 20% pectin 4400 during the last 4 weeks (HFD-P, n = 8). Diet composition can be consulted in Supplementary Material (Table Spl.1). The supplemented amount of pectin was according to those proposed by Ferreira-Lazarte et al. (2021).

2.6.1. Blood pressure measurements

Systolic blood pressure (SBP) was measured in awake rats using the tail-cuff method (NIPREM 645, Cibertec S.A., Madrid, Spain) (Llévenes et al., 2020; Blanco-Rivero et al., 2011) in all animals at week 7 of the study (prior to pectin administration) and again before sacrifice.

2.6.2. Oral glucose tolerance test

The oral glucose tolerance test (OTT) was performed one week prior to euthanasia according to a standard protocol (de Queiroz et al., 2015; Amor et al., 2019). Blood glucose levels were measured from the tail vein after a 4-h fasting period, using test strips and a commercial glucometer (FreeStyle Optium®, Abbott Laboratories S.A., Madrid, Spain). A single dose of glucose (2 g/kg of body weight) was administered by intragastric gavage, and glycemia was measured at 15, 30, 60, and 120 min.

2.6.3. Animal euthanasia and sample collection

Animals were sacrificed after 11 weeks by CO_2 inhalation followed by decapitation after a 4-h fasting period. Visceral and epididymal fat pads were weighed. Body and adipose tissue weights were normalized using the left tibia to ensure that weight and fat gain at the end of the study is due to feed intake and not animal size. Blood samples were kept at room temperature for 2 h and then centrifuged (3000×*g*, 10 min, 4 °C). The supernatant (serum) was collected and stored at −80 °C until use.

2.6.4. Liver histology

The liver was removed, and the left lateral lobe was cryoprotected by embedding it in optimum cutting temperature (OCT) compound for cryosectioning and then frozen at −80 °C until use. 5 μm cryostat sections were stained with the lipid dye Oil Red O, and nuclei were counterstained with hematoxylin. Images were captured at 20 × magnification, and the red staining was quantified using Image-Pro Plus 7 software. (Media Cybernetics, MD, USA).

2.6.5. Metabolic hormones

Serum concentrations of leptin (10,007,609, Cayman Chemical, Michigan, USA), insulin (RAB0904, Sigma Aldrich, Madrid, Spain), and adiponectin (ab108784, Abcam, USA) were measured by ELISA following the manufacturer's instructions. Absorbance was measured using a BioTek Synergy HT spectrometer (Winooski, VT, USA). The insulin values were used to calculate the Homeostatic Model Assessment Insulin Resistance (HOMA-IR) index using the following formula:

$$\text{HOMA} - \text{IR Index} = \text{fasting glucose}\left(\frac{\text{mg}}{\text{dL}}\right) \times \text{fasting insulin}\left(\frac{\text{ng}}{\text{mL}}\right) \Big/ 450$$

2.6.6. Lipid profile

The serum concentrations of cholesterol (TC), triglycerides (TG) and high-density lipoproteins (HDL) were measured with the RAL LC5 biochemical analyser (Barcelona, Spain). Low-density lipoprotein (LDL) values were calculated according to Sampson et al. (2020).

$$LDL = TC\ /\ 0.984 - HDL\ /\ 0.971 - \left(TG\ /\ 8.56 + TGx\ Non - HDL\ /\ 2140 - \mathrm{TG}^2\ /\ 16100\right) - 9.44$$

Oxidized LDL (OxLDL) were determined by ELISA (orb1199806, Biorbyt, UK). Absorbance was measured using a BioTek Synergy HT spectrometer (Winooski, VT, USA). Results were normalized using the serum albumin concentration (Ctrl = 6.46 ± 0.33; HFD = 6.03 ± 0.15; HFD-P = 6.25 ± 0.27).

2.7. Data analysis

Data were represented and analysed using GraphPad Prism 8.0.2 software (CA, USA). Results were expressed as mean ± SD and were

Table 1
Composition (%, over total carbohydrates) of the monosaccharides present in the pectin structure and compositional and structural properties of the pectin based on the monosaccharide composition.

Xyl + Arab	Rha	Gal	Glu	GalA	GalA/ Rha
1.98 ± 0.75	3.14 ± 1.24	14.01 ± 4.14	0.90 ± 0.03	80.06 ± 10.6	25.49
Contribution of HG (CH %)	Contribution of RG-I (CH %)	Contribution of RG-I to the Pectin Backbone (−)	Braching or RG-I (−)		
76.92	**22.18**	**25.49**	**5.06**		

Table 2
Emulsifying properties of pectin at different concentration. *Statistical analysis One-Way ANOVA * $p < 0.05$ vs. 4 %;# $p < 0.05$ vs. 1 %; (Tukey post-hoc test).*

Concentration % (m/v)	EC (%)	ES (%)
4 %	45 ± 7.1	2.3 ± 1.4
2 %	71.5 ± 4.9*	30.9 ± 0.5*#
1 %	63.5 ± 2.1	13.7 ± 0.5*

