

Influence of the extraction conditions on the carbohydrate and phenolic composition of functional pectin from persimmon waste streams

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ABSTRACT

Persimmon fruit (*Diospyros kaki* Thunb.) production suffers great losses (15–20%) due to the inefficient over-ripening process. In order to valorise this waste, a compositional characterization of different fruit stages of the residues was done, and immature fruit was selected due to its high pectin and very high polyphenol content. A 3-level full factorial design was carried out to study the effect of temperature (70–95 °C) and low pH (0.5–1.5), on yield, degree of esterification, carbohydrate constituents, phenolic content and antioxidant capacity of the extracted pectin, and a complete polyphenol profile (UPLC-MS/MS) was performed on selected extracts. All responses could be accurately adjusted to the models ($R^2 > 80$; lack of fit). Pectin yield, phenolic compounds and antioxidant activity ranged from 1.4 to 4.5%, 53.3 ± 2.27 to 111.7 ± 9.74 mg GAE/g pectin and 0.29 ± 0.01 to 2.77 ± 0.04 TEAC (Trolox $\mu\text{mol}/\text{mg}$ pectin), respectively. A strong pectin-polyphenol interaction was found, which significantly enhanced acid resistance of both the pectin and polyphenol constituents, with optimum yield and polyphenol content at pH 1 and 95 °C. These new pectin-based ingredients might have a great potential as functional foods or natural food ingredients enhancing the quality and shelf-life.

1. Introduction

The future of food and agriculture faces a number of uncertainties that raise significant concerns about its performance and sustainability. While food demand is expected to increase anywhere between 59% and 98% by 2050 (Berbel & Posadillo, 2018), more than 1.3 billion tonnes of food are wasted every year, which is around 1/3 of the world's production (FAO, 2016). With a global aim towards sustainability, it is necessary to drive a change and look for different ways to valorise by-products or residues from the food chain with potential to become new ingredients improving the quality and/or preservation of foods (Carocho, Morales, & Ferreira, 2018).

Persimmon (*Diospyros kaki* Thunb.) is a fruit tree originating from China, with a global production of 4.3 million tons in 2019 (FAOSTAT, 2021). Over the last decade, Spain has become the second global producer and the first exporter owing to the “Rojo Brillante” variety with registered designation of origin in the Valencian province (FAOSTAT, 2021; Martínez-Las Heras, Amigo-Sánchez, Heredia, Castelló, & Andrés,

2016). This especially resistant and high quality variety must be subjected to an overripening process with 1-methylcyclopropene and ethylene for 18–24 h to reduce its astringency before consumption. Although great efforts have been carried out in post-harvesting techniques and storage conditions to improve the quality and minimize losses, immature untreated fruits are severely affected by small physiological changes causing a poor CO₂ diffusion, which results in a low anaerobic respiration rate, reducing acetaldehyde accumulation which in turn causes a decrease in its interactions with soluble tannins, these being responsible for astringency and rejection (Munera et al., 2019). The seasonal production and this inefficient overripening result in huge amounts of discarded fruit (about 15–20% of the fruit harvested) (Munera et al., 2019), representing ~ 9500 Tn/year in the Valencian region only (data provided by the second grade cooperative ANECOOP).

Apart from free sugars, persimmon contains significant amounts of pectin and very high contents in polyphenols. Pectin is a family of polysaccharides built with several structural elements, where the most studied and highly identified are the homogalacturonan (HG) and type I

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rhamnogalacturonan (RG-I). HG, known as the smooth region, is constituted by galacturonic acid (GalA) units which can be naturally methylesterified at the C-6 carboxyl group (Prasanna, Prabha, & Tharanathan, 2007). The degree of methylesterification governs much of the properties in pectin, depending on which pectins are able to form gels with high amounts of soluble solids and/or the presence of divalent cations (Wai, Alkarkhi, & Easa, 2010) and constitute a valuable texturizing ingredient used in foods, such as dairy products, juices, sauces, marmalades, and even meat products or cheese (Adetunji, Adekunle, Orsat, & Raghavan, 2017; Virk & Sogi, 2004). Polyphenols are widely recognized for their health benefits as antioxidants, among other bioactivities (antimicrobial, prebiotic, etc) (Hamaizu, 2018; Pérez-Burillo, Oliveras, Quesada, & Rufián-Henares, 2018); .

In fact, several works have been focused on their extraction and characterization from persimmon (Butt et al., 2015; Hamaizu and Suwannachot, 2019), since its antioxidant activity has been reported to be significantly higher than other antioxidant-rich fruits like apple, grape or tomato (Chen, Fan, Yue, Wu, & Li, 2008). Furthermore, these polyphenols have been reported to be linked to pectin moieties, reducing both astringency and firmness during the ripening process (Mamet, Ge, zhen, Zhang, & Li, 2018; Taira, Ono, & Matsumoto, 1997). This unique characteristic offers a great potential for the valorization of functional pectins as new functional food ingredients with all benefits from the polyphenols adding up to the intrinsic properties of pectin.

