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Anti-inflammation effects of highly purified low-M_w RG-I pectins on LPS-activated macrophages

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ABSTRACT

Pectic oligosaccharides are viewed as excellent immunomodulator, but the specific molecular weight (M_w) range required for anti-inflammatory effects remains unclear. In this study, citrus pectins was depolymerized by the ultrasound/H2O2/Vc system and the pectic mixtures was separated through the superdex 30 chromatography column. Three highly purified pectin fractions (USP1, USP2, USP3) were obtained, with the M_w of 2713 g/mol, 3683 g/mol, and 7469 g/mol. All of them were RG-I pectins and USP3 contained more arabinan sidechains than USP1 and USP2. In vitro anti-inflammation assays based on LPS-induced RAW264.7 macrophages indicated that these pectins could suppress the NO release, ROS production and down-regulated the level of pro-inflammatory cytokines IL-1β, IL-6 and TNF-α in a M_w-dependent manner. The inhibitory effects increased in the order of USP1<USP2<USP3. Meanwhile, the regulation of pro-inflammatory cytokines were closely related to the content of arabinan sidechains in pectin. Thus, low-M_w pectins with M_w>3KD should be effective as a prophylactic agent against inflammatory diseases in functional food.

1. Introduction

Pectin, found in primary cell walls of higher plants, is a nontoxic, biocompatible, and natural polysaccharide with a high molecular weight and complex structure. It mostly composed of three main well-characterized structural motifs: homogalacturonan (HG), type-I rhamnogalacturonan (RG I), and type-II rhamnogalacturonan (RG II). Pectic polysaccharides have exhibited a variety of health effects for human beings, including alleviating obesity, controlling diabetes and hyperlipidemia, preventing virus infection, inhibiting tumor growth, and particularly regulating systematic inflammation (Wu et al., 2020b, 2021). However, the size of natural pectin (NP) is extremely large, limiting its ability to be absorbed by human bodies. Apart from the tendency of clumping during dissolution, the concentrated pectin solution shows extremely high viscosity, which imposes a lot of obstacle in experiment. Thus, several depolymerization methods such as acid, alkali, heat, and oxidation degradation have been employed to produce pectic oligosaccharides (DP), reducing the molecular size and improving the solubility. Previous studies have reported NP shows low inhibitory effects on the proliferation and migration of cancer cells, while DP exhibits stronger bioactivity (do Prado et al., 2019; Hao et al., 2013).

Depolymerized pectic polysaccharides of reduced molecular weight (M_w) possesses a variety of health-promoting properties, reported by extensive studies and clinic trials (Eliaz & Raz, 2019). Modified citrus pectin (MCP) suppresses tumor growth and angiogenesis via blocking the association between galactin-3 and its receptors (Zhang, Xu, & Zhang, 2015). Also, pectic oligosaccharides can reduce the atherosclerotic lesions (Lu et al., 2017) and restrict fat digestion (Aguilera Angel, Espinal Ruiz, & Narváez Cuenca, 2018). The commercial MCP, Pectasol-C®, has entered the phase III clinical trial for the treatment of

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prostate cancer (Keizman et al., 2019). Another pectic oligosaccharides product, GCS-100®, was effective in preventing fibrotic diseases an ACE inhibitor (Li, Li, Hao, Zhang, & Deng, 2019). Moreover, many studies have confirmed the anti-inflammatory properties (Ramachandran, Wilk, Melnick, & Eliaz, 2017; Vogt et al., 2016). However, most of these studies were performed using the mixture of pectic oligosaccharides, the M_w modifications and the explanation of M_w-function relationship still need to be elucidated. do Nascimento, Winnischofer, Ramirez, Iacomini, and Cordeiro (2017) have revealed that molecular weight was important structural feature of pectins involved the immunomodulatory properties. A medium-size HG oligosaccharides have been confirmed to be a promising plant immune system elicitors (degree of polymerization of 9–18) (Cao, Yang, Wang, Lu, & Yue, 2020). However, there is a lack of systematic discussion of specific M_w range of RG-I pectic oligosaccharides required for anti-inflammatory effects, which need to prepare highly purified pectic oligosaccharides of different sizes.

In our previous study, we have recovered a RG-I-enriched pectin (WSP) from basic canning processing water (Wu, Zheng, Hu, et al., 2020). Moreover, we have established an effective ultrasound/H2O2/ascorbic acid system to depolymerize pectic polysaccharides in an ultrafast and green way (Li, Li, Hao, et al., 2019). Here, we obtained three pure pectic oligosaccharides with different M_w through the ultrasound degradation and sephadex chromatography purification. The mice macrophage RAW264.7 cell lines were used to establish the inflammatory cell model. The aim of this study is to explore the effects of M_w on the anti-inflammatory effects of pectic oligosaccharides and clarify the M_w range for further development of functional food ingredient.