Table 3
Inhibition of the DPPH radical of pectin 4400, expressed as a percentage of inhibition of the antioxidant capacity obtained by TROLOX. *Statistical analysis One-Way ANOVA* $p < 0.05$ vs. 1 %;# $p < 0.05$ vs. 1.5 %; (Tukey post-hoc test).*

Concentration of pectin 4400 (mg/mL)	1	1.5	2
	50.2 ± 1.3	50.9 ± 2.0	54.5 ± 2.7

Table 4
Chow intake in grams/day, kcal/day ingested, final body weight, left tibia lenght and final body weigth (normalyzed using the left tibia) after 11 weeks. *Statistical analysis: One-Way ANOVA * $p < 0.05$ vs. Ctrl; # $p < 0.05$ vs. HFD; (Tukey post-hoc test).*

	Ctrl	HFD	HFD-P
Chow intake (g/day)	22.72 ± 1.46	15.91 ± 1.61*	14.06 ± 3.56*#
Chow intake (Kcal/ day)	70.2 ± 8.83	82.53 ± 8.55*	73.56 ± 18.37#
Final body weigth (g)	418.12 ± 17.76	461.50 ± 39.48*	411.37 ± 27.06#
Left tibia lenght (cm)	5.28 ± 0.15	5.17 ± 0.17	5.07 ± 0.26
Final body weigth (tibia normalyzed) (g)	79.07 ± 3.35	89.17 ± 2.25*	81.05 ± 2.99#

Table 5
Triglycerides (TG), total cholesterol (TC), High density lipoproteins (HDL), Low density lipoproteins (LDL) and Oxidized Low density lipoporteins (OxLDL) levels in blood serum. *Stastiscal analysis: One-Way ANOVA * $p < 0.05$ vs Ctrl; # $p < 0.05$ vs HFD. (Tukey post-hoc test).*

Serum Parameters	Ctrl	HFD	HFD-P
TG (mg/dL)	185.85 ± 33.42	255.37 ± 69.24*	251.25 ± 57.68*
TC (mg/dL)	99.62 ± 7.78	108.75 ± 4.71*	98.75 ± 7.49#
HDL (mg/dL)	47.78 ± 4.47	40.81 ± 4.50*	51.79 ± 5.35#
LDL (mg/dL)	20.24 ± 9.58	32.30 ± 12.06*	10.57 ± 6.01#
OxLDL	1.81 ± 0.56	2.25 ± 0.47*	2.83 ± 0.75*

compared using either the One-Way ANOVA for Tables 2–5 and Figs. 2–4 (4. A, 4. B and 4. C) and 5 or the Two-Way ANOVA for statistical test in Fig. 4D (Tukey post-hoc test, respectively), where $p < 0.05$ was considered a significant difference. Correlations were assessed using Pearsons's correlation tests.

3. Results and discussion

3.1. Caracterisation and evaluation of pectin

3.1.1. Composition and structural characterization of pectin

The physicochemical properties of the assayed pectin showed a pH of 3.19 ± 0.02, protein content of 1.08 ± 0.001, and moisture content of 10.3 ± 0.43. The degree of esterification (DE) value indicated that the pectin was high-methoxyl pectin (HMP), with over 78% of its carboxyl groups esterified. The estimated molecular weight (Mw) of the pectin was 429 kDa, consistent with the results of Muñoz-Labrador et al. (2018).

Pectin was composed of xylose, arabinose, galactose, and GalA (Table 1). The presence of glucose may have originated from cellulose and hemicellulose that could have been carried along during extraction. The contributions of HG (%) and RG-I (%) and other structural ratios were calculated based on Audenhove et al. (Van Audenhove et al., 2021). As shown in Table 1, the HG content and the GalA/Rha ratio indicate that HG is the predominant structure of this pectin, suggesting a lineal architecture with minimal branching. It is important to highlight the presence of other carbohydrates, such as galactose, arabinose, and xylose, in RG-I and RG-II. The presence of arabinose and galactose in pectic compounds could enhance their prebiotic potential (Ferreira-Lazarte et al., 2018). These results align with those obtained by Muñoz-Labrador et al. (2018) for pectin derived from citrus by-products. The amount of GalA with respect to the total carbohydrates constituting the pectin structure was 80.06%, which meets the additive (E−440) standard, which requires a minimum of 65% (Regulation (EU) No. 1129/2011) (Muñoz-Almagro et al., 2021b).

Fig. 1A represents the effect of viscosity on the shear stress of the pectin solution. The pectin solution exhibited non-Newtonian, pseudoplastic fluid behavior, characterized by a decrease in viscosity with an increase in shear rate. This phenomenon is common among polysaccharides, as viscosity increases with polymer concentration. Higher molecular weight (Mw) is also associated with greater viscosity. This can be explained by the reduction in intermolecular distances between pectin molecules, which increases the number of intermolecular interactions, as well as by the larger number of effective bonds formed per chain in the longer galacturonan chains and the significant increase in the hydrodynamic size of pectin chains when hydrated (Muñoz-Almagro et al., 2021a; Jong et al., 2023). Viscosity is also influenced by the DE of pectins. In this case, since it is HMP, the presence of methyl groups reduces electrostatic interactions between pectin molecules, thereby decreasing viscosity. Furthermore, the methyl groups in HMP tend to adopt a more compact configuration, which also contributes to lower viscosity (Sriamornsak, 2003).