Some authors have previously reported on the extraction of pectin from persimmon peel, (Jiang, Xu, Li, Li, & Huang, 2020; Nguyễn and Savage, 2013). Nevertheless, little information is available on how the extraction conditions influence the structural and functional properties of persimmon pectin, and especially, about how polyphenol compounds can interact with it (Mamet et al., 2018; Taira et al., 1997). To the best of our knowledge, there is no existing literature on how pectin extraction can modify these pectin polyphenol interactions and the physicochemical, structural and functional properties of persimmon pectin from discarded fruits.

This work presents a compositional analysis of persimmon discarded fruits of varying ripeness and, following response surface methodologies and a detailed polyphenolic profile, thoroughly investigates the effect of pH and temperature on the compositional, structural and functional properties of these potential new functional pectin ingredients.

2. Materials and methods

2.1. Material

Persimmon (*Diospyros kaki* Thunb) “Rojo brillante-Ribera del Xuquer” fruits in three ripeness stages (commercial immaturity –CI–, commercial mature not treated –CM– and commercial mature treated with 1-methylcyclopropene and ethylene –CMT–) were kindly supplied by Anecoop S. Coop. during the autumn season of 2019 in Spain. The fruits were processed removing the calyx and peduncle, cut into pieces of around 0.5–2.5 cm and stored in protective bags at - 20 °C until their evaluation. Citrus (CP) and apple (AP) pectin (Sigma-Aldrich) were used for comparative purposes, as they are typical pectin sources of wide industrial use and well defined structure and properties. Folin-Ciocalteu's reagent, Sodium bicarbonate, Hydrochloric acid (37% (v/v)), β -carotene ($\geq 97\%$), linoleic acid ($\geq 99\%$), Tween® 40, Butylated hydroxytoluene (BHT) ($\geq 99\%$), Trolox (97% purity), gallic acid ($\geq 98.0\%$ purity), phenol red (ACS grade), sodium hydroxide (pharma grade) and ABTS⁺ (HPLC grade) were supplied by Sigma-Aldrich (Stenheim, Germany). Acetone (99%) was from WVR chemicals. N-hexane ($>95\%$ purity) and ethanol (96% (v/v), USP grade) were purchased from Panreac Applichem (Darmstadt, Germany)

2.2. Proximate analysis

Persimmon fruits in all the ripeness stages were firstly characterized.

Moisture was determined according to the approved method 935.29, (AOAC, 1996). Protein determination was carried out by the Dumas combustion method (N conversion factor 5.35, Fujiyama, Kasuga, & Aoyagi, 2001) according to ISO/TS, 16634–2 (2016). Lipid content was quantified by lipid extraction with a Soxhlet apparatus according to Somashekar et al. (2001). Ash content was determined according to the standard TAPPI T211 om-07. Carbohydrates were estimated as the sum of all monosaccharide components (section 2.8). All the analyses were done in triplicate.

2.3. Experimental design

The effect of temperature and pH on the chemical and structural properties of pectin extracted from immature persimmon fruit (CI) was studied through a 3-level full factorial design (3^2) with 4 centered points. The solid-liquid ratio was fixed at 1:20 for a balanced extraction yield and solvent use according to results on pectin extraction from persimmon peel and other fruits (Campbell, 2006; Dranca & Oroian, 2018; Jiang et al., 2020; Prakash Maran, Sivakumar, Thirugnanasambandham, & Sridhar, 2014; Petkowicz, Vriesmann, & Williams, 2017; Rodsamran & Sothornvit, 2019a). Although time is usually an important factor for pectin extraction, the difference in extraction yield between 30 or 90 min using HCl as acidic solvent was not significant in persimmon fruit (e.g., 30 and 90 min extraction at 95 °C, pH 1.5, yielded 4.28 ± 0.15 wt% and 4.34 ± 0.09 wt% pectin, respectively). Individual and interactive effects of process variables (temperature (X_1) and pH (X_2)) over pectin extracts were evaluated (Table 1).

Analysis of variance (ANOVA) following by means comparison using Tukey's multiple comparison test, quality of fit (coefficient of determination (R^2), adjusted coefficient of determination ($\text{adj-}R^2$)), were attained using the software Minitab, version 15 (Minitab Inc.,

Table 1

3-level full factorial design, factors: temperature (X_1) and pH (X_2), and experimental values for of yield and chemical properties (degree of esterification -DE %, β - Carotene/linoleic acid bleaching antioxidant activity -%AA- and Trolox Equivalent Antioxidant Capacity -TEAC-) (on a dry weight basis).