2. Material and methods

2.1. Materials

The crude pectic polysaccharides WSP was recovered from citrus canning processing water in a citrus fruit canning factory (Ningbo, China). The Ultrahydrogel 250 gel-filtration column and the Superdex™ Peptide 10/300 GL column were purchased from Waters and GE. Deuterium oxide (D_2O), hydrogen peroxide, ascorbic acid, sodium chloride, and HPLC-grade methyl alcohol were acquired from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The 95% (v/v) ethanol (food grade) and other chemical reagent were obtained from Aladdin Chemical Reagent Co., Ltd. (Shanghai, China). Monosaccharide standards were purchased from Sigma-Aldrich (Shanghai, China). All of other chemicals were of analytical grade.

2.2. Degradation by the ultrasound/H_2O_2/ascorbic acid system

Ultrasound treatments were performed (Scientz-IID, Ningbo Scientz Biotechnology Co., Ningbo, China) with the following parameters (Li, Li, Hao, et al., 2019): maximum ultrasound power output, 900 W, ultrasound intensity, 3.8 W/mL, frequency, 22 kHz, and horn micro tip diameter, 10 mm. WSP (0.5% m/v) was added to a solution of H_2O_2 (50 mM) and ascorbic acid (10 mM). The sample solution were ultrasound-treated at the temperature of 30 °C for 60 min. Sodium hydroxide was added to neutralize the solution. Then, the depolymerized products were dialyzed (MWCO 500 Da) for 72 h. Dialyzed solutions were concentrated and subsequently lyophilized.

2.3. Fractionation of ultrasound-degraded pectic polysaccharides(USP)

The USP mixtures was further fractionated on a Superdex 30 column (5 × 200 cm) using 0.3 M NH_4HCO_3 as eluent at a flow rate of 0.5 mL per min. The eluents were collected in test tubes (16 min per tube). Then, the carbohydrate fractions in every tube were determined by high performance size exclusion chromatography (HPSEC) equipped with the Ultrahydrogel 250 gel-filtration column and a refractive index detector (Yan et al., 2017). These tubes of carbohydrate fractions with single peak were collected for next determination. Three major fractions were collected and lyophilized to obtain three purified pectic fragments: USP1, USP2 and USP3.

2.4. Chemical composition

The monosaccharide composition of each USP fractions was determined by high-performance anion-exchange chromatography with a pulsed amperometric detection (HPAEC-PAD) according to the method of Hu, Ye, Chantapakul, Chen, and Zheng (2020). The ^{1}H NMR spectra were recorded on Agilent DD2-600 MHz spectrometer (Agilent, USA) at room temperature.

2.5. Molecular weight determination

The HPLC equipped with two size exclusion columns (a OHpak SB-G guard column, SB-806 HQ and SB-804 HQ column, 7.8 × 300 mm, Shodex, Japan), a multi-angle laser light scattering detector (DAWN HELEOS II, Wyatt Technology, USA) and a refractive index detector (HPSEC-MALLS-RI) were used to detect the molecular information of USP fractions at 25 °C (Wei et al., 2019). Pectic fractions were dissolved in 0.15 M NaCl solution and then filtered through a syringe-filter membrane of 0.22 μm. 30 μL of sample solution was injected and the running time was 60 min. The molar mass was calculated with the dn/dc value of 0.138 mL/g.

2.6. Cytotoxicity assay

Anti-proliferative activity of pectic fractions was determined by cell viability of mouse macrophage RAW264.7 cells using the MTT assay (Cordeiro Caillot et al., 2018; Wu, Zheng, Hu, et al., 2020). RAW264.7 cells were seeded in 96-well cell culture plates (2×10^5 cells/well) and incubated at 37 °C overnight, and then incubating with sterilized pectic fractions (10–200 μg/mL) for 48 h. Cells cultured without pectin treatment were designated as blank control. 20 μL of MTT (5 mg/mL) was added into each well and the RAW264.7 cells were placed in incubator for 3–4 h to form formazan crystals. At the end of the period, the absorbance of the cells was measured at 490 nm using a microplate reader.

2.7. Determination of nitric oxide (NO) release

The content of released NO from active RAW 264.7 cells induced by lipopolysaccharide (LPS) was determined by NO assay kit (Beyotime Technology, Shanghai, China). Cells were plated and incubated with different pectic fractions at different concentrations and LPS (5 μg/mL, positive control) for 24 h. After incubation, the culture supernatants were collected and the NO content was analyzed according to the instructions (Yang et al., 2018).