The zeta potential of pectin, as shown in Fig. 1B, indicated that the isoelectric point was reached at a pH close to 1.5, with higher stability observed between pH 2.5 and 4.5. This behavior is associated with the fact that acidic pH levels increase the electronegativity of pectin, causing the particles to repel each other and remain suspended in the aqueous medium (Pacheco et al., 2019). The GalA content is also related to pectin stability; higher stability in pectin gels has been linked to higher GalA levels (Wang et al., 2021).

3.1.2. Properties of pectin

The WHC of pectin was 1.06 g/g. This property is related to structural factors. Higher DE values indicate the presence of more hydrophobic pectins due to a lower number of free hydroxyl groups, resulting in reduced water retention (Yang et al., 2024). observed a similar WHC for citrus pectins with similar DE to our pectin. However, Bayar et al. (2018) observed higher WHC values for fig pectins with lower DE (30 %). The extraction process, origin, and pH of the pectin can also affect its WHC (Martínez-Martí et al., 2023).

The OHC value was 0.96 g/g, similar to those reported by Bayar et al. (2018) for commercial citrus pectin. This techno-functional property is

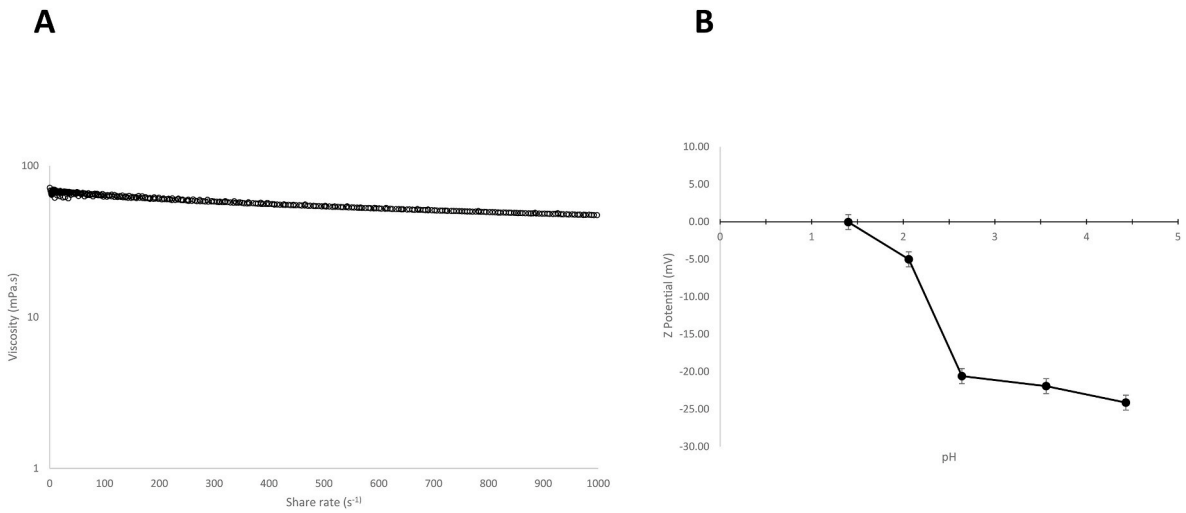

Fig. 1. (A) Viscosity curve at different shear rates of pectin 4400 2 wt % solution. (B) Zeta Potential (ζ) of curves of citrus pectins.