run	X_1 (°C)	X_2 (pH)	% Yield	mg GAE/ g pectin	DE (%)	%AA	TEAC (μmol Trolox/mg pectin)
1	70	1	1.76	83.14 \pm 2.54	67.29 \pm 0.26	27.57 \pm 3.21	1.74 \pm 0.08
2	82.5	0.5	3.98	61.36 \pm 1.32	54.5 \pm 0.44	17.87 \pm 2.74	1.04 \pm 0.05
3	95	0.5	4.02	53.26 \pm 2.27	44.05 \pm 2.42	4.42 \pm 7	0.29 \pm 0.01
4	82.5	1.5	2.49	111.71 \pm 9.74	71.42 \pm 0.45	28.41 \pm 4.22	2.4 \pm 0.05
5	70	1.5	1.37	103.41 \pm 0.76	74.24 \pm 0.74	25.95 \pm 9.94	2.65 \pm 0.08
6	82.5	1	3.46	100.28 \pm 0.74	67.48 \pm 0.82	25.15 \pm 1.84	2.21 \pm 0.09
7	82.5	1	3.54	100.21 \pm 0.62	66.72 \pm 0.43	29.74 \pm 0.81	2.72 \pm 0.06
8	95	1.5	4.32	101.96 \pm 1.78	70.06 \pm 0.63	45.87 \pm 7.91	2.36 \pm 0.12
9	82.5	1	3.44	100.11 \pm 0.50	66.44 \pm 0.49	29.55 \pm 5.35	2.77 \pm 0.04
10	82.5	1	3.39	100.07 \pm 0.92	67.21 \pm 1.96	29.39 \pm 4.73	2.65 \pm 0.06
11	95	1	4.50	79.48 \pm 0.78	62.91 \pm 1.7	14.91 \pm 4.87	1.52 \pm 0.06
12	82.5	1	3.65	98.67 \pm 1.87	67.5 \pm 1.16	27.94 \pm 3.4	2.66 \pm 0.12
13	70	0.5	2.18	65.85 \pm 0.82	41.94 \pm 0.14	27.21 \pm 3.34	1.16 \pm 0.03
AP			–	N.D.	78.57 \pm 3.47	N.D.	N.D.
CP			–	N.D.	58.25 \pm 0.62	N.D.	N.D.

Philadelphia, U.S.A.). Differences were considered significant at $p \leq 0.05$.

2.4. Extractions

Frozen persimmon was weighed, and the suitable amount of water was added taking into account the initial moisture content of the fruit. The pH was adjusted with 1 M HCl and solutions were heated under magnetic stirring on a hotplate during 30 min. After that, the pectin-based solution was filtered with a muslin cloth followed by vacuum filtration using Whatman filter paper n° 4 at 60 °C. Pectin in solution was coagulated by adding 2 vol of ethanol and the mixture was left overnight in the freezer. The coagulated pectin was centrifuged (23450 g for 20 min) and washed three times with 96% (v/v) ethanol and two times with acetone to remove less polar compounds and small molecules. The wet pectin was left to dry at 60 °C in a hot air oven until constant weight, ground and stored in a desiccator chamber (0% RH) until further use. Pectin yield (Y%) was calculated from the following equation:

$$Y(\%) = \frac{m_0}{m} \times 100$$

where m_0 (g) is the weight of dried pectin and m (g) is the weight of dried persimmon powder.

2.5. Esterification degree

Degree of esterification (DE) of the extracted pectin was measured according to the method described by (Méndez, Fabra, Gómez-Mascaraque, López-Rubio, & Martínez-Abad, 2021). Pectin samples of 0.05 g were weighed and wetted with 0.5 mL of ethanol (96% (v/v)) and dissolved in 10 mL of a 10% (w/v) sodium chloride solution overnight under magnetic stirring. Two drops of phenol red were added to the dissolved pectin as indicator and the solution was titrated with 0.1 M NaOH (V1). Then, 25 mL of 0.25M NaOH was added and mixed vigorously to de-esterify pectin and left at room temperature for 30 min. Next, a volume of 25 mL at 0.25 M HCl to neutralize NaOH was added and titrated again with 0.1 M NaOH until color change (V2). Calculations were performed according to equation (Eq. (1)), in triplicate and compared to citrus and apple pectin as references.

$$DE = \frac{V2}{V2 + V1} \times 100 \quad (\text{Eq. 1})$$

2.6. FTIR

FTIR measurements were recorded in transmission mode in a controlled chamber at 21 °C and dry air in order to avoid humidity and CO₂ using a Cary 630 FTIR spectrometer (Agilent, USA). The spectra were taken at 4 cm⁻¹ resolution in a wavelength range of 400–4000 cm⁻¹ and averaging a minimum of 32 scans. Spectra acquired were processed using Origin pro 2019 software (OriginLab Corporation, Northampton, MA, USA).