2.8. Measurement of TNF-α, IL-6, and IL-1β in RAW 264.7 cells

RAW 264.7 cells (2×10^5/mL) were plated, induced with LPS previously dissolved in RPIM1640 (5 μg/mL). Then 24 h later, the cells were treated with 200 μL of USPs solution at 60 μg/mL. The next day, supernatant was collected and submitted to cytokine assays. The concentration of IL-6, IL-1β and TNF-α were measured using immunoenzymatic assay (ELISA) kits (mlbio Biotechnology, Shanghai, China), according to the manufacturer's protocol.

2.9. Measurement of reactive oxygen species (ROS)

The determination of ROS generation of RAW 264.7 cells after pectins or LPS treatment was performed using the commercial kit (Beyotime Bio-Technology, Shanghai, China). RAW 264.7 cells were incubated by

the pectic samples or LPS at different concentrations (50 μg/mL, 100 μg/mL, 200 μg/mL, 400 μg/mL, 800 μg/mL) for 24 h. After that, 100 μL of DCFH-DA diluted in medium was added to each well and then incubated for another 30 min. The cells were washed three times with PBS solution and covered with 100 μL of fresh medium. The intracellular ROS levels were measured using a fluorescence microplate reader (SpectraMax® iD5, Molecular Devices, Shanghai, China) with an excitation wavelength at 485 nm and an emission wavelength at 525 nm.

2.10. Statistical analysis

All experiments were carried out in triplicate. Statistical analysis was performed with one-way analysis of variance (ANOVA) using SPSS 17.0 software (SPSS Inc. Chicago, IL, USA). Statistical significances were set at $p<0.05$ (*) and $p<0.01$ (**).

3. Results and discussion

3.1. Preparation of USP fractions

The recovered pectic polysaccharide WSP was depolymerized using ultrasound/H_2O_2/ascorbic acid system and produced a high yield of USP. Monosaccharide composition analysis revealed that USP mainly consisted of 23% galacturonic acid (GalA), 6% rhamnose (Rha), 21.3% galactose (Gal) and 40.5% arabinose (Ara), which was similar to that of WSP in the published studies (Wu, Zheng, Hu, et al., 2020). USP showed a broad distribution of molecular weight (M_w) on SB-806 HQ and SB-804 HQ column, with an average M_w of 14 KDa (Fig. S1). The depolymerized pectic oligosaccharides were separated by gel filtration chromatography on a Superdex 30 column, eluted with 0.3M NH_4HCO_3 solution, and the M_w distribution of these samples obtained were determined by high performance size exclusion chromatography (HPSEC), equipped with Ultrahydrogel 250 gel-filtration column (Fig. 1). Based on the M_w profiles according to the HPSEC, we observed that from the 30th tube, pure pectic fractions were collected. We combined the tubes with similar M_w and obtained three pure USP fractions: 1 KDa < USP1 (35–36T)<3 KDa, 3 KDa < USP2 (33–34T) < 5 KDa, 5 KDa < USP3 (31–32T) < 12 KDa. The recovery rate of the three USP fractions was 4%, 4.8% and 3.4%. As shown in Fig. 1, every fraction presented a single narrow and symmetrical peak. All of these polydispersity values decreased from that of USP (2.367), suggesting higher purity of these three fractions. The M_w of USP1, USP2, and USP3 were 2113 g/mol, 3683 g/mol, 7469 g/mol, respectively.

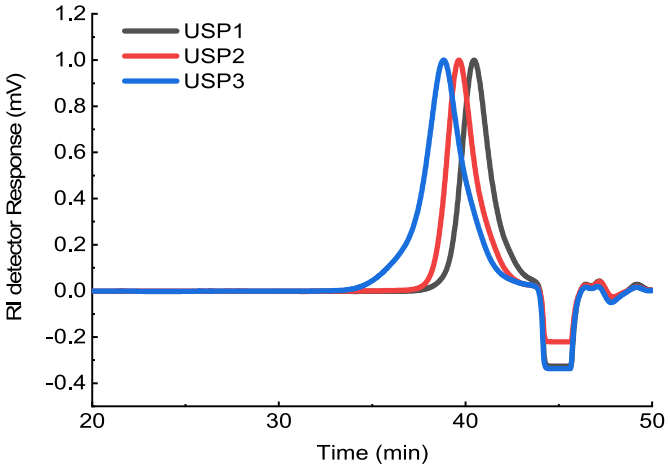

Fig. 1. The HPSEC chromatography of USP1, USP2, and USP3.