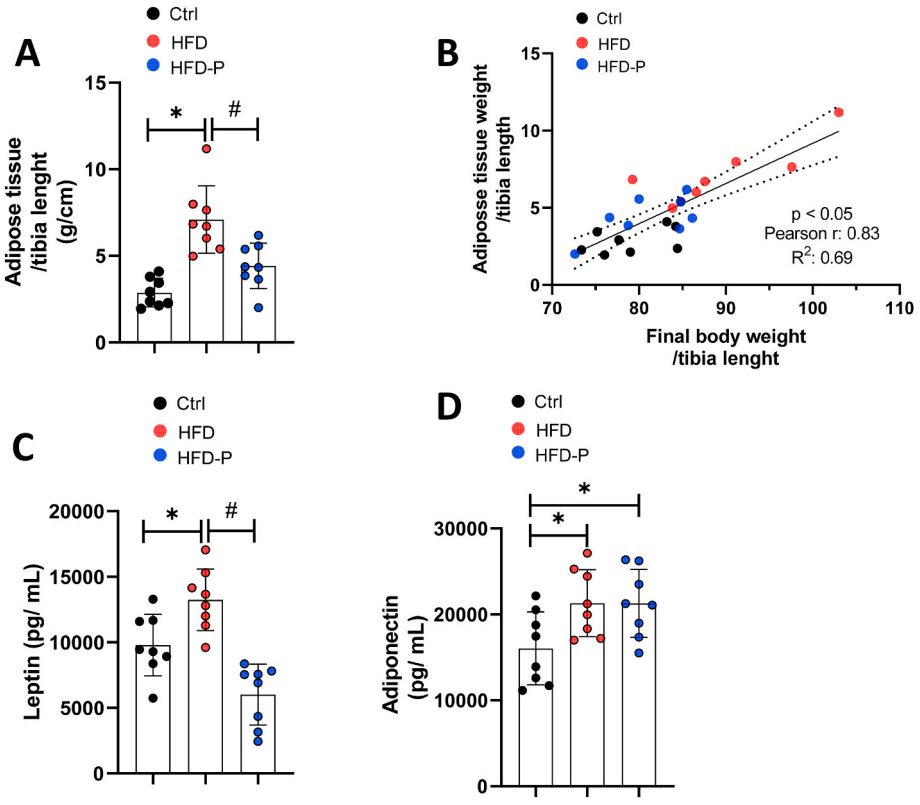

Fig. 2. (A) Relationship between adipose tissue weight and tibia length in animals from the three experimental groups. Statistical analysis: One-Way ANOVA * $p < 0.05$ vs. Ctrl; #$p < 0.05$ vs. HFD; (Tukey post-hoc test) $n = 8$ animals each group. (B) Correlation between adipose tissue weight/tibia length ratio and final body weight/tibia length ratio. Serum levels of leptin (C) and adiponectin (D). Statistical analysis: One-Way ANOVA * $p < 0.05$ vs. Ctrl; #$p < 0.05$ vs. HFD; (Tukey post-hoc test). $n = 8$ animals each group.

influenced by the structure, DE, pectin origin, pH, temperature, and interactions with other substances. Cui et al. (2023) observed that pectins with higher DE values exhibited better OHC compared to those with lower DE due to the presence of hydrophobic regions (fewer free hydroxyl groups). DE and pH are particularly interesting from a digestive perspective; in more acidic pH levels, such as those found in the stomach and duodenum, pectins with a higher DE exhibit a better capacity for retaining fats and bile salts, thereby contributing to improved cholesterol regulation (Chandel et al., 2022; Rubio-Senent et al., 2015).

Regarding EC and ES (Table 2), 2% samples exhibited the best values for these properties. Higher concentrations, due to their elevated viscosity, hindered proper emulsion formation because they did not mix well with corn oil, resulting in poorer EC and ES values. As with WHC and OHC, the structural characteristics of pectin affect EC and ES. Pectins with a high DE demonstrate better emulsifying capacity due to the interactions between their esterified groups and oil. The HG blocks provide better water retention and support the formation of more stable networks, enhancing emulsifying properties. Additionally, the neutral sugar side chains (arabinose and galactose) may create a three-dimensional network that stabilizes the emulsion by interacting with