2.7. Monosaccharide composition

The sugar composition of the extracts was determined after acidic methanolysis as previously described (Martínez-Abad, Giummarella, Lawoko, & Vilaplana, 2018). Freeze-dried samples (1 mg) were incubated with 1 mL of 2 M HCl in dry methanol for 5 h at 100 °C. Samples were then neutralized with pyridine, dried under a stream of air, and further hydrolyzed with 2 M TFA at 120 °C for 1 h. The samples were again dried under a stream of air and re-suspended in milliQ, filtered and injected. The monosaccharides were analyzed using high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) with a ICS-3000 system (Dionex) equipped with a

CarboPac PA1 column (4 × 250 mm, Dionex). Control samples of known concentrations of mixtures of glucose (Glc), fucose (Fuc), rhamnose (Rha), galactose (Gal), arabinose (Ara), xylose (Xyl), mannose (Man), galacturonic acid (GalA) and glucuronic acid (GlcA) were used for calibration (Merck, Germany). Due the high lability of fructose (Fru) during methanolysis, the free Fru and Glc were measured spectrophotometrically using a sucrose, D-fructose and D-glucose (K-SUFRG) Assay Kit (Megazyme, Bray, Ireland), according to the manufacturer's instructions. All measurements were carried out in triplicate.

2.8. Total phenolic content (TPC)

The phenolic compounds from the pulp fruit were first extracted according to Pellegrini et al. (2017) from the freeze-dried material and measured with an spectrophotometric method (Singleton, Orthofer, & Lamuela-Raventós, 1999). In the case of pectin extracts, 0.2 mL of the pectin solutions in distilled water (5 mg/mL) were mixed with 1 mL of 10% Folin-Ciocalteu's reagent and 0.8 mL 7.5% NaHCO₃. The samples were thereafter incubated at 50 °C for 10 min and the absorbance was measured at 750 nm. Gallic acid was used for calibration and the content of phenolic compounds in the enriched-pectin extracts and phenolic extracts from pulp fruit was expressed in terms of gallic acid equivalents (mg GAE/g pectin). Assays were performed in triplicate.

2.9. Phenolic profile

A complete phenolic profile of immature persimmon fruit (CI) and selected pectin extracts obtained after less severe (pH 1.5, 70 °C, run 5 and pH 1.5, 82 °C, run 4), averagely severe (pH 1, 90 °C, run 11) or extreme acid conditions (pH 0.5, 82 °C, run 2) was performed according to (Mattila & Kumpulainen, 2002). The free phenolic fraction was recovered after liquid-liquid extraction. 0.5 g of sample were dissolved in 7 mL of solution A (85 mL of methanol + 15 mL of acetic acid at 10% (v/v) + BHT at 0.2% (w/v)), sonicated for 30 min, and further diluted in distilled water to 17 mL. Then, the pH was adjusted to 2.0 and 15 mL of solution B (50 mL of diethyl ether + 50 mL of ethyl acetate) were added. After gentle shaking, the organic phase was separated and dried overnight at room temperature. The aqueous solution was further diluted with distilled water (12 mL), and 10 mL of 10 M NaOH was added. Alkaline-hydrolysis was performed under anaerobic conditions by stirring the mixture overnight at room temperature. Monomer aglycone phenolics were then recovered by the same procedure using solution B in order to obtain the alkaline fraction, corresponding to esterified phenolics. The aqueous phase was then acidified with HCl (2.5 mL) and heated (85 °C) for 30 min. The mixture was cooled down to room temperature and processed in the same way using solution B to obtain the acid fraction, corresponding to conjugated phenolics. The detection and quantification of the individual phenolic compounds was performed by UPLC-MS/MS on an AcQuity Ultra-Performance™ liquid chromatography/tandem mass spectrometry equipment (Waters, USA) with a flow rate of 0.3 mL/min and the injection volume 2.5 µL. The UPLC-MS/MS detection conditions are previously described elsewhere (Yuste et al., 2018). The quantification of the individual phenolic compounds was determined by comparison with calibration curves of commercial standards. Due to lack of commercial standards of all the individual phenolic compounds, some of these compounds were tentatively quantified by using the calibration curve of another phenolic compound with a similar structure.

2.10. Trolox Equivalent Antioxidant Capacity (TEAC)

The Trolox Equivalent Antioxidant Capacity (TEAC) was only determined in the pectin-enriched extracts using a modification of the original TEAC method (Re et al., 1999). Samples were dissolved in distilled water for 12 h and analyzed for ABTS^{•+} (2,2-azinobis (3-4-ethylbenzothiazoline)-6-sulfonic acid) radical scavenging activity. The

ABTS⁺ solution with an initial absorbance at 734 nm of 0.70 ± 0.08 was freshly prepared, 20 μ L of the enriched-pectin extract solution were added to 230 μ L of the ABTS⁺ solution and the absorbance was registered after 6 min. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) solutions were used as a standard for calibration. The TEAC of the enriched-pectin extracts was determined by comparing the corresponding percentage of absorbance reduction at 6 min with the Trolox concentration–response curve. All the determinations were carried out, at least six times using a CLARIOstar (BMG LABTECH, Germany) spectrophotometer with water as blank.