3.2. Monosaccharide composition analysis

The chemical composition of these pectin fractions were listed in Table 1. Sugar composition analysis indicated that USP1 was composed of 45% GalA, 39% Ara, 8% Gal, 2% Rha and minor Glc and Xyl, which was similar to that of USP2. While USP3 mainly contained 50% Ara, 21% GalA, 13% Gal, and 11% Rha, suggesting that Ara dominated in this fraction. The ratio of Rha/GalA of theses fractions were calculated to be 0.05, 0.08, 0.54, respectively, which indicated that USP1 and USP2 had relatively higher HG content than USP3 (Arnous & Meyer, 2009). Interestingly, the content of RG-I increased (51.53%, 59.07%, 86.37%) with the increasing of molecular weight, which might be due to the rich sidechains structure. The ratio of (Ara + Gal)/Rha was used to evaluate the degree of branching in rhamnogalacturonan backbone in RG-I. The ratio of 21.53 and 14.93 in USP1 and USP2 indicated the presence of high ratio of branched points and relatively short sidechains. While the value of 5.69 in USP3 suggested there was low ratio of branched Rha residues and long neutral sidechains. These results revealed that all of these pectin fractions were RG-I type pectin and USP contained much more long neutral sidechains with high molecular weight.

3.3. FT-IR spectra analysis

As shown in Fig. 2, the infrared spectra of these pectin fractions were identical. The broadly stretched brand at 3420 cm^{-1} was attributed to hydroxyl groups, and the weak absorption peak at about 2927 cm^{-1} was the characteristic signal of C-H stretching vibrations of these pectins (Farhadi, 2017). The degree of methylation of pectins usually can be evaluated by the proportion of signal corresponding to carboxylic ester to the sum of signals ascribed to carboxylic acid groups and carboxylic ester. The important wavenumber 1601 cm^{-1} denoted the high amount of ionic carboxyl groups, and no absorption peak at 1740 cm^{-1} indicated nearly no carboxylic ester could be found, which suggested all of these pectic fractions were of low DM, which was consistent with previous studies (Chen et el., 2017; Li, Li, Hao, et al., 2019). The absorption band at around 1405 cm^{-1} was attributable to -COO^- symmetric stretching. The O-C-O asymmertric stretching of pectin were confirmed by the absorption at 1144 cm^{-1} (Szymanska-Chargot & Zdunek, 2013). The two absorption bands at 1099 cm^{-1} and 1022 cm^{-1} suggested the presence of pyranose (Li, Li, Hao, et al., 2019). The absorption band around 956 cm^{-1} suggested the presence of α-type glycosidic bonds, which was the main glycosidic linkage in these pectins (Farhadi, 2017). According to the IR spectra, all of these pectin fractions presented similar structural features. However, IR is relatively insensitive to fine structural differences, so we detected the ^1H spectra of these pectin samples.

3.4. ^1H NMR analysis

The 1H NMR spectra of these three pectins and the assignment of peaks were presented in Fig. 3. The typical peaks in the region of 4.87–5.34 ppm confirmed the presence of α configurations in pectins (Li et al., 2019b; Xu et al., 2019). In the spectrum of USP3, the high peak signals at 5.14 ppm, 5.20 ppm, and 5.23 ppm were attributed to the H-1 of Ara, and the lower peak at 5.17 ppm was assigned to H-1 of GalA (Amaral et al., 2019; Xu, Qi, Goff, & Cui, 2020; Zhou et al., 2018). Additionally, the intense peak at 4.85 ppm was assigned to H-5 of GalA (Xu et al., 2019). The signal at 5.31 ppm was corresponding to H-1 of Rha residues and the peaks at around 1.29 ppm confirmed the -CH_3 groups of Rha. The highest peaks at 5.11 ppm in the spectra of USP-2 and USP-1 were attributed to the H-1 of GalA, suggested the amount of GalA in USP1 and USP2 were higher than that of USP3. The well-resolved signal at 5.19 ppm, 5.14 ppm and 5.08 ppm in the spectra of USP2 and USP1 were assigned to the H-1 of Ara residues (Barbieri et al., 2019; Xu et al., 2020). The anomeric proton signal of Gal residues was detected at 4.67 ppm (Siu, Xu, Chen, & Wu, 2016). Moreover, no proton signal of carboxylic ester groups (3.69 ppm) and acetyl groups (2.07 ppm) were

Table 1
Monosaccharide composition and molecular properties of USP fractions.

USP fractions	Monosaccharide composition (mol%)						M_w (Da)	Polydispersity
	Ara	Gal	Rha	GalA	Glc	Xyl		
USP1	39.30±0.7a	7.85±1.15a	2.19±0.21a	45.15±0.15b	4.1±0.5c	1.59±0.09a	2713±0.15a	1.286±0.21a
USP2	44.3±3.7a	7.79±0.69a	3.49±0.19a	42.31±2.30b	0.85±0.15a	0.78±0.68a	3683±0.07b	1.226±0.10a
USP3	50.47±2.47b	13.44±1.44b	11.23±2.77b	20.68±2.31a	2.8±1.0b	0.78±0.68a	7469±0.04c	1.449±0.09a

Ara: arabinose; Gal: galactose; Rha; rhamnose; GalA: galacturonic acid; Glc: glucose; Xyl: xylose; Means with the different letter are significantly different between lines.

