International Journal of Biological Macromolecules 246 (2023) 125505

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Pectin from sunflower by-products obtained by ultrasound: Chemical characterization and *in vivo* evaluation of properties in inflammatory bowel disease

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ARTICLE INFO

Keywords:
Sunflower pectin
Structural characterization
Galacturonic acid
Degree of methyl-esterification
Low-methoxyl pectin
Anti-inflammatory effect

ABSTRACT

Inflammatory bowel disease (IBD) is a public health challenge and the use of pectin for symptom amelioration is a promising option. In this work, sunflower pectin has been extracted without (CHP) and with assistance of ultrasound (USP) using sodium citrate as a food-grade extracting agent. At optimal conditions (64 °C, 23 min) the highest yield was obtained with ultrasound application (15.5 *vs.* 8.1 %). Both pectins were structurally characterized by ^{1}H NMR, HPSEC-ELSD, FT-IR and GC-FID. Unlike CHP, USP showed a lower molecular weight, higher galacturonic acid, lower degree of methyl-esterification and, overall, higher viscosity. These characteristics could affect the anti-inflammatory activity of pectins, evaluated using DSS-induced IBD model mice. So, USP promoted the defence (ICAM-1) and repair of the gastrointestinal mucosa (TFF3, ZO-1) more effectively than CHP. These results demonstrate the potential amelioration of acute colitis in IBD mice through USP supplementation. Taking into account the biomarkers analysed, these results demonstrate, for the first time, the positive impact of sunflower pectin extracted by ultrasound under very soft conditions on inflammatory bowel disease that might open up new possibilities in the treatment of this serious pathology.

1. Introduction

Inflammatory bowel disease (IBD) (ulcerative colitis and Crohn's disease) has emerged as one of the major chronic disorders in the world [1,2]. Current therapies (biological agents, antibiotics, immunosuppressive and anti-inflammatory drugs) involve unpredictable side effects [1,3,4]. Thus, there is a growing interest in the development of effective treatments that repair the intestinal barrier and mitigate symptoms. In this sense, pectins seem to be a promising polysaccharide as an adjuvant in the treatment of IBD, exhibiting anti-inflammatory effects *in vitro* and *in vivo* [5–7].

Pectins are heteropolysaccharides composed mainly of a linear polymer of (1 → 4) linked galacturonic acid (GalA), known as homogalacturonan (HG) which can be partially methyl esterified in C-6. This domain can be interrupted by rhamnose residues that bear side chains (arabinans, galactans and arabinogalactans) to form rhamonogalacturonan-I (RG-I) [8]. Pectins are classified as high-methoxyl pectins (HMP; degree of methyl-esterification (DM) > 50 %) or low-methoxyl pectins (LMP, DM < 50 %). The former derive almost exclusively from by-products from juice manufacturing (citrus peel and apple pomace), being the most used commercial pectins [9]. The industry produces artificial LMP by acid, alkaline and/or enzymatic de-esterification methods that are costly for large-scale commercial production. An alternative to de-esterification processes is the use of cheap unconventional sources of natural LMP such as sunflower heads (SH) that can contain up to 20 % of this polysaccharide [9,10]. Unlike HMP, LMP has a net negative charge from the free carboxyl groups of the GalA residues, which interact with positively charged amino acids, located at

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https://doi.org/10.1016/j.ijbiomac.2023.125505
Received 8 March 2023; Received in revised form 8 June 2023; Accepted 20 June 2023
Available online 22 June 2023

the C- and N-termini of mucin. In this way, LMP penetrate more easily the mucin layer of the intestinal wall by direct interaction with the intestinal epithelia, stimulating small intestinal mucin secretion [11–13].

Industrially, pectin extraction requires the use of strong acids during long periods at high temperatures, which is time- and energy-consuming and leads to pectin degradation [14]. However, the use of sodium citrate as an extraction agent can favour the quality requirements for food-grade products, and also contribute to reducing the environmental risks associated with treating chemical effluents [15]. In addition, Ultrasound Assisted Extraction (UAE) has received considerable attention due to its numerous advantages including reductions in energy costs and, mostly, increasing the yield and quality of the recoverable pectin with a shorter extraction time [16]. The mechanism of this technique is based on the periodic production and collapse of numerous cavitation bubbles that cause cell disruption, contributing to the higher penetration of the solvent into the target material [17,18]. Besides, this cavitation effect can cause, among other effects, the breakage of glycosidic linkages, reducing the molecular weight of the pectin (Mw), and consequently, facilitating its metabolism in the colon, resulting in an increase in its bioactivity [19,20].

Ponmurugan et al. [14] studied the recovery of pectin from SH by UAE, focusing on the process variables used in the optimization of sunflower pectin extracted with ultrasound (US), although its chemical composition was scarcely characterized, and its biological properties were not evaluated. In the present study, the anti-inflammatory activity of sunflower pectins obtained with US was evaluated in mice with colitis induced by dextran sulfate sodium (DSS). Moreover, an effort to elucidate the relationship between pectin structure, its rheological properties and its bioactivity was made. Therefore, a structural analysis of obtained pectins, using different analytical techniques, was carried out. In addition, we optimized the extraction parameters of US through the response surface methodology (RSM) in order to reach the maximum pectin yield. In this way, we propose that these pectins could be ready-to-operate through a scaling process for their industrialization, without endangering the environment.

2. Materials and methods

2.1. Materials and chemicals

Sunflower (*Helianthus annuus*) heads (SH) without seeds (Fig. 1 Supplementary) were harvested in Albacete (Spain) and supplied as a generous gift by Syngenta (Madrid, Spain). The SH samples were grinded and sieved (particle size $\leq$ 100 μm). Standard monosaccharides (glucose, mannose, rhamnose, arabinose, galactose, GalA and xylose), Pullulan Standard Set (0.34–805 kDa), β-phenyl-glucoside, hexamethyldisilazane, trifluoroacetic acid, sodium citrate tribasic dihydrate, citric acid monohydrate and RNAlater® were purchased from Sigma Aldrich (St. Louis, MO, USA). Carbon adhesive tabs and aluminium mount were acquired from Aname (Madrid, Spain). Deuterated water was obtained from Symta (Madrid, Spain). Ammonium acetate was acquired from Panreac Applichem (Darmstadt, Germany) and ethanol 96 % was obtained from VWR (Barcelona, Spain). DSS (36–50 kDa) was obtained from MP Biomedicals (Santa Ana, CA, USA). Tri-Reagent® was provided from Thermo Fisher Scientific (Invitrogen, USA). The oligo (dT) primers (Promega, Southampton, UK) and KAPA SYBRsFAST qPCR Master Mix (Kapa Biosystems, Inc., Wilmington, MA, USA) were used to perform the qPCR analyses.

2.2. Pectin extraction

Before extraction, 7.5 g of ground SH powder were washed with 300 mL MilliQ water at room temperature in order to remove impurities. Afterward, the mixture was centrifuged at 3700*g* (Heraeus Multifuge 3SR Plus, Termo Scientific, Massachusetts, USA) for 10 min. The wet solid was mixed with 0.74 % (*w/v*) sodium citrate giving an original solid/liquid ratio 1:20 w/v; this mixture was adjusted with citric acid at pH 3.3. These conditions were selected based on a previous study [21] whose parameters were optimized by a response surface methodology (RSM) taking into account the yield of extraction.

The extraction method was assisted by US using an ultrasonic bath with internal dimensions of 24.0 cm × 14.0 cm × 10.0 cm, a capacity of 3 L and a frequency of 45 kHz (Sonica Sweep System EP 2200, Soltec, Milan, Italy). The US power used was determined using the following equation (Eq. (1))

$$\text{Power density} = \text{mCp}\frac{\text{dT}}{\text{dt}} \quad (1)$$

where m is the water mass (kg), Cp is the specific heat of water [J/(kg °C s)] and dT/dt is the temperature change along with the whole time range (°C/s) determined by a polynomial curve fitting. Power density used was 0.17 W/cm^3.

After the extraction reactions with sodium citrate with the assistance of US, samples were centrifuged and the supernatants were treated with ethanol to precipitate pectins. After two cycles of washing with acidulated ethanol, pectins were freeze-dried for further analysis.

The pectin yield was calculated according to the formula:

$$\text{Pectin yield}\ (\%) = \frac{\text{m}\ (\text{g})}{\text{m}_0(\text{g})} \times 100 \quad (2)$$

where m is the weight of dried pectin (g) and m_0 is the initial weight of SH (g).

2.3. Statistical experimental design and analysis

Optimization of pectin extraction from SH was done applying a Centred Composite Rotatable Design (CCRD) using "Design-Expert®10 trial version" (Stat-Ease Inc., Minneapolis, USA). The influence of temperature (X_1, 40–60 °C) and time (X_2, 40–120 min) was studied obtaining surface responses for pectin yield. CCRD consist of four axial, 4 factorial and 5 central points, and totalizing 13 trials for each type of extraction (Table 1) at the 0.05 significance level. The experiments were performed in a randomized order to avoid systematic errors. The quadratic model for predicting the optimal point was expressed as follows:

$$Yi = \beta_0 + \beta_i X_i + \beta_j X_i^2 + \beta_{ij} X_i X_j \quad (3)$$

Table 1
Centered composite design with independent variables, temperature (°C, X_1) and time (min, X_2) for yield of sunflower pectin. General conditions 0.74 % (w/v) sodium citrate, pH 3.3, solid/liquid ratio 1:20 w/v; using an ultrasonic bath (45 kHz).