2.11. β -Carotene/linoleic acid bleaching

The antioxidant activity of the enriched-pectin extracts was determined according to the β -carotene bleaching method described by Fontes-Candia, Erboz, Martínez-Abad, López-Rubio, and Martínez-Sanz (2019) with some modifications. Briefly, 4 mL β -carotene solution (20 mg in 10 mL chloroform), 50 mg linoleic acid and 400 mg Tween 40 were transferred to a round-bottomed flask. The mixture was then dried with air flow during 5 min to remove chloroform. Then, 100 mL of oxygenated distilled water was slowly added and vigorously stirred resulting in a stable emulsion. 10 μ L of the pectin solutions (2 mg/mL in PBS) were added to 250 mL aliquots of the β -carotene/linoleic acid emulsion and the absorbance was measured (Abs^0) at 470 nm using a microplate spectrophotometer CLARIOstar (BMG LABTECH, Germany). Microtiter plates were incubated in the dark at 45 °C and the absorbance of each sample measured again after 120 min (Abs^{120}). PBS and *tert*-Butyl hydroxytoluene (BHT) (2 mg/mL) were analogously used as blank and positive control, respectively. All samples were assayed in triplicate. The antioxidant activity (AA) was expressed as the percentage of inhibition relative to the degradation rate (DR) using the following formula:

$$DR = \ln(Abs^0 / Abs^{120}) \times 1/120 \text{ min AA\%} = [(DR_{control} - DR_{sample}) / DR_{control}] \times 100$$

3. Results and discussion

3.1. Proximate analysis and sugar composition at different maturity stages

The proximate composition of the persimmon fruit at the three ripeness stages is shown in Table 2. Moisture content was significantly different ($82.42 \pm 0.5\%$, $79.45 \pm 0.12\%$ and $78.39 \pm 0.41\%$ for CI, CM and CMT persimmon fruits) depending on the ripeness of the fruit, which is in agreement with previous literature (Chen et al., 2016; Phytochemicals et al., 2015). Changes in moisture content often represent the humidity loss during storage, which can affect the polysaccharide composition (Tsuchida, Sakurai, Morinaga, Koshita, & Asakura, 2004). The ripeness stage did not significantly alter the overall carbohydrate, protein, ashes or fat content, these values being also similar to those reported for persimmon fruit by other authors (Phytochemicals et al., 2015; Yaqub et al., 2016). However, a significant change was noticed in the carbohydrate composition of the different samples, highly affected by the physiological and chemical changes due to the climacteric nature of the persimmon fruit, and related to ripening (Taira et al., 1997). As expected, a positive correlation was observed between ripeness and increased fructose and glucose content (Table 2), typical for astringent persimmon species and the “Rojo Brillante” variety (Novillo, Salvador, Crisosto, & Besada, 2016). The amount of GalA content, which is the main pectin constituent for persimmon, was significantly higher in CI

Table 2

Chemical composition of persimmon fruit at different ripeness stages commercial immaturity (CI), commercial mature non treated (CM), commercial mature treated with 1-methyl cyclopropane and ethylene (CMT).

Fruit composition (dry wt. %)	CI	CM	CMT
Ash	3.63 ± 0.58^a	2.42 ± 0.47^{ab}	2.70 ± 0.6^{ab}
Lipids	3.64 ± 0.94^a	2.8 ± 0.43^a	3.4 ± 0.59^a
Protein	3.45 ± 0.01^a	3.6 ± 0.01^a	3.53 ± 0.02^a
Total polyphenol content (mg GAE/g)	25.91 ± 2.28^a	17.13 ± 0.94^b	1.97 ± 0.04^c
Carbohydrates ^a	78.95 ± 8.50^a	75.82 ± 8.8^a	88.94 ± 7.90^a
of which (μ g/mg dry basis)			
GalA ^b	60.64 ± 13.56^a	$44.12 \pm 2.76^{a,b}$	37.35 ± 3.33^b
Gal ^b	9.16 ± 0.95^a	6.09 ± 0.48^b	5.74 ± 0.24^b
Ara ^b	10.15 ± 1.79^a	6.35 ± 0.08^b	6.61 ± 0.35^b
Xyl ^b	36.37 ± 8.44^a	6.40 ± 0.05^b	5.42 ± 0.42^b
Glu ^c	385.25 ± 0.40^b	387.60 ± 0.42^b	451.09 ± 12.60^a
Fru ^c	288.00 ± 7.99^b	307.67 ± 13.20^b	383.00 ± 12.08^a

Means of each characteristic followed by different letters in the same row are significantly different ($p \leq 0.05$).

Moisture content of fresh samples was $82.42 \pm 0.5\%$, $79.45 \pm 0.12\%$ and $78.39 \pm 0.41\%$ for CI, CM and CMT persimmon fruits.

^a As the sum of all detected monosaccharide constituents.

^b Monosaccharide composition determined by HPAEC-PAD.