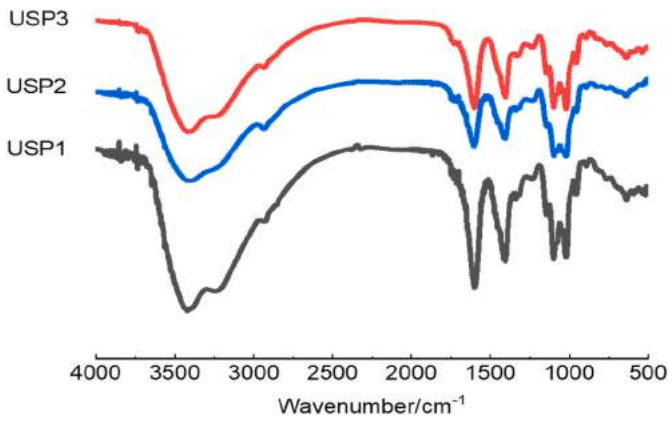

Fig. 2. IR spectra (% transmittance as a function wavenumber) of USP1, USP2, and USP3.

detected in these spectra, indicated these pectins were non-esterified. Collectively, USP1 and USP2 showed more similar composition of monosaccharides, while USP3 contained more arabinan sidechains and less GalA. These results were consistent with the monosaccharide composition analysis.

3.5. Effects on cell viability of macrophages

The cytotoxicity of these pectic fractions were determined using MTT assays. As shown in Fig. 4, murine macrophage RAW264.7 cells exhibited above 92% cell viability under all the tested concentrations of these pectins, indicating that all of these pectins had no significant cytotoxicity in macrophages at concentrations less than 200 μg/mL.

3.6. Effects on NO level of inflammatory cell model

After activated by the bacterial lipopolysaccharides (LPS), the production of NO in immune cells increased significantly during inflammatory response. As shown in Fig. 5, all of these pectins alleviated the NO release of LPS-treated RAW264.7 cells in a dose-dependent manner.

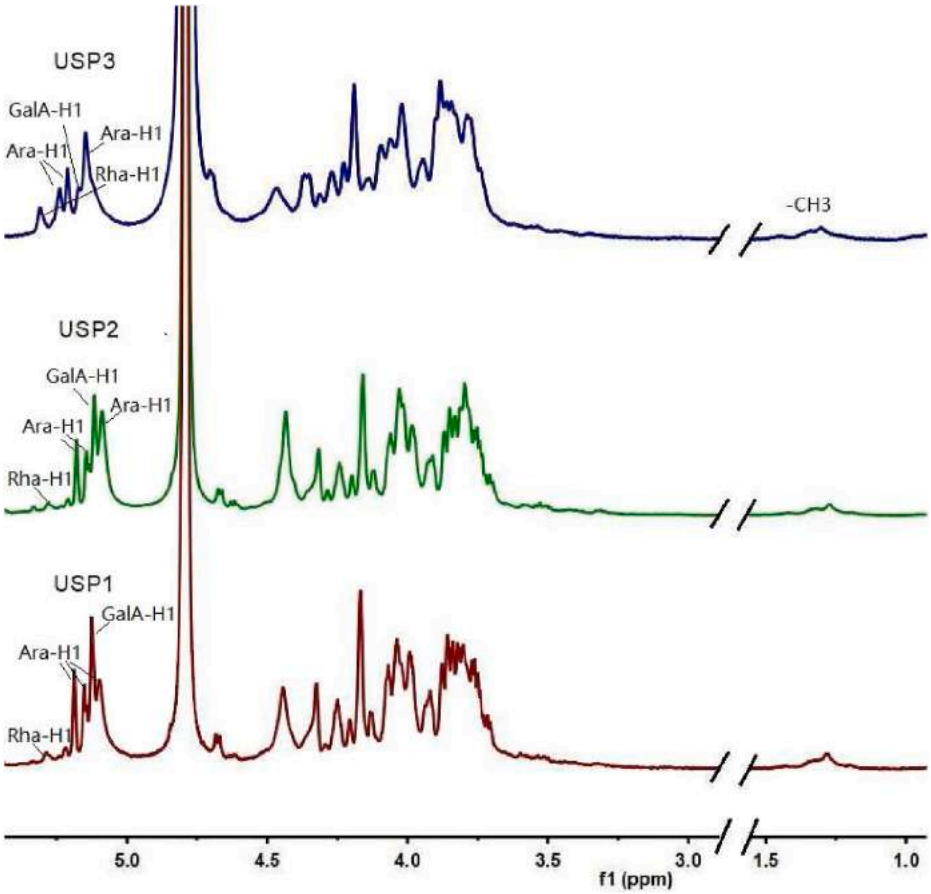

Fig. 3. The 1H NMR spectra of USP1, USP2, and USP3.