Experiments	Design points	Temperature (°C)	Time (min)	Pectin yield (%)
1	Factorial point	40.0	40.0	2.0
2	Factorial point	60.0	40.0	8.7
3	Factorial point	40.0	120.0	8.7
4	Factorial point	60.0	120.0	7.9
5	Axial (star) point	35.9	80.0	1.4
6	Axial (star) point	64.1	80.0	13.1
7	Axial (star) point	50.0	23.4	2.5
8	Axial (star) point	50.0	136.6	2.0
9	Centre (middle) point	50.0	80.0	1.5
10	Centre (middle) point	50.0	80.0	1.6
11	Centre (middle) point	50.0	80.0	1.4
12	Centre (middle) point	50.0	80.0	1.4
13	Centre (middle) point	50.0	80.0	1.3

where Y_i is the predicted response for each experience (i = 1–13) and each experimental response (X_{1-2}), β_0 is the intercept, β_i, are linear coefficients, βii are squared coefficients, and βij are the interaction coefficients for each independent variable. The quality of fit of the second-order model equation was expressed by the coefficient of determination R^2. Also, R^2-Adjusted (percentage of explained variance) and R^2-Predicted (indicator of how well the regression model predicts responses for new observations) were taken into account to evaluate uniformity of the model. Statistical significance was determined by *P*-value. The significance of the regression coefficients was tested by t-value.

2.4. Structural characterization of sunflower pectin

The estimation of Mw distribution of pectin samples was determined according to the method described by Muñoz-Almagro et al. [22]. Samples (50 μL) were eluted with 0.01 M NH_4Ac at a flow rate of 0.5 mL/min for 50 min at 30 °C in a HPSEC-ELSD.

Pectins extracted were hydrolyzed and derivatized forming their thimethylsilyl oximes before GC-FID analysis following the method of Muñoz-Almagro et al. [21]. Analyses were carried out using a DB-5HT capillary column (30 m × 0.32 mm × 0.10 μm) (J&W Scientific, Folson, California, USA). Oven temperature program was increasing from 150 °C to 165 °C at 1 °C/min, held for 17 min, then increased at a rate of 10 °C/min to 200 °C and up to 380 °C at a heating rate of 50 °C/min.

Freeze-dried samples were analysed by FT-IR according to the method described by Muñoz-Almagro et al. [21]. The DM of pectin was determined as the average of the ratio of the peak area at 1747 cm^{-1} (COO-R) over the sum of the peak areas of 1747 cm^{-1} (COO-R) and 1632 cm^{-1} (COO^-).

Morphological observations of the sunflower pectins extracted without and with UAE at optimal conditions (CHP, USP), were performed using a DSM 950 scanning electron microscope (SEM) (Zeiss Iberia, Madrid, Spain) at 7 kV accelerating voltage, a magnification of 350× and a distance of 10 mm.

For Nuclear Magnetic Resonance (NMR) spectroscopy analysis, ^1H NMR spectra were acquired at 30 °C. The samples were solubilized in D_2O and the chemical shifts were expressed as δ (ppm), using this solvent as internal references at 4.64 ppm. NMR spectra were recorded on a Varian Unity VXR-300.

2.5. Rheological properties of sunflower pectins

Rheological measurements were done using an AR 2000 rheometer (TA Instruments Ltd., Crawley, U.K.). Data were analysed with the computer software Rheology Advantage (TA Instruments, Waters Co., Ltd). A cone (40 mm diameter, 1°) was employed for viscosity (0.1–2 % *w/v*) using a gap size of 85 μm at 25 °C. For steady flow studies, the shear rate changed from 0.1 to 1200 s^{-1}. Rheological tests were performed in the range 0.05–10 Hz (0.5 % strain).

2.6. Assessment of anti-inflammatory activity in DSS-treated mice

2.6.1. Experimental design

C57BL/6 male mice (7–9 weeks old) were obtained from Janvier (St Berthevin Cedex, France). This study was carried out in accordance with the Guide for the Care and Use of Laboratory Animals as promulgated by the National Institutes of Health. The experimental protocol was approved by the Commission of Ethics in Animal Experimentation (Protocol CEEA 2010-286) of the University of Granada (Spain). They were housed in Makrolon cages, maintained under an air-conditioned atmosphere with a 12 h light–dark cycle, and provided with free access to tap water and a standard rodent diet (Panlab A04 diet, Panlab S. A., Barcelona, Spain).

Mice (23 ± 2 g) were maintained under specific pathogen-free conditions in the facilities of Animal Experimentation Unit of the University of Granada, and were randomly assigned to four groups (n = 10): healthy, DSS control, sunflower pectin extracted by UAE at optimal conditions (USP) and its corresponding control heating pectin (CHP), sunflower pectin extracted at optimal conditions without US. The pectins were diluted with water and administered by oral gavage (100 μL per day) corresponding to a daily dose of 40 mg/kg per mouse. This dose was chosen taking into account previous studies [6]. Induction of colitis was performed 15 days after the start of the treatment with pectins by adding 3 % (*w/v*) DSS to the drinking water for seven days, following the time schedule showed in Fig. 2 Supplementary.

2.6.2. Macroscopic indicators

Weight variation and Disease Activity Index (DAI) were considered to be macroscopic indicators. DAI was assigned as the sum of body weight, the presence of gross blood in the feces and the stool consistency according to the Zhao et al. [23] (Table 1 Supplementary).

2.6.3. In vivo intestinal permeability

The day before sacrifice, mice were fasted for 12 h and administered DX-4000–FITC (Sigma-Aldrich) by oral gavage (350 mg/kg body weight). 4 h after administration, just before sacrifice under isoflurane anaesthesia, blood was collected by cardiac puncture and centrifuged at

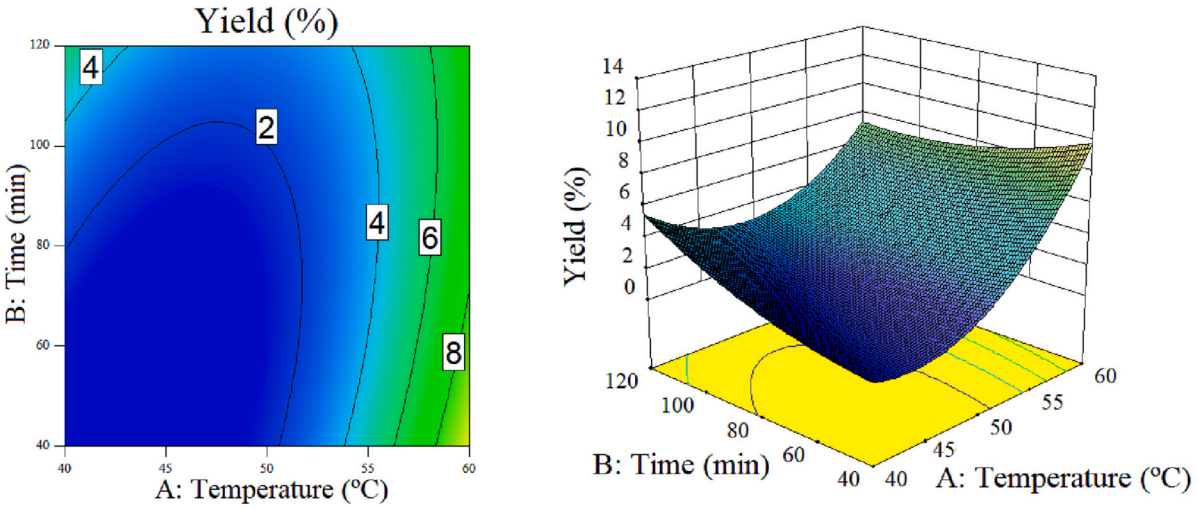

Fig. 1. Response surface plot (2D, right) and contour plot (3D, left) of sunflower pectin yield (%) as a function of significant interaction between extraction time and extraction temperature.

Table 2
Analysis of variance (ANOVA) of the quadratic model for the yield of sunflower pectin.

Source	Sum of squares	DF	Mean square	F-value	P-value	
Model	5	163.569	32.7138	8.1	0.008	Significant
Linear	2	66.35	33.1752	8.21	0.015	
Temperature	1	62.98	62.9795	15.58	0.006	
Time	1	3.371	3.3708	0.83	0.391	
Square	2	83.156	41.5781	10.29	0.008	
Temperature * temperature	1	81.545	81.5448	20.18	0.003	
Time * time	1	5.936	5.9361	1.47	0.265	
2-Way interaction	1	14.062	14.0625	3.48	0.104	
Temperature * time	1	14.062	14.0625	3.48	0.104	
Error	7	28.288	4.0411			
Lack-of-fit	3	28.236	9.412	724	0.000	Significant
Pure error	4	0.052	0.013			
Total	12	191.857				
R^2					85.26 %	
R^2adj					74.72 %	

Table 3
Molecular parameters and sugar composition of pectins extracted from SH under optimum conditions on yield extraction, DM and Mw (mean ± SD).