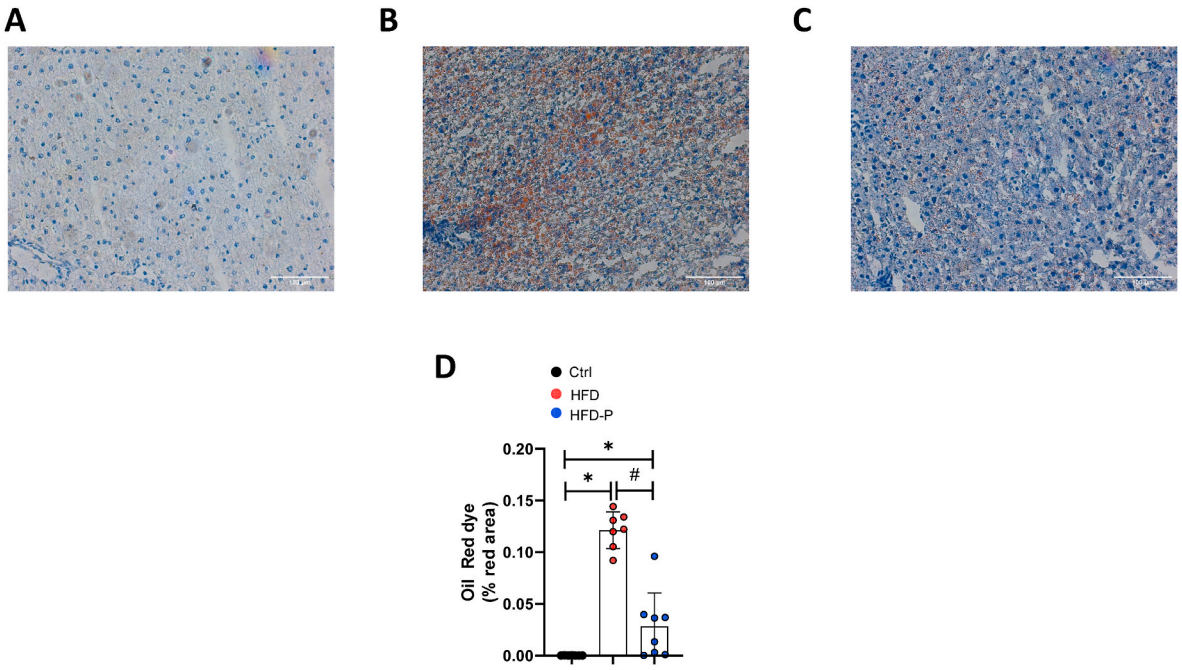

Fig. 3. Liver sections stained with Oil Red lipid dye. Representative images (20× magnification; scale: 100 μm) from Ctrl (A),HFD (B) and HFD-P (C) rats are shown. Pannel D shows the quantitative analysis of Oil Red lipid dye staining in liver sections from all the experimental groups. Statistical analysis: One-Way ANOVA * $p < 0.05$ vs. Ctrl; #$p < 0.05$ vs. HFD; (Tukey post-hoc test). $n = 8$ animals each group.

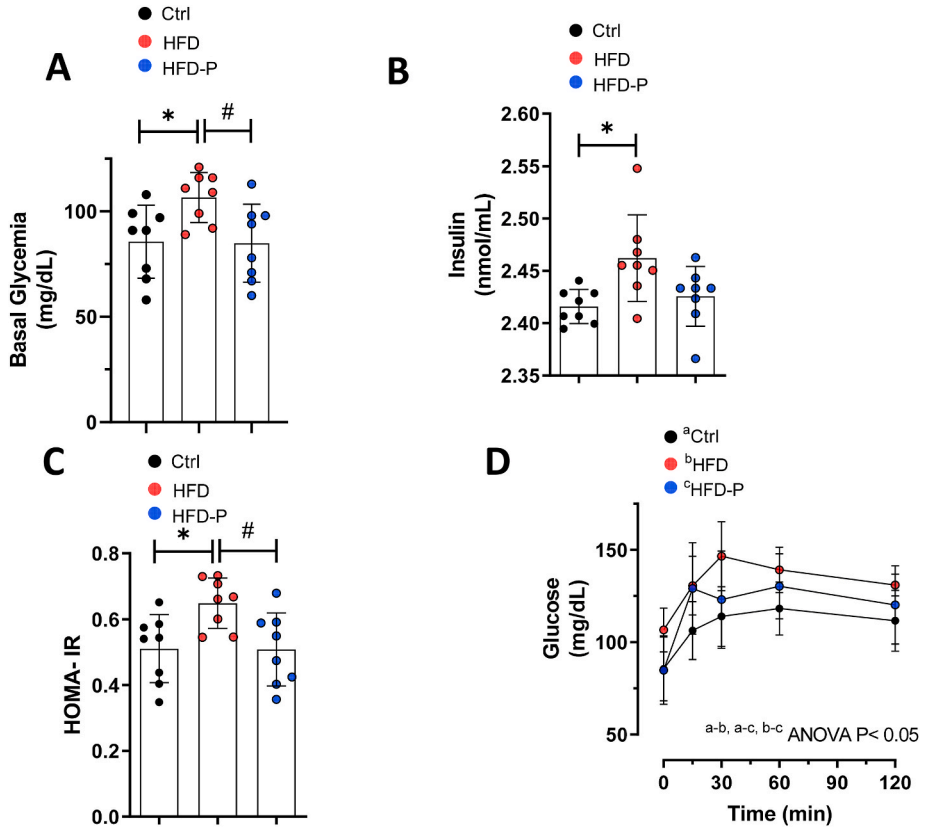

Fig. 4. Fasting glycemia (A), circulating insulin levels (B) and Homeostatic Model Assessment Insulin Resistance (HOMA-IR) index (C) in Ctrl, HFD, and HFD-P animals. Statistical analysis: One-Way ANOVA * $p < 0.05$ vs. Ctrl; #$p < 0.05$ vs. HFD; (Tukey post-hoc test). $n = 8$ animals each group. (D) Evolution of insulin resistance measured by OGTT in the experimental groups at 11 weeks of study. Statistical analysis: Two-Way ANOVA.

both water and oil. Lastly, pectins with higher Mw present better emulsifying abilities due to their capacity to form more extensive networks that effectively encapsulate oil droplets (Bayar et al., 2018; Cervantes-Paz et al., 2017).