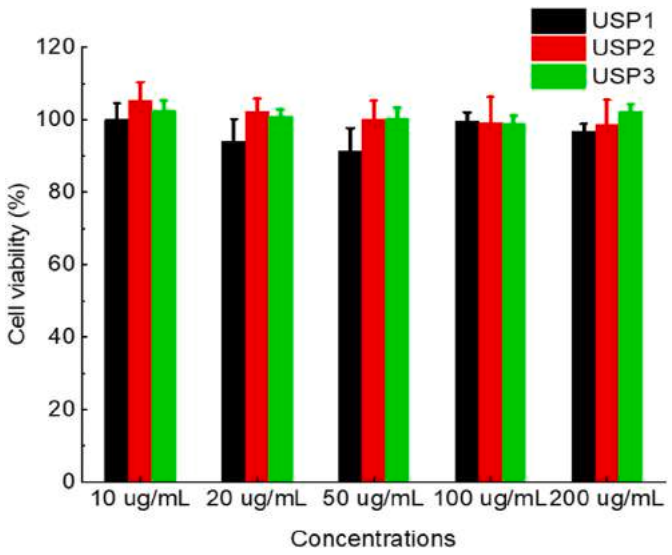

Fig. 4. The cytotoxicity of USP fractions on RAW264.7 cell lines. Groups with the different letter are significantly different the others.

At the dosage range of 10–100 μg/mL, there was no significant differences among the these samples. USP2 and USP3 showed better effects than USP1 with significant differences at higher concentration (100–200 μg/mL), suggested that pectin of higher M_w (>3KD) might possessed better protective effects against NO and the complete sidechains played an essential role (Wu et al., 2021). The previous studies have confirmed the significant role of arabinogalactan sidechains of blackberry wine RG-I in reducing the NO production in LPS-treated macrophages (Cordeiro Caillot et al., 2018). Together, high amount of neutral sidechains could improve the immunomodulatory function of pectins.

3.7. Effects on ROS production of inflammatory cell model

Reactive oxidative species (ROS) are generally considered to be deleterious to host cells. When macrophages are stimulated by LPS, signaling from the cell surface TLRs will induce ROS generation (West et al., 2011; Zhang et al., 2013). During inflammatory response, accumulation of ROS can lead to oxidative damage and external antioxidative substances can protect macrophages against oxidative stress

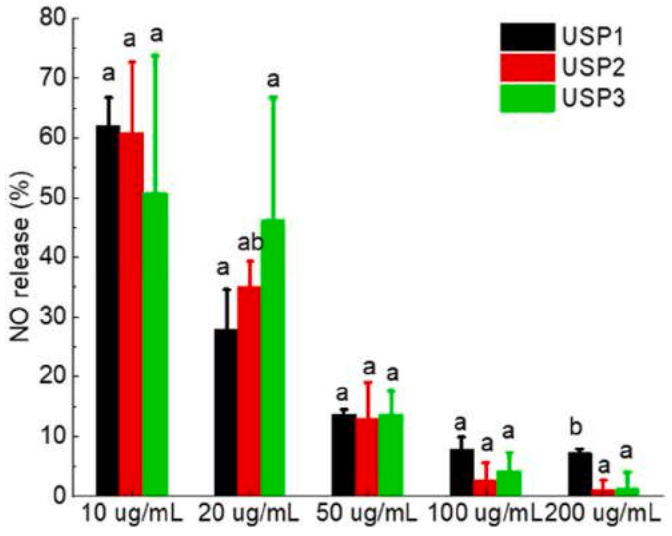

Fig. 5. NO release of RAW264.7 cells after incubated with USP1, USP2, and USP3. Groups with the different letter are significantly different the others.

(Uttara, Singh, Zamboni, & Mahajan, 2009). As shown in Fig. S2, stimulation of LPS at 20 μg/mL significantly promoted the ROS level of macrophages, compared to the control group. After pretreatment of pectins at the concentration of 30 μg/mL, 60 μg/mL and 120 μg/mL, the intracellular ROS levels were significantly reduced. No significant differences were observed among different pectins groups at the concentration of 60–120 μg/mL in Fig. 6. However, at the lower concentration (30 μg/mL), USP3 showed remarkably stronger effects on inhibiting the production of ROS, suggesting that pectin of higher M_w exhibited better cytoprotective effects against oxidative damage. RG-I pectins exerted the anti-ROS activities in a dosage-dependent manner, and the large RG-I molecules exhibited better protective effects under the low dosage. Previous studies have reported that there was no significant differences between raspberry pectin and ultrasound-depolymerized pectin on inhibiting ROS production, which might be due to the different cell lines and the higher dosage (Chen et al., 2020). And apart from the molecular weight, the architecture of sidechains still affected the activity of pectin. Thus, both of the long neutral sidechains and the high molecular weight in USP3 are important structural factors.