	CHP	USP
Yield (%)	8.1 ± 0.2^a	15.5 ± 0.4^b
GalA (%)	84.2 ± 0.6^a	88.0 ± 0.9^b
Rha (%)	3.8 ± 0.1^a	4.0 ± 0.2^a
Gal (%)	3.9 ± 0.1^b	3.1 ± 0.1^a
Xyl (%)	2.4 ± 0.1^b	0.2 ± 0.0^a
Ara (%)	2.9 ± 0.2^a	3.0 ± 0.1^a
Man (%)	1.2 ± 0.1^b	0.8 ± 0.2^a
Glc (%)	1.6 ± 0.2^b	0.9 ± 0.3^a
Mw (kDa)	776 ± 12^b	500 ± 8^a
DM (%)	42 ± 0.5^b	35 ± 0.2^a
$\frac{GalA}{Rha}$	22.2 ± 0.6^a	22.0 ± 0.9^a
$\frac{Gal + Ara}{Rha}$	1.8 ± 0.1^b	1.6 ± 0.1^a

Note: values with different small case superscript letters (a–b) in the same column within each solvent and each pectin indicate significant differences as estimated by Tukey's test ($P < 0.05$); Xyl: xylose; Ara: arabinose; Rha: rhamnose; Gal: Galactose; Man: mannose; Glc: glucose; GalA: galacturonic acid.

3000 rpm for 10 min at 4 °C. Plasma was diluted (1:10) in PBS, and FITC-dextran concentration was determined with a fluorescence spectrophotometer (Fluorostart, BMG Lab Technologies) at an emission wavelength of 535 nm and an excitation wavelength of 485 nm. A standard curve was obtained by serial dilutions of FITC-dextran in PBS.

2.6.4. Biochemical markers

The expressions of pro-inflammatory cytokines such as tumor necrosis factor (TNF-α), interleukins (IL-6, IL-1β), inducible nitric oxide synthase (iNOS), monocyte chemoattractant protein-1 (MCP-1), receptors as toll-like receptor 4 (TLR4) as well as barrier intestinal proteins such as trefoil factor peptide 3 (TFF3), intercellular adhesion molecule (ICAM-1), mucin (MUC-1, MUC-2, MUC-3), occluding, villin and zonula occludens-1 (ZO)-1, were evaluated. The colon tissue was longitudinally divided into different fragments and stored at −80 °C in RNAlater®. Total RNA from colonic samples was isolated using Tri-Reagent® following the manufacturer's protocol. All RNA samples were quantified with the Thermo Scientific NanoDrop™ 2000 Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) and 2 μg of RNA was reverse transcribed using oligo (dT) primers (Promega). Real-time quantitative PCR was carried out on optical grade 48-well plates in an Eco™ Real-Time PCR System (Illumina, CA, USA) with 20 ng of cDNA,

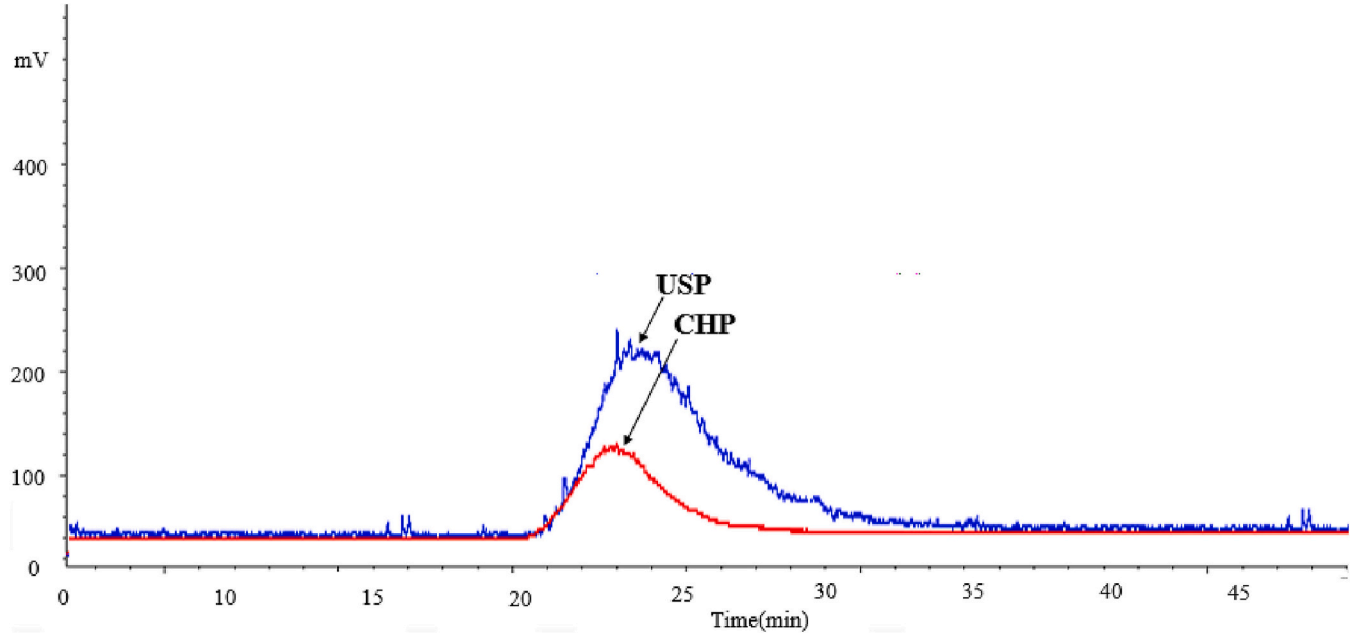

Fig. 2. Chromatographic HPSEC-ELSD profiles for sunflower pectin extracted without (CHP) and with ultrasound (USP) at optimal conditions (64 °C, 23 min).

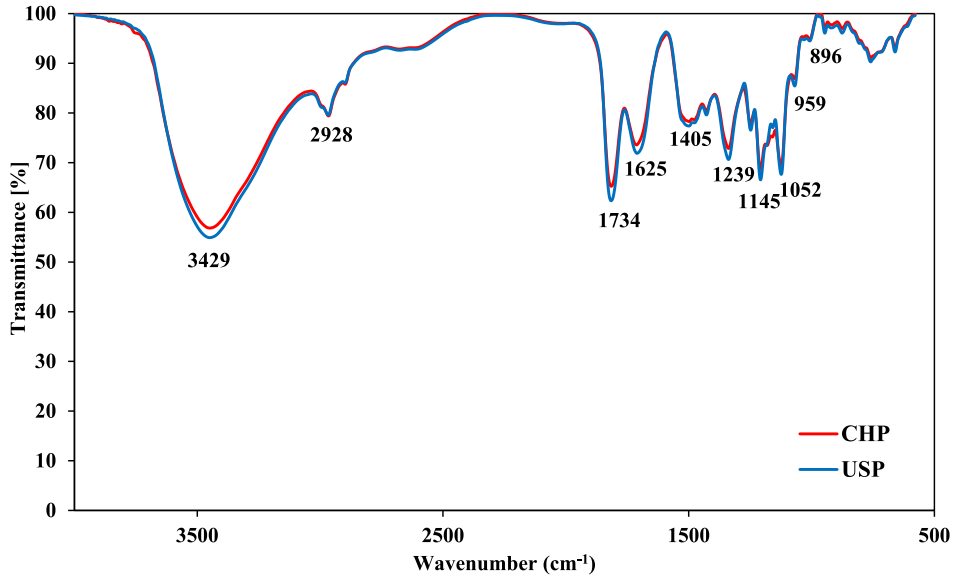

Fig. 3. FT-IR spectra of the sunflower pectin extracted without (CHP) and with ultrasound (USP) at optimal conditions (64 °C, 23 min).

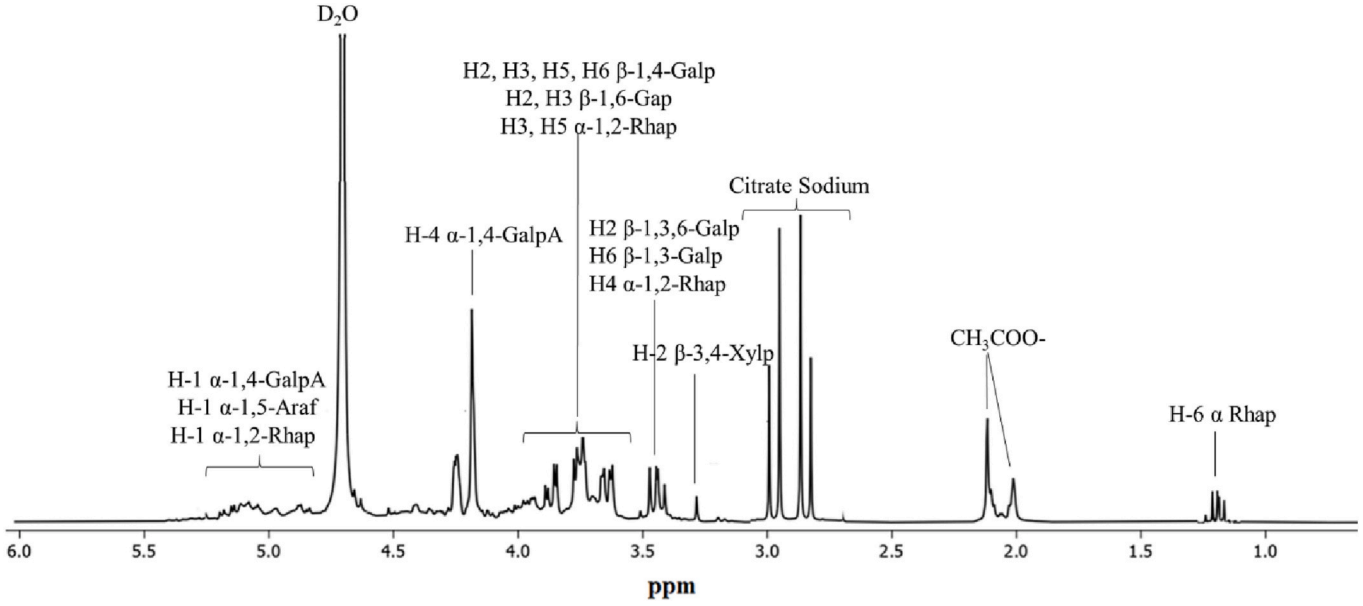

Fig. 4. ^{1}H NMR spectra of USP.