3.1.3. Antioxidant activity and total phenolic content

We observed DPPH inhibition (Table 3) that was similar regardless of the concentration of pectin used, demonstrating that this pectin possesses antioxidant activity, likely due to the hydroxyl groups in its structure. This effect is more pronounced when the concentration of the pectin solution is not too high, as higher viscosity may hinder effective interaction between the hydroxyl groups in pectin and free radicals. Additionally, during the extraction of pectin, polyphenols, carotenoids, and proteins can be co-extracted, contributing to additional antioxidant capacity (Gerschenson et al., 2021; Chandel et al., 2022). However, we found insignificant TPC values which may be inherent to the extraction process, as these compounds could have been separated from the pectin during ultrafiltration. Therefore, the antioxidant activity of the tested pectin cannot be attributed to phenolic compounds. Instead, it seems to be more closely related to its Mw, GalA content, the presence of hydroxyl groups, and the RG-I region (Shafie and Gan, 2020). Liu et al. (2022) observed that low Mw pectins derived from citrus fruits exhibited greater antioxidant activity compared to high Mw pectins, likely due to their higher electron donation capacity and greater interaction with free radicals. These authors reported good antioxidant activity for a citrus pectin with a Mw of 422 kDa, similar to the pectin analysed in this study. Moreover, a high GalA content may enhance the antioxidant capacity of pectins, as the carboxyl groups present can chelate metal ions (iron and copper), which act as catalysts in free radical formation reactions (Djaoud et al., 2022; Shafie and Gan, 2020). Finally, pectins with lower DE tend to exhibit higher antioxidant activity due to the presence of a greater number of free hydroxyl groups that can interact with free radicals. Nevertheless, high-methoxyl pectins (HMP), despite having lower antioxidant activity than low-methoxyl pectins (LMP), still show some antioxidant activity. These findings highlight the complex relationship between the structure of pectin and its antioxidant activity.

3.2. Effect of pectin supplementation in the experimental model of MtS

3.2.1. Body weight gain and fat deposition

An increase in body weight was observed in rats fed a high-fat diet (HFD) for 7 weeks, which was greater than that of the control (Ctrl) group (Supplementary Material Table Spl. 3). After dividing the HFD group to introduce pectin supplementation, the final body weight remained greater in the HFD group compared with both the Ctrl group and the high-fat diet supplemented with pectin (HFD-P) group (Table 4). This difference might be attributed to food intake. The Ctrl group had higher food intake compared to the HFD group, but caloric intake was greater in the HFD group. The satiating effect and the reduction in gastric emptying produced by a high-calorie diet could explain these differences (Llévenes et al., 2020). Additionally, we found lower food and caloric intake in the HFD-P group compared to HFD animals (Table 4). As previously mentioned, pectin's high viscosity and emulsifying and WHC capacities likely contribute to the formation of a viscous gel at the gastrointestinal level. This gel could delay gastric emptying and enhance satiety, resulting in lower food and caloric intake and reduced body weight gain in the HFD-P group (Wei et al., 2021).

Regarding the accumulation of body fat, at the end of the experimental procedure, a higher accumulation of adiposity in the animals fed an HFD was observed compared to the Ctrl rats (Fig. 2A), likely due to the high caloric intake exceeding their energy expenditure. In the case of HFD-P rats, body fat accumulation was lower compared to the HFD group. These values correlate with the observed variations in body weight (Fig. 2B) and suggest a potential beneficial effect of pectin intake, likely due to its satiating effect and the OHC of pectin, which traps lipids and prevents their accumulation in adipose tissue (Rubio-Senent et al., 2015).

3.2.2. Adipokine release

When alterations in adipose tissue occur, the release of adipokines can be modified, potentially acquiring a deleterious role that disrupts the organism's homeostasis. Leptin, an adipokine with an anorexigenic role, can modulate adipogenesis, lipolysis, and glucose metabolism by suppressing food intake or increasing energy expenditure, playing an important role in regulating body weight and adipose tissue mass (Obradovic et al., 2021; Yu et al., 2022). In this study, we observed higher serum leptin levels in the HFD group compared to Ctrl animals (Fig. 2C), which could be related to the higher adiposity present in the HFD animals. Elevated leptin levels may lead to leptin resistance, reducing satiety, promoting overconsumption, and contributing to increased body mass, thereby exacerbating the problems associated with MtS and its related complications (Obradovic et al., 2021). We also observed a reduction in circulating leptin levels in the serum of HFD-P animals, which might contribute to decreased caloric intake, body weight, and adiposity in these animals. As previously reported by Yu et al. (2022), the reduction of leptin levels after weight loss is greater in obese individuals compared to those of normal weight.

Deregulation of adiponectin is associated with multiple cardiometabolic diseases, including insulin resistance, cardiovascular disease, and diabetes (Luo and Liu, 2022). While obesity related changes in adiponectin release are controversial, with reports of both increased and decreased levels following HFD intake, we observed an increase in serum adiponectin in HFD animals (Fig. 2D). In addition, the HFD-P group showed greater adiponectin levels than Ctrl animals, which may be due to the positive effect of pectin on insulin sensitivity, decreased adipose tissue, and reduction of inflammation-related components (Amor et al., 2019; Pacheco et al., 2018).

3.2.3. Lipid profile and liver steatosis

The HFD group showed elevated levels of TG, TC, and LDL, along with reduced HDL levels (Table 5), consistent with the lipid profile alterations present in MtS. The accumulation of TG and elevated cholesterol levels are associated with liver steatosis, which results from the irregular accumulation of TG in hepatocytes (Lian et al., 2020). In this sense, we observed liver steatosis in the HFD group (Fig. 3), as previously reported (Llévenes et al., 2020). In contrast, the HFD-P group demonstrated hypertriglyceridemia but showed lower TC and LDL and increased HDL values compared to the HFD group, reaching values similar to those of the Ctrl group. Similarly, Evans et al. (Evans, 2020) has shown that pectin and other soluble fibers can lower both TC and LDL cholesterol levels. The physical properties of pectin could be responsible for these effects: as an HMP, the acidic conditions of the stomach, combined with pectin's ability to absorb water and lipids in the small intestine and its high viscosity in aqueous solutions, may allow it to form gels that reduce the intestinal absorption of bile salts and, consequently, the action of lipases. This would lead to a reduction in serum cholesterol in the HFD-P group (Chan et al., 2017).