3.8. Effects on pro-inflammatory cytokines production

The disorder of inflammatory cytokines is involved in the development of immune decreases (Kaunitz & Nayyar, 2015). TNF-α, IL-6 and IL-1β are considered to be the dominant pro-inflammatory cytokines, which are mainly secreted by monocytes, macrophages, neutrophils and lymphocytes. These pro-inflammatory cytokines could cause colon damage, damage the epithelial barrier and induce cell apoptosis (Bevivino & Monteleone, 2018). To further identify the immunomodulatory properties of the bioactive fractions, these three pectins were incubated with LPS-activated RAW264.7 cells. As shown in Fig. 7, LPS induced significant increase of IL-1β, IL-6, TNF-α amounts in macrophages. All of these three pectic fractions remarkably reduced the level of these cytokines. USP-1 was capable to down-regulate the IL-1β, IL-6, TNF-α production by 13%, 45%, 22%. USP2 achieved 21%, 55%, 24% of the cytokines inhibition, and the three inhibitory rates of USP3 were 26%, 63% and 37%, respectively. Interestingly, the IL-6 response to these pectins was much more higher than that of IL-1β and TNF-α. This might be due to the higher amount of arabinan in these RG-I pectins. Previous studies have confirmed that sidechains of orange pectins could directly interact with the Toll like receptors in macrophages and suppress the IL-6 secretion (Ishisono, Mano, Tomio, & Kitaguchi 2019). It has been reported that sidechains can suppress the production of IL-6 by stimulating TLR1/2 and TLR4 in macrophages. The responses of IL-6 to linear arabinan was higher than that of branched arabinan (Meijerink et al., 2018). Thus, the level of IL-6 level was particularly regulated by long linear arabinan sidechains in pectins. Nevertheless, the binding mechanism between RG-I pectin and the TLRs still remained unclear, which structure domains in RG-I was involved in the molecule interaction need to be further investigated. Additionally, all of these cytokines were down-regulated by pectin fractions in a M_w-dependent manner. The inhibitory effects represented in the decreasing order of 2 KD<3 KD<7 KD. This results were similar to the published studies. Vogt et al. (2016) have confirmed that intact sugar chain structure played an important role in the activation of immune cells and the decrease of M_w led to a loss of activity. Also, the reduction of molecular weight could induce lower IL-10 secretion in THP-1 macrophages (do Nascimento et al., 2017).

4. Conclusions

In conclusion, three highly purified low-M_w pectins (USP1, USP2, USP3) were prepared from the depolymerized pectic oligosaccharides. USP1 with M_w 2713 g/mol consisted of almost the same chemical composition as USP2 with M_w 3683 g/mol. USP3 possessed the highest M_w of 7469 g/mol, and contained more long arabinan sidechains. All of these pectins exhibited low degree of methylation. Also, this study is the