KAPA SYBR®FAST qPCR Master Mix (Kapa Biosystems), and specific primers at their annealing temperatures (Table 2 Supplementary). In order to normalize mRNA, the expression of the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), was measured. The relative quantitation of mRNA was calculated using the ΔΔCt method.

2.7. Statistical analysis

Data were expressed as mean values ± SD. A Tukey's test to evaluate the differences was performed using IBM® SPSS® Statistics 25 (IBM Corporation, Armonk, NY, USA).

3. Results and discussion

3.1. Optimization and validation of pectin extraction

To investigate the influence of time and temperature on the extraction yield of sunflower pectins, a CCRD was carried out with an US bath (45 kHz). In general, the higher the temperature the better the yields of pectin extraction (Table 1). The lowest yields (<1.5 %) were found at temperatures below 50 °C, whereas the highest yield values were obtained at the highest temperature (64.1 °C).

The optimal conditions obtained to reach the highest extraction recovery of pectin from SH were 23 min and 64 °C with a desirability of >0.999. In these conditions, the mean experimental pectin yield (15.5 %) was near the predicted value (14.4 %), demonstrating the validation of the optimized conditions. It is noteworthy that the optimal values found for these experiments were located in the edges of the surface of study for all evaluated variables, which suggests that the optimal region

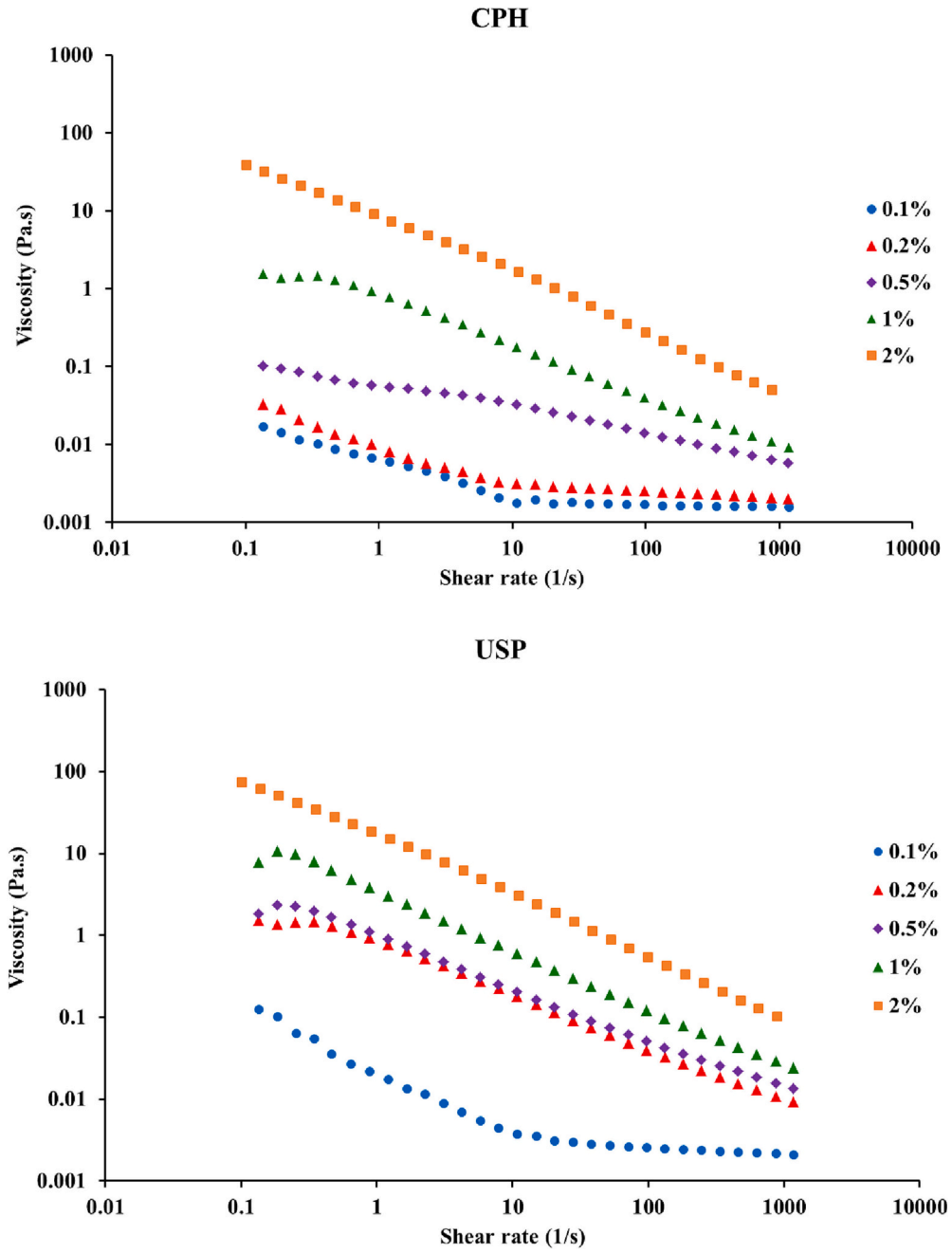

Fig. 5. Viscosity-shear rate profiles of sunflower pectins at different concentration (T = 25 °C, 0.1–2 % (*w/v*)).

might be located at high temperatures and short reaction times (Fig. 1), but always taking into account that the maximum value was very close to 60 °C. According to the literature, the maximum mechanical effect of US is obtained at temperatures near 60 °C, since this appeared to be the maximum temperature for cavitation bubbles to coalesce with vapour bubbles, suggesting that values above this temperature might interfere with the US effect on pectin extraction [24].

Temperature had a significant effect ($p < 0.05$) for linear and interaction in the model parameters, which means it plays an important role in pectin extraction. For all cases, R^2 was above 85 %, which indicates a good fit of data to the model. R^2-adjusted values were close to R^2, which is desired for the model. According to the ANOVA results shown in Table 2, temperature had an important effect ($p < 0.05$) on the yield of pectin extraction for linear and quadratic parameters.

At the same optimal conditions, sunflower pectin was also obtained but without the use of US (control heating pectin, CHP). Thus, as indicated in Table 3 the yields obtained using sodium citrate at 64 °C, 23 min and pH 3.2 without US were 8.1 %, around 47 % lower than the yield obtained under the same conditions but with ultrasonic assistance. The UAE increases the rupture of the sunflower cell wall matrix leading to better interactions between the solvent and extracted material [25]. In addition to that, our results were also above those reported by Ponmurugan et al. [14] who obtained 8.9 % of sunflower pectin by UAE. These discrepancies could be attributed to the different solid-liquid rate used (1:15 *vs* 1:20). Bayar et al. [25] found that the highest yields are reached by the increase of the liquid-solid ratio, which improves the contact area between the raw material and the extracting agent. In other words, an attenuation of the US intensity occurs with increasing solid particle contents.

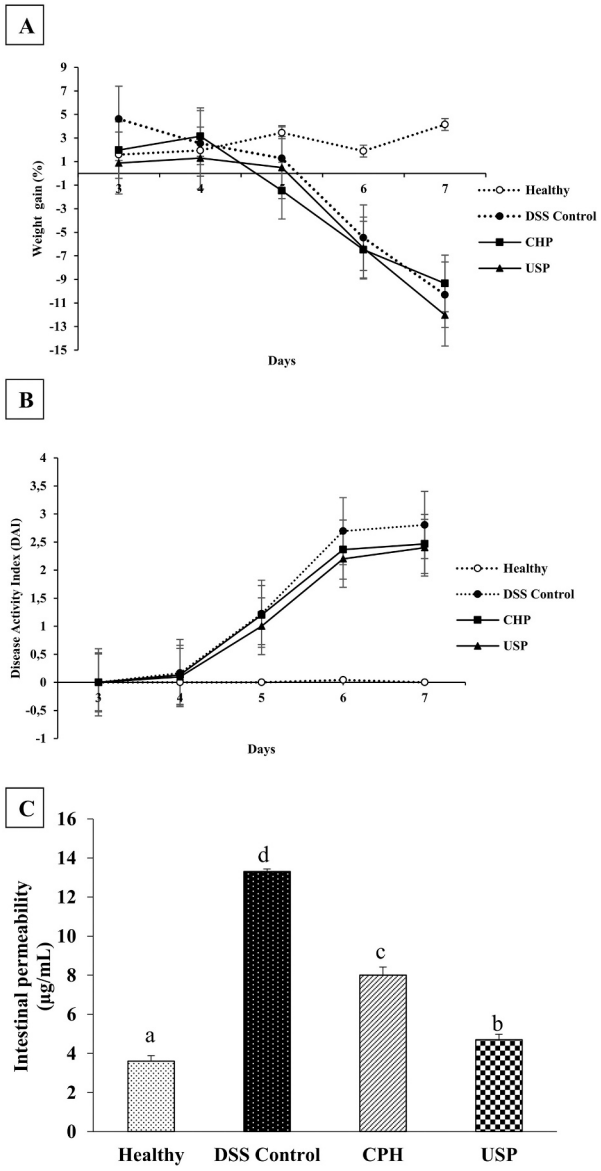

Fig. 6. Effects of CHP and USP on the macroscopic indicators. (a) weight gain; (b) Disease Activity Index (DAI); (c) intestinal permeability. CHP: control heating pectin. USP: pectin extracted by ultrasound.