^c Measured with D-fructose and D-glucose (K-SUFRG) Assay Kit.

fresh fruits than in CMT samples, showing not significant differences between CMT and CM persimmon samples, the latter being in agreement with values reported for different persimmon cultivars at full-ripeness stage (0.69 – 2.39 g pectin/100 g fresh weight) (Chen et al., 2016). Persimmon fruit had a higher pectin content than other fruits, such as cherry, tomato, passion fruit or peach, although still lower than typical pectin sources, such as lemon or apple, or other alternative sources, such as watermelon or mango (Méndez et al., 2021; Prasanna et al., 2007). The decreasing pectin content with ripening is mainly caused by collective action of several pectin-degrading enzymes (Posé et al., 2019). Likewise, the significant reduction in the xylose content for the ripest fruits suggests further depolymerization of hemicellulose polysaccharides (Han et al., 2015). Also, a reduced moisture content in CM and CMT fruits may promote the reduction of some neutral sugars present in pectin, such as Ara and Gal (Tsuchida et al., 2004). Considering the results of the different stages and its composition, the preferred ripeness stage for pectin extraction was the CI stage, due to its significantly higher pectin and polyphenol content (Table 2).

3.2. Characterization of pectin extracts

Second order polynomial equations including linear, interactive and quadratic terms were employed to generate mathematical models to evaluate the relationship between process variables and responses (cf. Table 3, Eqs. (3)–(14)). Different responses were selected according to the potential functionality they may confer to the pectin extracted. Yield (%) is related to the extraction efficiency while DE is related to

Table 3

Polynomial equations generated and statistical results for responses evaluated depending on (X_1) temperature and (X_2) pH.

Polynomial equations		R^2	R^2 - adj
$Y(\%) = 15.08 + 0.390X_1 - 2.64X_2 - 0.00202X_1^2 + 0.0444X_1X_2 - 0.844X_2^2$	Eq. 3	94.88	91.22
$DE(\%) = -151.9 + 3.99X_1 + 91.92X_2 - 0.0232X_1^2 - 0.252X_1X_2 - 23.04X_2^2$	Eq. 4	94.89	91.23
<i>Carbohydrate composition</i>			
$GalA (\mu g/mg) = 1459 + 16.3X_1 - 751X_2 + 0.093X_1^2 - 8X_2^2 + 5.99X_1X_2$	Eq. 5	87.25	78.15
$Ara (\mu g/mg) = -325.8 + 7.43X_1 + 69.6X_2 - 0.0417X_1^2 - 0.906X_1X_2 + 13.47 X_2^2$	Eq. 6	91.90	86.11
$Xyl (\mu g/mg) = -18.7 + 1.03X_1 - 31.9X_2 - 0.00910X_1^2 + 0.438X_1X_2 - 3.51X_2^2$	Eq. 7	74.20	55.77
$Rha (\mu g/mg) = -27.6 + 2.10X_1 - 51.2X_2 - 7.09 X_1^2 + 0.754X_1X_2 - 7.09X_2^2$	Eq. 8	80.90	67.26
$Gal (\mu g/mg) = -303 + 10.98X_1 - 38.7X_2 - 0.0776X_1^2 + 1.278X_1X_2 + 0.0024X_2^2$	Eq. 9	81.29	67.93
$RL = 105.3 - 3.08X_1 + 32.2X_2 - 0.0257X_1^2 + 10.08X_2^2 - 0.765X_1X_2$	Eq. 10	85.35	74.88
$RB = -23 + 0.553X_1 + 7.03X_2 - 0.00314X_1^2 + 0.4X_2^2 - 0.068X_1X_2$	Eq. 11	78.57	63.26
<i>Antioxidant capacity</i>			
$TPC (mg GAE/g \text{ pectin}) = -451 + 12.21X_1 + 64.7X_2 - 0.0782X_1^2 - 27.9 X_2^2 + 0.446 X_1X_2$	Eq. 12	96.60	94.18
$TEAC (\mu Mol \text{ trolox}/mg) = -21.58 + 0.541X_1 + 3.41X_2 - 0.0035X_1^2 - 1.859X_2^2 + 0.0236X_1X_2$	Eq. 13	89.27	81.61
$AA = 63 + 0.58X_1 - 120.3X_2 - 0.0151X_1^2 - 1.9X_2^2 + 1.708X_1X_2$	Eq. 14	86.00	76.00

Abbreviations: Yield (Y), degree of esterification (DE), galacturonic acid (GalA), Arabinose (Ara), Xylose (Xyl), Rhamnose (Rha), Galactose (Gal), ratio linearity (RL), Ratio branching (RB), Total phenolic content (TPC), Trolox Equivalent Antioxidant Capacity (TEAC), β - Carotene/linoleic acid bleaching antioxidant activity (AA%).

thickening, gelling and rheological properties. The main mono-saccharide constituents (GalA, Gal, Ara, Rha and Xyl ($\mu g/mg$)) were analyzed as a means to describe compositional changes in pectin, which in turn may govern their mechanical and functional properties, as well as the presence of hemicellulose impurities (Table 3). More important, the polyphenol content and antioxidant capacity were also introduced in the model, as a means to evaluate the potential of these pectins as functional ingredients.