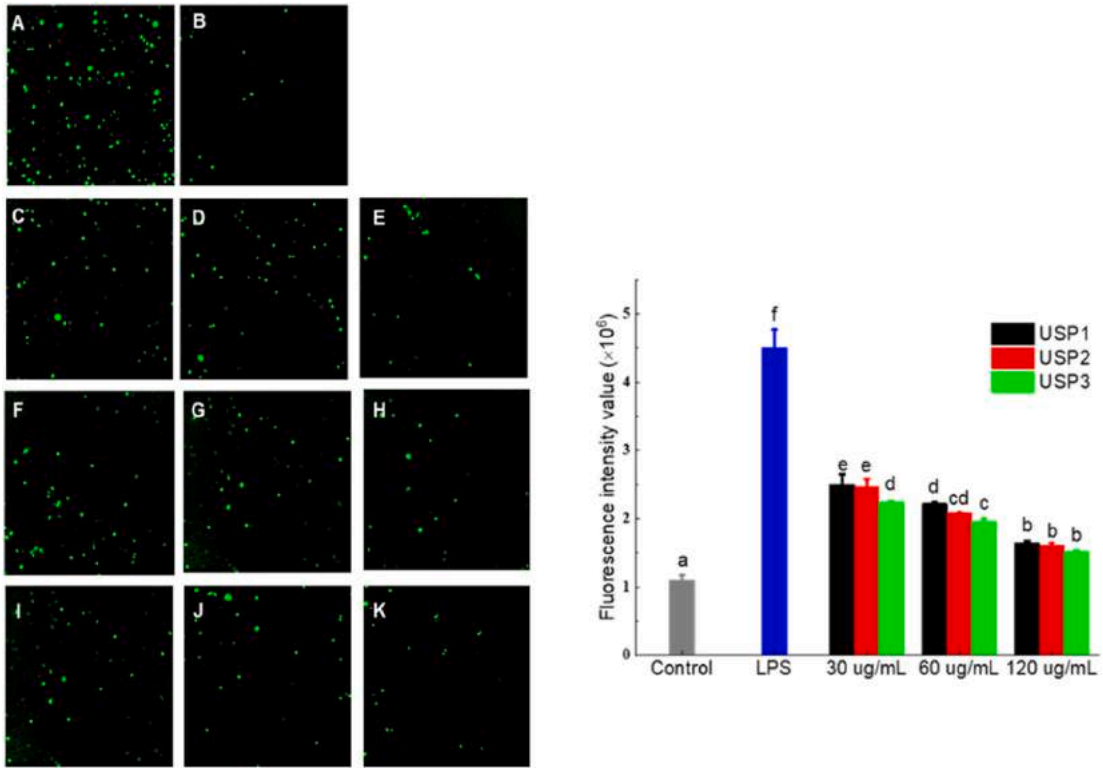

Fig. 6. Imaging of ROS level using laser scanning confocal microscopy. (A) Positive control; (B) normal cells; (C) USP1 at 30 μg/mL; (D) USP2 at 30 μg/mL; (E) USP3 at 30 μg/mL; (F) USP1 at 60 μg/mL; (G) USP2 at 60 μg/mL; (H) USP3 at 60 μg/mL; (I) USP1 at 120 μg/mL; (J) USP2 at 120 μg/mL; (K) USP3 at 120 μg/mL (left); Effects of USP1, USP2, and USP3 on the intracellular ROS levels of RAW264.7 cells (right). Groups with the different letter are significantly different the others.

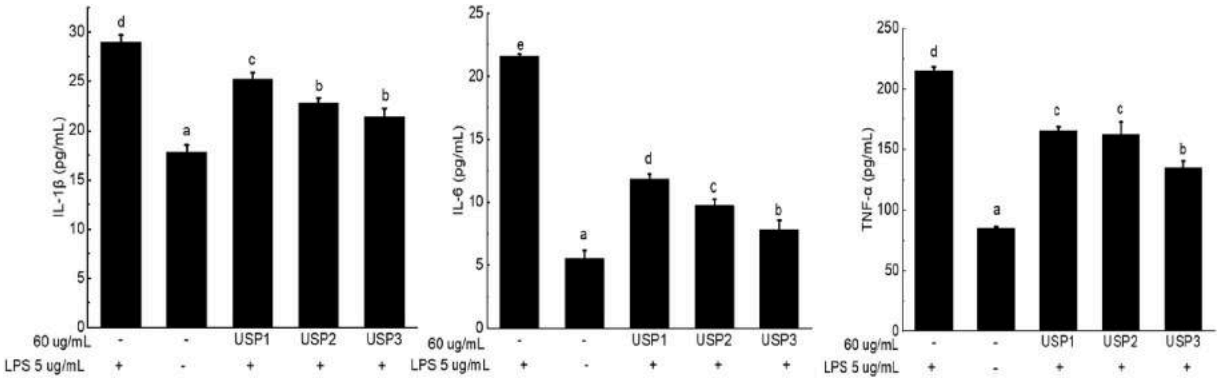

Fig. 7. The IL-6, IL-1β and TNF-α secretion of LPS-induced RAW264.7 cells after incubated with USP1, USP2 and USP3. Groups with the different letter are significantly different the others.

first time to compare the anti-inflammatory effects of pectic oligosaccharides with different M_w on LPS-treated macrophages. All of these pectins can exerted the activities via inhibiting NO release, ROS production, and the pro-inflammatory cytokines. Moreover, the inhibitory effects increased with the increasing molecular weight, especially on the secretion of IL-6. And the long arabinan sidechain architecture of RG-I pectin was the key factor to down-regulated at least one pro-inflammatory cytokines. Thus, pectin rich in arabinan sidechains of M_w>3KD might be a promising prophylactic agent against inflammatory diseases. Neverthless, in which way pectins of different molecular weights affect immune cells, whether it will affect the function of receptors in the surface of immune cells, and how pectins interact with the receptors need more investigation. Further studies about the mechanisms on inhibiting pro-inflammatory cytokines secretion in vivo are underway.

CRediT authorship contribution statement

Dongmei Wu: Conceptualization, Investigation, Methodology, Writing – original draft. **Xiaoliang Zheng:** Methodology. **Weiwei Hu:** Software. **Kai Zhu:** Software. **Chengxiao Yu:** Software. **Qiaojun He:** Resources. **Robert J. Linhardt:** Writing – review & editing. **Xingqian Ye:** Funding acquisition, Resources. **Shiguo Chen:** Methodology, Funding acquisition, Writing – review & editing, All authors have seen and approved the submission of the manuscript.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

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