3.2. Characterization of extracted pectin under optimal selected conditions

The compositional and structural characteristics of the pectin recovered with and without the assistance of US are shown in Table 3. As expected, GalA was the most abundant monosaccharide in both samples, being significantly higher in samples extracted by US, in agreement with Dranca and Odorian [26] who stated, in apple pomace, that sonication has a statistically significant influence on the GalA content of the extracted pectin. Regarding the content of GalA and neutral sugars (Table 3), it is evident that the structure of pectin consists mainly of HG, as well as a small part of RG-I (arabinan and arabinogalactan) chains [8]. As a consequence of sonication, hardly any change was observed in the degree of branching (GalA/Rha) and complexity of branch distribution (Ara + Gal/Rha), with only a slight decrease in the latter.

Another noteworthy aspect is that the content of the monosaccharides (glucose and mannose) derived from non-pectic polysaccharides such as cellulose and hemicellulose, were considerably lower in the pectin obtained after sonication. Ponmurugan et al. [14] showed similar trends in sunflower pectin recovered by UAE under different extraction conditions. A plausible explanation for this fact could be that the US treatment cleaved the covalent linkages of non-pectic and pectic polysaccharides [14]. This could corroborate that US treatment improves the purity of the obtained pectin in comparison with the traditional method.

Moreover, a study on the effect of US on the Mw of sunflower pectins was carried out by HPSEC-ELSD. Fig. 2 depicts the chromatographic profiles of pectin extracted with and without US in the presence of sodium citrate. The pectin distribution in the CHP sample showed the earliest elution and, in the case of USP, a displacement of the peaks to be right could indicate that the pectin extracted with US treatment was modified to species of lower Mw (776 *vs* 500 kDa, Table 3), in agreement with Soria, Villamiel and Montilla [27] who revised the main structural effect of US on polysaccharides, including pectins.

FT-IR, ^{1}H NMR and SEM (Figs. 3–4 and Fig. 3 Supplementary) were applied to elucidate if the different extraction methods used to obtain pectin could affect the functional groups and their bonding configurations. The FT-IR spectra of both sunflower pectins are presented in Fig. 3. The wide and marked absorption band at 3429 cm^{-1} corresponds to stretching vibration of hydroxyl groups due to inter- and intramolecular hydrogen linkages located in the main chain of GalA [25]. The band that appears on 2928 cm^{-1} assigned to the vibrations of C—H (—CH, —CH_2, —CH_3), exhibited the same area in all pectin samples, indicating that the main linear domain (HG) of the USP did not suffer any breakage of glycosidic bond during the sonication. These results can also be corroborated with the peaks 1405, 1239, 1145 and 1052 cm^{-1} corresponding to the stretching vibrations of C—OH side groups and the C—O—C glycosidic bond vibration, which could be due to the minor presence of glycosidic linkages between sugar units [28]. From the analysis of the DM (Table 3), it is observed that slight but significant changes were registered between the pectin obtained after US treatment and its corresponding control (35 % *vs* 42 %). These data might indicate that the de-esterification of pectin chains that occurs under US extraction was not along with variations in the main structural HG chain of pectin [29].

The ^{1}H NMR spectrum corresponding to USP (Fig. 4) showed different doublet around 1.20 ppm corresponding to methyl protons of H-6 rhamnoses. The following peaks appearing at 2.01 and 2.12 ppm corresponded to methyl protons from acetyl group binding [30,31]. Most of the pectin signals appear between 3.0 and 5.5 ppm. 3,4-β-Xylp gave a H2 signal at 3.31 ppm [32]. The signal at 3.45 ppm was assigned to H2 of 1,3,6-β-Galp, H6 of 1,3-β-Galp and H4 of 1,2-α-Rhap. The signals overlapping between 3.60 and 3.80 ppm were attributed to 1,4-β-Galp: H2, H3, H5 and H6; 1,6-β-Galp: H2 and H3 and 1,2-α-Rhap: H3 and H5 [33]. The signal for H2 of 1,4-α-GalpA was observed at 3.83 ppm in the spectrum. The signal at 3.88 ppm was attributed to H5 and H6 of 1,6-β-Galp and H5 of 1,5-α-Araf. The intensity signal at 4.23 was assigned to H4 of 1,4-α-GalpA. At around 5 ppm appear the signals of H1 of 1,4-α-GalpA, 1,5-α-Araf and 1,2-α-Rhap [33]. These results were in agreement with the aforementioned characterization of pectin.

The microstructure of CHP and USP analysed by SEM is illustrated in Fig. 2 Supplementary (a–b). As is depicted, both pectins consisted of larger, irregular, smooth and flake-shaped surfaces. A slight and increasing compaction of the structure seems to be observed in USP, probably due to the higher content of GalA that favours the formation of a network of pectin [34].

3.3. Steady-shear measurements of sunflower pectins

As shown in Fig. 5, the apparent viscosity of the studied sunflower pectins depended on the shear rate at different concentrations. CHP and USP solutions showed nearly Newtonian flow behaviour at concentrations inferior to 0.2 and 1 % *w/v*, respectively. On the contrary, a pseudoplastic flow became dominant when the concentration increased. The viscosity of pectin decreased rapidly with increasing shear rate

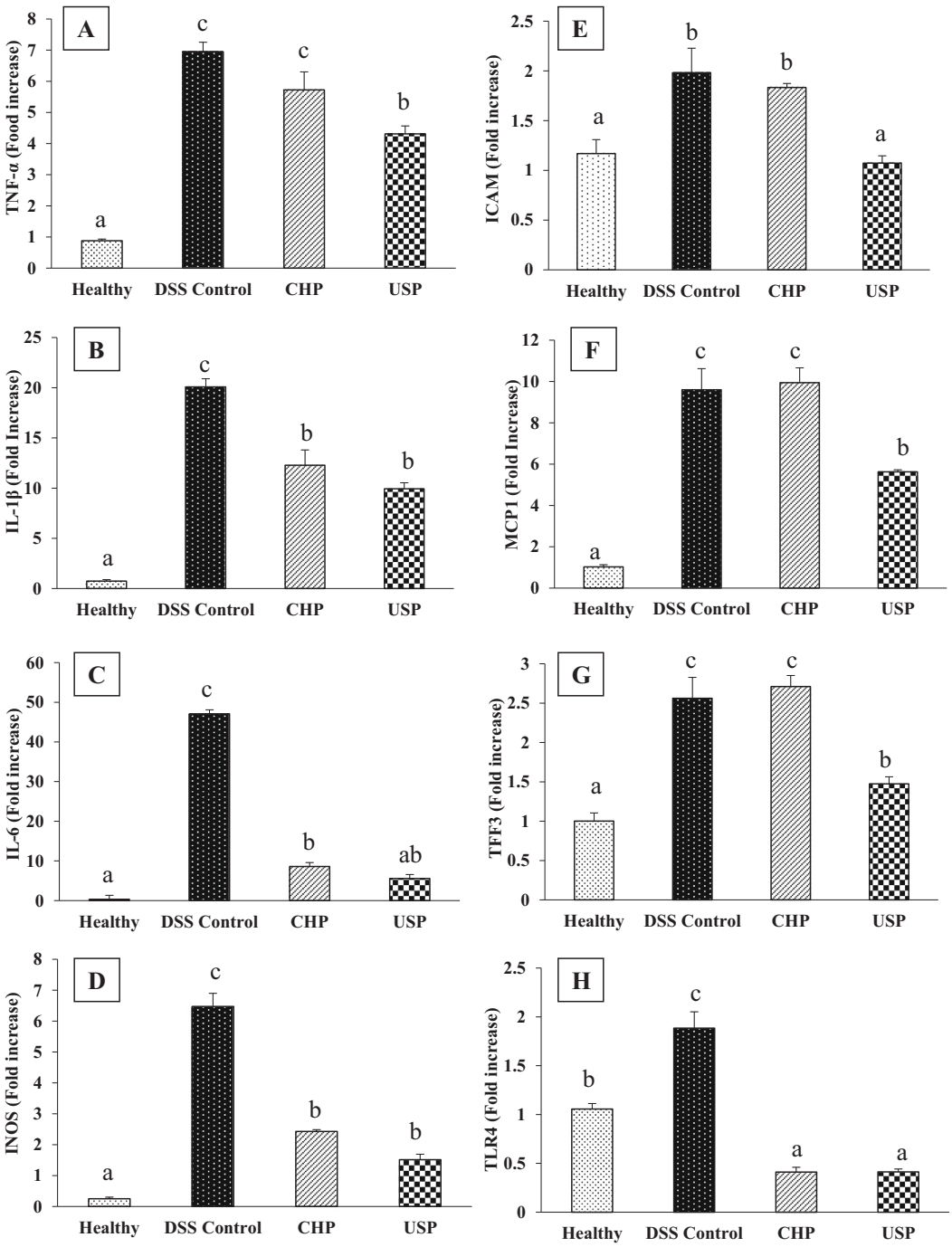

Fig. 7. Effects of CHP and USP on the expression of pro-inflammatory cytokines and chemokines in colonic tissue of the DSS colitis mice. Different letters on the bars indicate statistical differences ($p < 0.05$) among groups.