3.2.1. Yield

The yield values of the enriched-pectin extracts were properly fitted (R^2 94.88; Table 3), indicating a good reliability for the experimental predictions. To investigate the effect of pH and temperature on the yield, the response surface plots were also obtained for the pectin enriched extracts (Fig. 1a). In general, the response surface plot illustrates that the highest yield was obtained for extractions performed at the highest temperature ($p < 0.05$) and pH 1.0, which correspond to the average conditions for pectin extraction from other fruits (Denman & Morris, 2015). At high temperatures and under extreme acidic conditions (pH 0.5), yields decreased, most probably due to hydrolytic depolymeration resulting in the loss of this low molecular weight pectin after washing (see Fig. 1a). It should be noted that the maximum yield values in this work (4.5% dry weight, Table 1) corresponds to 75% of all pectin being extracted from the fruit, which evidences high extraction efficiency. These values are slightly higher than those previously reported by other authors for freeze-dried persimmon (Nguyễn and Savage, 2013). Most often, the strong hydrogen bonds generated during the freeze-drying process much detrimentally affect the extraction yield (Pettolino, Walsh, Fincher, & Bacic, 2012), suggesting higher yields might be achieved with fresh residues.

Even though the aim of this work was to obtain higher added-value functional pectin and lower yields compared to traditional pectin sources were expected, due to the higher pectin content in both dried citrus peel (20–30%) and dried apple pulp (15–20%, compared to persimmon fruit, (Lara-Espinoza, Carvajal-Millán, Baladrán-Quintana, López-Franco, & Rascón-Chu, 2018), the extraction time in this work was significantly lower compared to traditional pectin extraction. Similar short extraction times also resulted in lower yields for orange (2.95%, Ognyanov & Kussovski, 2013) or melon peel (2.87%, Raji, Khodaiyan, Rezaei, Kiani, & Hosseini, 2017), pointing out the relative cost-efficiency of this process.

3.2.2. Pectin esterification

The degree of esterification in pectin much governs the rheological properties and gelling behavior. High methoxyl pectin (HM; DE > 50%) can form gels at a high sugar content, whilst low methoxyl pectin (LM) form gels at a lower sugar content in the presence of divalent cations (Lara-Espinoza et al., 2018; Wai et al., 2010). The degree of pectin esterification in the present work properly fitted to the polynomial model (R^2 94.89; Table 3, Equation (4)). The obtained response surface plot, shown in Fig. 1b, revealed that changes in the DE values of enriched pectin extracts were mainly influenced by pH ($p < 0.05$), while the correlation between Y and DE is also illustrated in Fig. S1 (in the Supplementary Material). Interestingly, the usual decrease in DE under severe acidic conditions is only patent at pH 0.5, while no significant decrease is observed at pH 1 or pH 1.5 (Jafari, Khodaiyan, Kiani, & Hosseini, 2017). The higher acid stability of pectin esterification in persimmon samples might be associated with the high abundance of pectin-polyphenol complexes, as commented below, which has interesting implications towards the potential application as functional bioactive pectin (Denman & Morris, 2015; Wai et al., 2010). Highest yields attained at pH 1 and high temperature also show a high DE (Table 1). These DE values are somewhat higher compared to pectin extracts from other pectin sources (Aina et al., 2012), but in line with reference AP or CP (see Table 1).

3.2.3. Sugar constituents

A quantitative sugar analysis was carried out in order to elucidate the composition of the pectin extracts (Table 4) and understand how it is affected by process parameters (pH and temperature). To get an overall perspective on the structural features of the pectin extracts, different sugar ratios were also calculated for each extraction condition, giving valuable information on the degree of arabinogalactan branching (RB), the linearity (RL), the severity of the extraction (RS) or the contribution of RGI, HG and their relative ratio (Alba, Bingham, Gunning, Wilde, & Kontogiorgos, 2018; Denman & Morris, 2015) (Table 5). The main sugar constituents in persimmon pectin extracts were GalA, Fuc, Rha, Ara, Gal and Xyl, which is in accordance with previous works carried out with persimmon peel (Jiang et al., 2020). Man, Glc and Fru were not detected or only present in trace amounts ($\leq 0.5 \mu g/mg$). The lack of free sugars in the extracts indicates a successful separation of pectin from the very abundant free sugar fraction and other potential low molecular weight compounds not bound to the polysaccharide (Houben, Jolie, Fraeye, Van Loey, & Hendrickx, 2011). The different degree of aggressiveness in the extractions results in a wide range of compositional characteristics and both the abundance of the different pectin constituents as well as the degree of branching and linearity, were analyzed as responses and properly fitted to the polynomial model ($R^2 > 75\%$ except for Xyl), enabling a reliable prediction of the pectin traits based on specific extraction conditions (Table 3, Equation (7)).