Other cholesterol-lowering effects of pectin may result from its interactions with bile acids. As pectin supplementation reduces bile acid reabsorption in the small intestine, more bile acids are transported to the colon and are partially dehydroxylated by microbiota enzymes. This increased bile acid excretion is associated with increased hepatic bile acid synthesis and depletion of cholesterol in the liver (Dongowski and Lorenz, 2004), which could explain the reduction in liver adiposity observed in HFD-P animals (Fig. 3). However, other authors have suggested that this reduction in liver steatosis might be due to the fermentation of pectin by gut microbiota, producing short-chain fatty acids that could serve as gluconeogenic precursors, inhibiting fatty acid synthase expression, and increasing fatty acid oxidation and glycogen storage in the liver (Hu et al., 2022). Nevertheless, no differences in OxLDL levels were found between HFD and HFD-P groups, which were significantly higher than in the Ctrl group. This indicates that despite the

antioxidant effect of the citrus pectin observed *in vitro*, it did not have repercussions in our *in vivo* model.

Structural factors dependent on the origin of the pectin, such as the DE and Mw, could also contribute to the lipid-lowering effects observed (Hu et al., 2022; Cervantes-Paz et al., 2017). As previously mentioned, the ability of pectin to absorb water during digestion results in high viscosity, which interferes with fat emulsification. This hinders the action of lipases, impeding lipid hydrolysis and absorption. Furthermore, the OHC of pectin may play a role in preventing lipid absorption, thereby improving the lipid profile and reducing adipose tissue accumulation. (Chandel et al., 2022).

3.2.4. Glucose homeostasis

Fasting blood glucose levels were higher in the HFD group (Fig. 4A) but returned to control values in the HFD-P animals, suggesting a hypoglycemic effect of pectin supplementation. This effect may be attributed to pectin's ability to form viscous gels that trap carbohydrates, preventing their absorption, as well as its inhibition of α-amylase, which hinders the digestion of complex carbohydrates and reduces hyperglycemia by preventing glucose absorption (Djaoud et al., 2022; Sánchez et al., 2008). MtS is also characterized by the development of insulin resistance. If insulin resistance persists over time, it can lead to the development of type 2 diabetes mellitus (T2DM). Elevated insulin levels are associated with increased adiposity, and the hypertrophy of adipose tissue observed in HFD rats leads to heightened secretion of adipokines, such as leptin, and pro-inflammatory cytokines. Notably, an increase in TNF-α can activate numerous intracellular signaling pathways that contribute to the inflammatory response, resulting in altered insulin function and, ultimately, insulin resistance (Rohm et al., 2022).

In addition to the greater adiposity observed in HFD animals, both plasma insulin concentrations and HOMA-IR (an indicator of insulin resistance) were elevated in this group (Fig. 4B and 4C), suggesting the presence of insulin resistance in animals fed an HFD diet. During the OTT, blood glucose levels increased in the Ctrl animals but returned to baseline after 2 h. However, this increase was more pronounced in the HFD group and persisted over the 2-h period, confirming the presence of insulin resistance in HFD rats (Fig. 4D).

The reduction in fasting glycemia observed in HFD-P animals suggests that pectin might exert a hypoglycemic effect. Additionally, pectin's capacity to retard peroxidation chain reactions could also contribute to this effect (Villamiel, 2021). The response to the oral glucose tolerance test was lower in HFD-P animals compared to the HFD group, although it did not reach the levels observed in Ctrl animals, indicating a partial improvement in insulin resistance due to pectin intake. While plasma insulin concentrations were similar between HFD-P and HFD animals, HOMA-IR was reduced by pectin intake to levels comparable to those of Ctrl animals (Fig. 4B and Fig. 4C). This improvement in insulin resistance may be related to the presence of galactose in pectin's structure, which helps mitigate inflammation and peroxidation (Muñoz-Almagro et al., 2021b; Amor et al., 2019; Pacheco et al., 2018). The reduction in leptin levels observed could also contribute to an anorexigenic effect in HFD-P animals.

Collectively, these effects may account for the reduction in adiposity and the improvement in glucose homeostasis.