(from 1 to 100 s^{-1}) but decreased less rapidly at higher shear rates (100–1000 s^{-1}) at higher concentrations. Unlike the USP sample, low viscosities were observed in CHP at concentrations lower than 0.2 % (*w/v*). In general terms, when pectin concentration was above 0.5 % (*w*/w) in both samples, the initial viscosity was significantly increased. This change indicated that the intermolecular distance had been shortened due to an increase in solid concentration facilitating the intermolecular interactions, especially hydrogen bonding with hydroxyl groups [35]. In all cases, USP was more viscous than CHP, which can be interpreted by the higher Mw of CHP in comparison with USP (776 *vs* 500 kDa). According to Hua et al. [35], the high Mw could be related to a small number of molecules which were too far apart to interact with another one. The fact that USP presented higher viscosity than CHP could be also ascribed to the significantly higher content of GalA and lower DM, indicated above. Hua et al. [35] observed that high Mw and methoxyl content means a small number of molecules, a greater distance between them and strong electrostatic repulsions along the chain, resulting in lower viscosity of pectin extracted from conventional heating. Following a Pearson correlation, Dranca and Oroian [26] found a negative correlation between the viscosity and 1634 cm^{-1} wavenumber transmittance

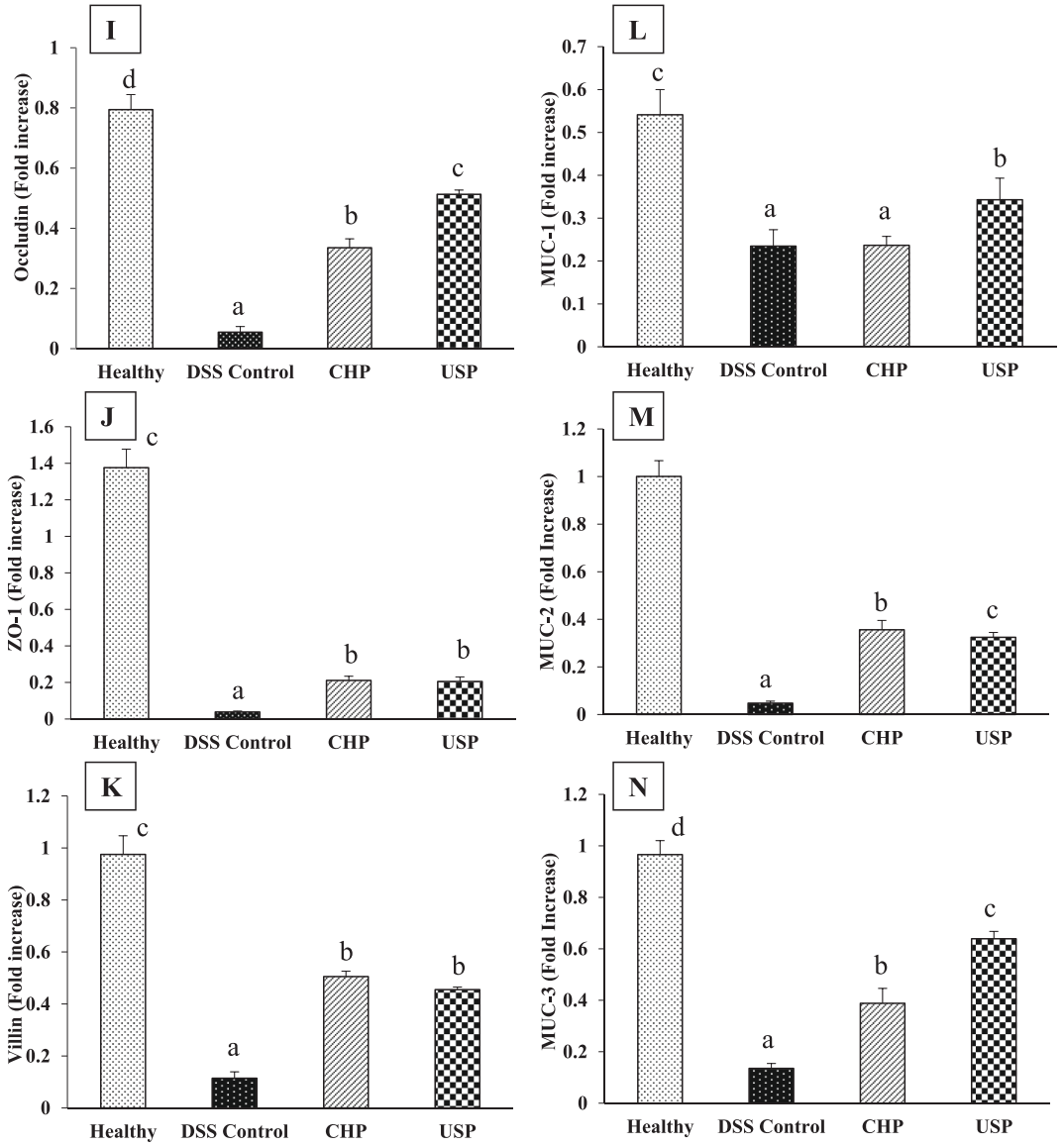

Fig. 7. (*continued*).

values ($r = -0.9991$), which was responsible for (C═O) stretching vibration of the carboxylate ion. Similarly, Polanco-Lugo et al. [36] observed in citrus pectin high methoxyl group content and lower viscosity. A highly positive correlation between viscosity and GalA content ($r = 0.992$) was also found, since a higher GalA content directly influences the formation of the pectin network [36].

Taking altogether the results indicated above, this might explain why the solutions obtained with the pectin extracted by US were more viscous.

3.4. Assessment of anti-inflammatory activity in DSS-treated mice

Based on the structural features mentioned above, USP exhibited a lower Mw which could improve its solubility, facilitating its metabolism in the colon. Moreover, previous studies demonstrated that the presence of neutral sugars (α-arabinoses and β-galactoses) in pectins is able to decrease pro-inflammatory cytokine expression in mice [6,37]. Given these promising results and the interesting chemical and rheological characteristics of USP, a study on its impact in inflammatory bowel disease was done.

First, different macroscopic indicators and symptoms of colitis induced in mice by the oral administration of DSS were investigated. Unlike the healthy control group, both samples showed a significant weight loss that was similar to the DSS control group (Fig. 6a). Pacheco et al. [5] and Sabater et al. [6] also found similar behaviour using citrus by-products and artichoke pectins, respectively, and attributed this fact to the anorexia observed in mice from the first day after the beginning of DSS administration. However, mice which have ingested CHP and USP did not reach the DAI values of the DSS control, which evidenced the protective effect of both pectins against DSS-induced colonic damage (Fig. 6b). As observed in Fig. 6c, during the DSS period intestinal permeability increased significantly in the DSS control group, which could contribute to the entry of commensal bacteria and decrease transepithelial resistance [38]. On the contrary, the concentration of this macroscopic indicator decreased significantly in animals treated with pectins, with the values corresponding to USP being significantly lower than CPH and close to the healthy group.

As expected, DSS-induced colitic inflammation was characterized by an altered immune response, which was evidenced by an increasing expression of TNF-α, IL-1β and IL-6 in comparison with healthy mice

(Fig. 7). The expression of these pro-inflammatory cytokines decreased significantly in the groups fed with both pectins, being notably lower in mice treated with USP ($p < 0.05$) (Fig. 7a–c). The same behaviour was found in the expression of the enzyme nitric oxide synthase (iNOS) which plays a role in the pathology of experimental colitis in the colon by mediating high NO production (Fig. 7d) [39].

Regarding inter-cellular adhesion molecule (ICAM-1) and monocyte chemoattractant protein-1 (MCP-1) which are related with the infiltration of immune cells to the inflammation site and contribute to tissue damage, only the USP intake decreased its expression in both chemokines, reaching the same level as the healthy group in the case of ICAM-1 (Fig. 7e and f). An analogous tendency was observed in the trefoil peptide 3 (TFF3) which is a key mediator in the defence and repair of the gastrointestinal mucosa [40] (Fig. 7g). The activity of the toll-like receptor 4 (TLR4) is to lead to the liberation of pro-inflammatory cytokines that could intensify gut inflammation (Fig. 7h) [41]. In this case, no changes were observed between both pectins, showing values significantly lower than the healthy group.

The intestinal mucosa is composed by epithelial cells that establish a barrier and protect the internal milieu from potentially harmful substances. As has been observed in patients with inflammatory bowel diseases, changes in this barrier increase the permeability of bacteria and promote damage on the epithelium. In fact, Dharmani et al. [42] reported a thin mucus layer and a decrease of the expression of intestinal barrier proteins such as MUC-1, MUC-2, MUC-3, occludin, zonula occludens-1 (ZO-1) and villin. In general, the levels of all these proteins (Fig. 7i–n) improved significantly in the mice treated with USP in comparison to the DSS control group ($p < 0.05$), demonstrating it to be the most effective treatment. These results indicated that the administration of USP improves the intestinal mucosal barrier function in mice, reducing the symptoms and demonstrating a preventive effect against a future colitis crisis [1,5].

Taking into account that conventional pharmacological treatments for human IBD include aminosalicylates, corticosteroids, immunosuppressants and biological agents, and although they show efficacy, in many patients they are not fully effective and can be associated with major adverse effects that limit their required chronic use [43]. Accordingly, attention has been given to dietary supplements and natural products as alternative therapies for patients who do not respond to standard medications. In this regard, dietary soluble fibres, including pectins, have many beneficial effects on gastrointestinal health. Pectins can be easily degraded by commensal bacteria in the gut with the production of short-chain fatty acids (SCFA), thus improving mucosal barrier functions and attenuating intestinal inflammation during colitis [44,45].

These positive effects on IBD of pectin from sunflowers and, particularly in the case of pectin extracted by US, could be ascribed to the viscosity. As indicated above, this parameter was higher in the sample subjected to UAE whose chemical characteristics lead to higher viscosity. Lee et al. [46] reported in an *in vitro* study a direct relationship between the viscosity of the polysaccharides of maca root and a reduction in inflammatory processes.

4. Conclusions

As indicated above, we have carried out the structural characterization and the evaluation of the functionality of sunflower pectin. We can infer that the sunflower pectins here obtained could have benefits as a preventive treatment to attenuate IBD, as shown in the *in vivo* assays with DSS-induced colitis in mice. It is noteworthy that the most effective pectin was the obtained by ultrasound. Ultrasound not only improved significantly the yield of sunflower pectin extraction and improved the pectin purity as compared to conventional treatment, but also gave rise to structures (lower Mw and DM, higher GalA) that could exert a more effective protection against this IBD presumably due to a higher viscosity. In the case of macroscopic indicators, the main effect was observed in a decrease in intestinal permeability. Regarding the immune response, the expression of pro-inflammatory cytokines, nitric oxide synthase and chemokines related to tissue damage was reduced and the intestinal protective barrier enhanced in comparison with pectin obtained by means of the conventional procedure. Although more research is needed to scale up the method and to carry out more *in vivo* assays, this work shows a soft, easy, green and effective procedure to obtain bioactive pectin from sunflower by-products with benefits in IBD.

Abbreviations

Ara	arabinose
CHP	control heating pectin at optimal conditions
DM	degree of methyl-esterification
DSS	dextran sulfate sodium
ELSD	Evaporative Light Scattering Detector
FAO	Food and Agriculture Organization of United Nations
FT-IR	Fourier-Transform Infrared Spectroscopy
Gal	galactose
GalA	galacturonic acid
Glc	glucose
HG	homogalacturonan
HMP	high-methoxylated pectins
HPSEC	High Performance Size Exclusion Chromatography
LMP	low-methoxylated pectins
Man	mannose
Mw	molecular weight
NMR	Nuclear Magnetic Resonance
RG-I	rhamnogalacturonan-I
Rha	rhamnose
RSM	Response Surface Methodology
SEM	Scanning Electron Microscope
SH	sunflower heads
UAE	ultrasound assisted extraction
USP	pectin extracted with ultrasound at optimal conditions
Xyl	xylose

CRediT authorship contribution statement

Nerea Muñoz-Almagro: Investigation, Methodology, Writing - original draft, review & editing. **Antonia Montilla:** Funding acquisition, Supervision, Writing - original draft, review & editing. **Jose Alberto Molina-Tijeras:** Investigation, Methodology, Writing. **Teresa Vezza:** Investigation, Methodology, Writing. **María Sánchez-Milla:** Investigation, Methodology, Writing. **Fabián Rico-Rodríguez:** Investigation, Methodology, Writing. **Mar Villamiel:** Funding acquisition, Supervision, Writing - original draft, review & editing.

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.

Data availability

Data will be made available on request.

Acknowledgments

This work has been funded by MINECO of Spain, Project AGL2014-53445-R and by Grant PID2021-123862OB-I00 funded by MCIN/AEI/10.13039/501100011033 and by ERDF A way of making Europe. J.A. Molina-Tijeras is the recipient of pFIS (FI20/00159) from the Instituto de Salud Carlos III. Muñoz-Almagro thanks to Ministry of Economy of Spain for providing her FPI predoctoral fellowship.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2023.125505.