3.2.5. Systolic blood pressure

Another characteristic of MtS is the presence of hypertension. Elevated levels of total cholesterol and LDL can accumulate in the endothelium of blood vessels, thereby promoting the development of atherosclerosis. This pathology is characterized by an oxidative and inflammatory environment in vascular tissue (Andersson and Hellstrand, 2012). The accumulation of these atheroma plaques produces a local increase in the release of proinflammatory cytokines such as C-reactive protein, IL-6, and TNF-α. In addition, LDL can undergo oxidation to form OxLDL. Together, these processes lead to the development of endothelial dysfunction and the onset of hypertension (Torres et al., 2019). In this study, the intake of an HFD for 7 weeks resulted in an increase in systolic blood pressure (Fig. 5A), with values exceeding 130 mm Hg, classified as hypertension (Egan et al., 2019). After randomly dividing the HFD group into two subgroups, we observed that

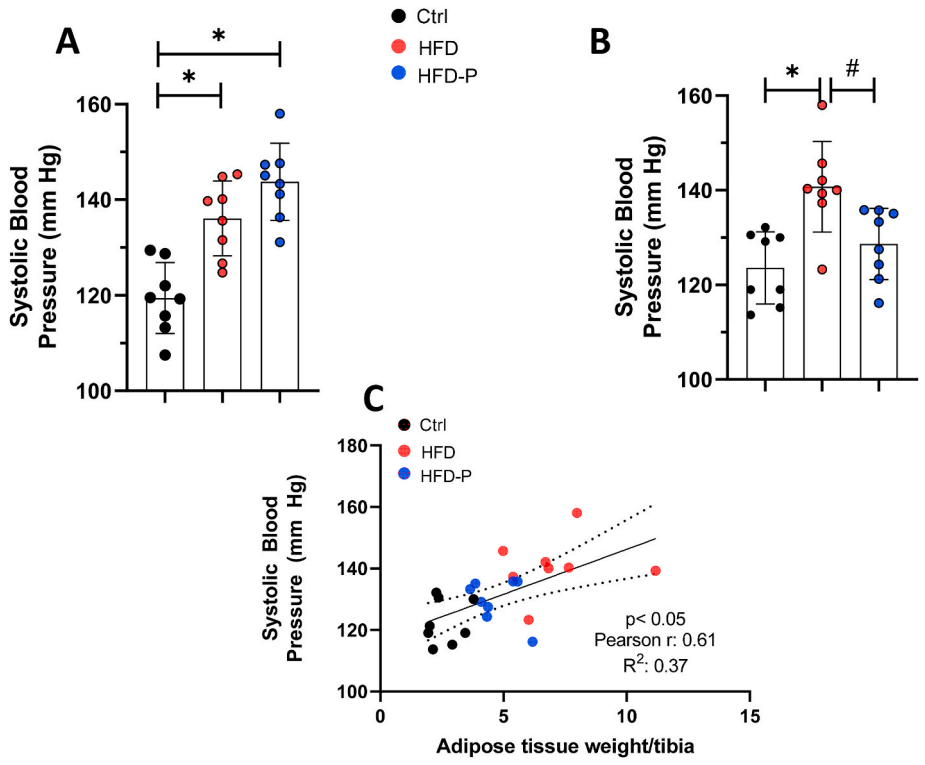

Fig. 5. Systolic blood pressure at week 7 (A) and 11 (B). Statistical analysis: One-Way ANOVA * $p < 0.05$ vs. Ctrl; #$p < 0.05$ vs. HFD; (Tukey post-hoc test). n = 8 animals each group. Correlations between final systolic blood pressure (week 11) and adipose tissue/tibia ratio (C).

pectin supplementation reduced blood pressure to levels consistent with normotension (Fig. 5B). As previously mentioned, pectin was effective in lowering both blood glucose levels and visceral fat accumulation. In line with this, we found significant positive correlations between systolic blood pressure and adipose tissue deposition at the end of the experimental procedure (Fig. 5C), further reinforcing the link between these variables and the reduction in arterial pressure (Llévenes et al., 2020; Evans, 2020). Moreover, the hypocholesterolemic effect, and the antioxidant capacity of soluble fiber produce a decrease in the adhesion of molecules on the endothelium, a decrease in TC and LDL levels, and a decrease in the oxidative environment. These effects highlight how soluble fiber consumption mitigates endothelial dysfunction, thereby generating a cardioprotective effect (Capomolla et al., 2019; Maki et al., 2007; Griendling et al., 2021).

4. Conclusions

This study is the first to investigate the relationship between the structure and physicochemical properties of pectin and its effects on key biomarkers of MtS in an obesity model associated with hypertension. The results obtained from this animal model suggest that citrus pectin supplementation may be beneficial for improving MtS induced by a HFD, particularly when combined with pharmacological treatment.

CRediT authorship contribution statement

Pablo Méndez-Albiñana: Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Methodology, Visualization, Validation. **Raquel Rodrigues-Díez:** Investigation, Methodology. **Pilar Rodríguez-Rodríguez:** Investigation, Methodology. **Rodrigo Moreno:** Investigation, Methodology, Resources. **David Muñoz-Valverde:** Investigation, Methodology. **Laura Casani:** Investigation, Methodology, Resources. **Mar Villamiel:** Funding acquisition, Conceptualization, Formal analysis, Investigation, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing, Methodology, Resources, Visualization. **Javier Blanco-Rivero:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing, All authors have read and agreed to the published version of the manuscript.

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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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2025.101014.

Data availability

Data will be made available on request.

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