References

[1] D. Yuan, C. Li, Q. Huang, X. Fu, H. Dong, Current advances in the anti-inflammatory effects and mechanisms of natural polysaccharides, Crit. Rev. Food Sci. Nutr. (n.d.) doi:https://doi.org/10.1080/10408398.2022.2025535.
[2] A.E. M'Koma, Inflammatory bowel disease: an expanding global health problem, Clin. Med. Insights Gastroenterol. 6 (2013) 33–47, https://doi.org/10.4137/CGast.S12731.
[3] L.E. Targownik, C.N. Bernstein, W.D. Leslie, Inflammatory bowel disease and the risk of osteoporosis and fracture, Maturitas. 76 (2013) 315–319, https://doi.org/10.1016/j.maturitas.2013.09.009.
[4] J.K. Triantafillidis, E. Merikas, F. Georgopoulos, Current and emerging drugs for the treatment of inflammatory bowel disease, Drug Des. Devel. Ther. 5 (2011) 185–210, https://doi.org/10.2147/DDDT.S11290.
[5] M.T. Pacheco, T. Vezza, P. Diez-Echave, P. Utrilla, M. Villamiel, F.J. Moreno, Anti-inflammatory bowel effect of industrial orange by-products in DSS-treated mice, Food Funct. 9 (2018) 4888–4896, https://doi.org/10.1039/C8FO01060A.
[6] C. Sabater, J.A. Molina-Tijeras, T. Vezza, N. Corzo, A. Montilla, P. Utrilla, Intestinal anti-inflammatory effects of artichoke pectin and modified pectin fractions in the dextran sulfate sodium model of mice colitis. Artificial neural network modelling of inflammatory markers, Food Funct. 10 (2019) 7793–7805, https://doi.org/10.1039/c9fo02221j.
[7] D. Wu, X. Ye, R.J. Linhardt, X. Liu, K. Zhu, C. Yu, T. Ding, D. Liu, Q. He, S. Chen, Dietary pectic substances enhance gut health by its polycomponent: a review, Compr. Rev. Food Sci. Food Saf. 20 (2021) 2015–2039, https://doi.org/10.1111/1541-4337.12723.
[8] H. Tian, J. Xiong, H. Yu, C. Chen, H. Xu, X. Lou, Characterize the physicochemical properties and microstructure of pectin from high-pressure and thermal processed cloudy hawthorn (*Crataegus pinnatifida*) juice based on acid heating extraction, Food Chem. 407 (2023), https://doi.org/10.1016/j.foodchem.2022.135199.
[9] L.R. Adetunji, A. Adekunle, V. Orsat, V. Raghavan, Advances in the pectin production process using novel extraction techniques: a review, Food Hydrocoll. 62 (2017) 239–250, https://doi.org/10.1016/j.foodhyd.2016.08.015.
[10] M.T. Iglesias, J.E. Lozano, Extraction and characterization of sunflower pectin, J. Food Eng. 62 (2004) 215–223, https://doi.org/10.1016/S0260-8774(03)00234-6.
[11] Y. Sun, Y. He, F. Wang, H. Zhang, P. de Vos, J. Sun, Low-methoxyl lemon pectin attenuates inflammatory responses and improves intestinal barrier integrity in caerulein-induced experimental acute pancreatitis, Mol. Nutr. Food Res. 61 (2017) 1–9, https://doi.org/10.1002/mnfr.201600885.
[12] S. Hino, K. Sonoyama, H. Bito, H. Kawagishi, S. Aoe, T. Morita, Low-methoxyl pectin stimulates small intestinal mucin secretion irrespective of goblet cell proliferation and is characterized by jejunum Muc2 upregulation in rats, J. Nutr. 143 (2013) 34–40, https://doi.org/10.3945/jn.112.167064.
[13] P. Sriamornsak, N. Wattanakorn, H. Takeuchi, Study on the mucoadhesion mechanism of pectin by atomic force microscopy and mucin-particle method, Carbohydr. Polym. 79 (2010) 54–59, https://doi.org/10.1016/j.carbpol.2009.07.018.
[14] K. Ponmurugan, N.A. Al-Dhabi, J.P. Maran, K. Karthikeyan, I.G. Moothy, N. Sivarajasekar, J.J.B. Manoj, Ultrasound assisted pectic polysaccharide extraction and its characterization from waste heads of *Helianthus annus*, Carbohydr. Polym. 173 (2017) 707–713, https://doi.org/10.1016/j.carbpol.2017.06.018.
[15] FEEDAP, Scientific opinion on the safety and efficacy of citric acid when used as a technological additive (preservative) for all animal species, EFSA J. 13 (2015) 2, https://doi.org/10.2903/j.efsa.2015.4010.
[16] M. Marić, A.N. Grassino, Z. Zhu, F.J. Barba, M. Brnčić, S.R. Brnčić, An overview of the traditional and innovative approaches for pectin extraction from plant food wastes and by-products: ultrasound-, microwaves-, and enzyme-assisted extraction, Trends Food Sci. Technol. 76 (2018) 28–37, https://doi.org/10.1016/j.tifs.2018.03.022.
[17] J.P. Maran, B. Priya, N.A. Al-Dhabi, K. Ponmurugan, I.G. Moorthy, N. Sivarajasekar, Ultrasound assisted citric acid mediated pectin extraction from industrial waste of *Musa balbisiana*, Ultrason. Sonochem. 35 (2017) 204–209, https://doi.org/10.1016/j.ultsonch.2016.09.019.
[18] I.G. Moorthy, J.P. Maran, S. Ilakya, S.L. Anitha, S.P. Sabarima, B. Priya, Ultrasound assisted extraction of pectin from waste *Artocarpus heterophyllus* fruit peel, Ultrason. Sonochem. 34 (2017) 525–530, https://doi.org/10.1016/j.ultsonch.2016.06.015.
[19] B. Du, H. Zeng, Y. Yang, Z. Bian, B. Xu, International journal of biological macromolecules anti-inflammatory activity of polysaccharide from *Schizophyllum commune* as affected by ultrasonication, Int. J. Biol. Macromol. 91 (2016) 100–105, https://doi.org/10.1016/j.ijbiomac.2016.05.052.
[20] W. Wang, X. Ma, P. Jiang, L. Hu, Z. Zhi, J. Chen, T. Ding, X. Ye, D. Liu, Characterization of pectin from grapefruit peel: a comparison of ultrasound-assisted and conventional heating extractions, Food Hydrocoll. 61 (2016) 730–739, https://doi.org/10.1016/j.foodhyd.2016.06.019.
[21] N. Muñoz-Almagro, M. Prodanov, P.J. Wilde, M. Villamiel, A. Montilla, Obtainment and characterisation of pectin from sunflower heads purified by membrane separation techniques, Food Chem. 318 (2020), 126476, https://doi.org/10.1016/j.foodchem.2020.126476.
[22] N. Muñoz-Almagro, F. Rico-Rodriguez, M. Villamiel, A. Montilla, Pectin characterisation using size exclusion chromatography: a comparison of ELS and RI detection, Food Chem. 252 (2018) 271–276, https://doi.org/10.1016/j.foodchem.2018.01.087.
[23] H.W. Zhao, Y.H. Yue, H. Han, X.L. Chen, Y.G. Lu, J.M. Zheng, Effect of toll-like receptor 3 agonist poly I:C on intestinal mucosa and epithelial barrier function in mouse models of acute colitis, World J. Gastroenterol. 23 (2017) 999–1009, https://doi.org/10.3748/wjg.v23.i6.999.
[24] A. Hernoux-Villière, U. Lassi, J.-M. Lévêque, An original ultrasonic reaction with dual coaxial frequencies for biomass processing, Ultrason. Sonochem. 20 (2013) 1341–1344, https://doi.org/10.1016/j.ultsonch.2013.04.011.
[25] N. Bayar, T. Bouallegue, M. Achour, M. Kriaa, A. Bougatef, R. Kammoun, Ultrasonic extraction of pectin from *Opuntia ficus* indica cladodes after mucilage removal: optimization of experimental conditions and evaluation of chemical and functional properties, Food Chem. 235 (2017) 275–282, https://doi.org/10.1016/j.foodchem.2017.05.029.
[26] F. Dranca, M. Oroian, Ultrasound-assisted extraction of pectin from *Malus domestica* 'Fălticeni' apple pomace, Processes. 7 (2019) 488, https://doi.org/10.3390/pr7080488.
[27] A.C. Soria, M. Villamiel, A. Montilla, Ultrasound effects on processes/reactions involving carbohydrates, in: M. Villamiel, J.V. García-Pérez, J. Cárcel, J. J. Benedito (Eds.), Ultrasound in Food Processing: Recent Advances, John Wiley & Sons, Chichester, West Sussex, United Kingdom, 2016, pp. 437–458, https://doi.org/10.1002/9781118964156.ch17.
[28] H. Zhang, J. Chen, J. Li, L. Yan, S. Li, X. Ye, D. Liu, T. Ding, R.J. Linhardt, C. Orfila, S. Chen, Extraction and characterization of RG-I enriched pectic polysaccharides from mandarin citrus peel, Food Hydrocoll. 79 (2018) 579–586, https://doi.org/10.1016/j.foodhyd.2017.12.002.
[29] L. Zhang, X. Ye, T. Ding, X. Sun, Y. Xu, D. Liu, Ultrasound effects on the degradation kinetics, structure and rheological properties of apple pectin, Ultrason. Sonochem. 20 (2013) 222–231, https://doi.org/10.1016/j.ultsonch.2012.07.021.
[30] J.-S. Yang, T.-H. Mu, M.-M. Ma, Optimization of ultrasound-microwave assisted acid extraction of pectin from potato pulp by response surface methodology and its characterization, Food Chem. 289 (2019) 351–359, https://doi.org/10.1016/j.foodchem.2019.03.027.
[31] K. Asgari, M. Labbafi, F. Khodaiyan, M. Kazemi, S.S. Hosseini, High-methylated pectin from walnut processing wastes as a potential resource: ultrasound assisted extraction and physicochemical, structural and functional analysis, Int. J. Biol. Macromol. (2019), https://doi.org/10.1016/j.ijbiomac.2019.10.224.
[32] P.V. Fernández, V.M. Zelaya, L. Cobello, A.S. Vega, M. Ciancia, Glucuronoarabinoxylans and other cell wall polysaccharides from shoots of *Guadua chacoensis* obtained by extraction in different conditions, Carbohydr. Polym. 226 (2019), 115313, https://doi.org/10.1016/j.carbpol.2019.115313.
[33] M.K. Chan, Y. Yu, S. Wulamu, Y. Wang, Q. Wang, Y. Zhou, L. Sun, Structural analysis of water-soluble polysaccharides isolated from *Panax notoginseng*, Int. J. Biol. Macromol. 155 (2020) 376–385, https://doi.org/10.1016/j.ijbiomac.2020.03.233.
[34] A.F. Fracasso, C.A. Perussello, D. Carpiné, C.L. de Oliveira Petkowicz, C.W. I. Haminiuk, Chemical modification of citrus pectin: structural, physical and rheologial implications, Int. J. Biol. Macromol. 109 (2018) 784–792, https://doi.org/10.1016/j.ijbiomac.2017.11.060.
[35] X. Hua, K. Wang, R.J. Yang, J.Q. Kang, J.N. Zhang, Rheological properties of natural low-methoxyl pectin extracted from sunflower head, Food Hydrocoll. 44 (2015) 122–128, https://doi.org/10.1016/j.foodhyd.2014.09.026.
[36] E. Polanco-Lugo, J.I. Martínez-Castillo, J.C. Cuevas-Bernardino, T. González-Flores, R. Valdez-Ojeda, N. Pacheco, T. Ayora-Talavera, Citrus pectin obtained by ultrasound-assisted extraction: physicochemical, structural, rheological and functional properties, CYTA J. Food. 17 (2019) 463–471, https://doi.org/10.1080/19476337.2019.1600036.
[37] C.L. Leivas, L.F. Nascimento, W.M. Barros, A.R. Santos, M. Iacomini, L.M. Cordeiro, Substituted galacturonan from startfruit: chemical structure and antinociceptive and anti-inflammatory effects, Int. J. Biol. Macromol. 84 (2016) 295–300, https://doi.org/10.1016/j.ijbiomac.2015.12.034.
[38] D.D. Eichele, K.K. Kharbanda, Dextran sodium sulfate colitis murine model: an indispensable tool for advancing our understanding of inflammatory bowel diseases pathogenesis, World J. Gastroenterol. 23 (2017) 6016–6029, https://doi.org/10.3748/wjg.v23.i33.6016.
[39] S.K. Mishra, J.-H. Kang, D.-K. Kim, S.H. Oh, M.K. Kim, Orally administered aqueous extract of Inonotus obliquus ameliorates acute inflammation in dextran sulfate sodium (DSS)-induced colitis in mice, J. Ethnopharmacol. 143 (2012) 524–532, https://doi.org/10.1016/j.jep.2012.07.008.

[40] S. Borniquel, C. Jädert, J.O. Lundberg, P8: dietary nitrite induces TFF3 and attenuates inflammation in experimental colitis, Nitric Oxide 31 (2013) S16, https://doi.org/10.1016/j.niox.2013.02.010.
[41] Y. Lu, X. Li, S. Liu, Y. Zhang, D. Zhang, Toll-like receptors and inflammatory bowel disease. frontiers in immunology, 9, toll-like receptors and inflammatory bowel disease, Front. Immunol. 9 (2018) 1–9, https://doi.org/10.3389/fimmu.2018.00072.
[42] P. Dharmani, P. Leung, K. Chadee, Tumor necrosis factor-a and Muc2 mucin play major roles in disease onset and progression in dextran sodium sulphate-induced colitis, PLoS One 6 (2011), https://doi.org/10.1371/journal.pone.0025058.
[43] C.A. Siegel, Review article: explaining risks of inflammatory bowel disease therapy to patients, Aliment. Pharmacol. Ther. 33 (2011) 23–32, https://doi.org/10.1111/j.1365-2036.2010.04489-x.
[44] J. Gálvez, M. Comalada, J. Xaus, Prebiotics and probiotics in experimental models of rodent colitis: lessons in treatment or prevention of inflammatory bowel diseases, in: R.R. Watson, V.R. Preedy (Eds.), Bioactive Foods in Promoting Health: Probiotics and Prebiotics, Academic Press, Cambridge, 2010, pp. 589–610, https://doi.org/10.1016/B978-0-12-374938-3.00035-9.
[45] A.L.M. Silveira, A.V.M. Ferreira, M.C. de Oliveira, M.A. Rachid, L.F. da Cunha Sousa, F. Dos Santos Martins, A.C. Gomes-Santos, A.T. Vieira, M.M. Teixeira, Preventive rather than therapeutic treatment with high fiber diet attenuates clinical and inflammatory markers of acute and chronic DSS-induced colitis in mice, Eur. J. Nutr. 56 (2017) 179–191, https://doi.org/10.1007/s00394-015-1068-x.
[46] Y.K. Lee, S.K. Jung, Y.H. Chang, Rheological properties of a neutral polysaccharide extracted from maca (*Lepidium meyenii* Walp.) roots with prebiotic and anti-inflammatory activities, Int. J. Biol. Macromol. 152 (2020) 757–765, https://doi.org/10.1016/j.ijbiomac.2020.02.307.