According to the definitions of the international scientific expert committee on Food Additives (JECFA) administered jointly by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), GalA is recognized as an indicator of the purity of pectin and HG is related to the quality of pectin which is associated to its intrinsic properties and functionality (Alba et al., 2018;

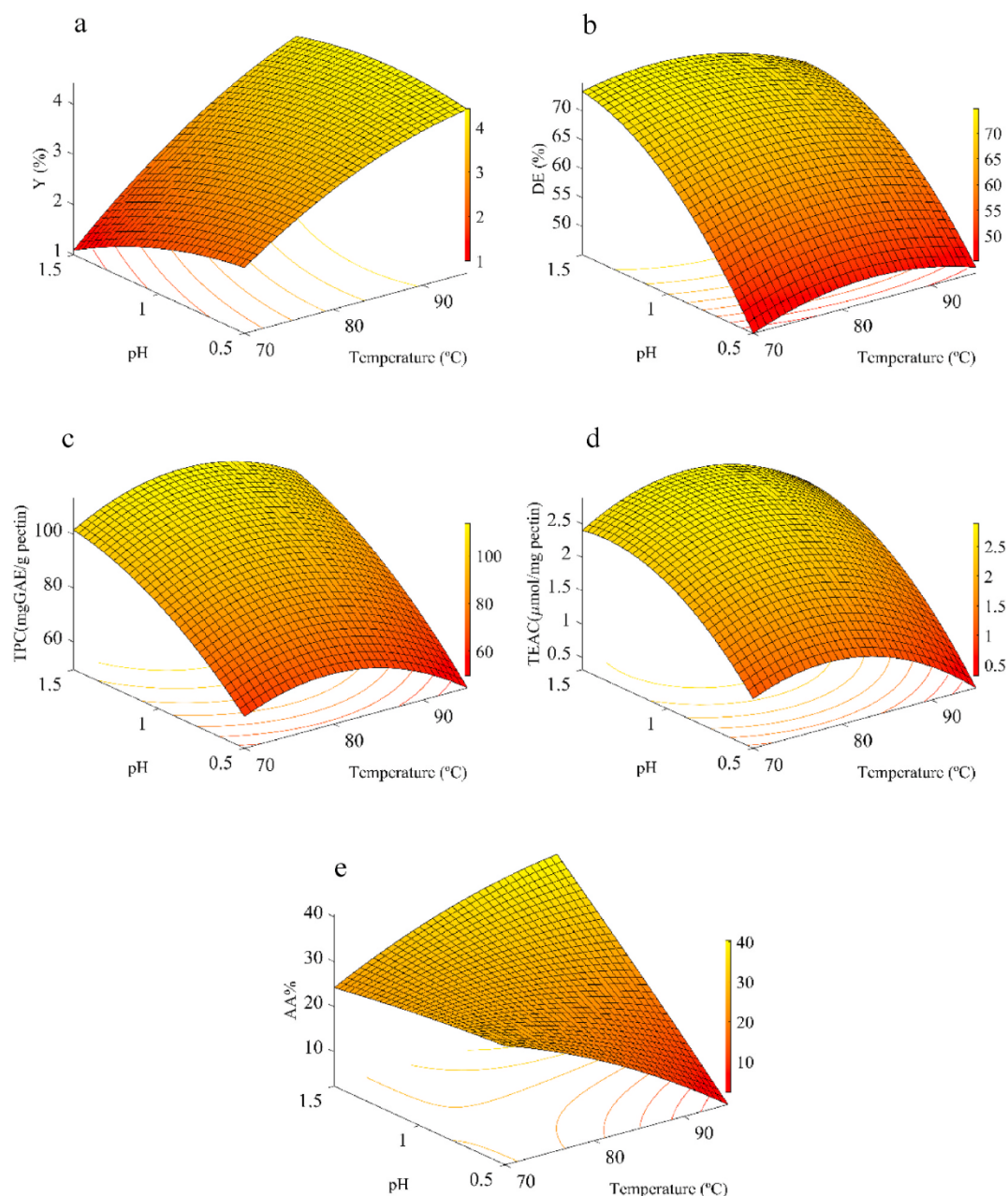


Fig. 1. Surface response for pectin (a) yield extraction (Y(%)), (b) degree of esterification (DE%), (c) total phenolic content (mg GAE/mg sample), (d) TEAC, and (e) AA% β -Carotene/linoleic acid bleaching from persimmon fruit pectin rich extracts with temperature and pH as main factors.

Müller-Maatsch et al., 2016). Both values for persimmon pectin produced under all tested conditions are in line with reference citrus and apple pectin (see Tables 4 and 5), except for the less severe (70 °C, pH 1.5, run 5), while extreme conditions (pH 0.5) resulted in purer HG fractions (62.7–63.4% compared to 45.7% and 54.3% for AP and CP, respectively) at the expense of yield, highest at pH 1 and 95 °C (run 11). Again, it is notable that only extreme acidic conditions are able to significantly degrade the pectin backbone (e.g. reduction in Rha and GalA; Table 5, Fig. 3a and b) (Denman & Morris, 2015; Kulkarni & Vijayanand, 2010). Extracted pectin under optimum conditions maximizing yield result in a theoretical 4.42% yield, 79.4 mg GAE/g at pH 0.93 and 95 °C, very much as run 11, and would thus generate a pectin complying with current standards for its use as texture modifier in the food industry, which would add up to its bioactive functionalities commented below.

Ara and Gal are the main neutral sugar constituents found in RGI, in

the form of arabinan, galactan or arabinogalactan (AGI) side chains. Their higher acid lability, especially of arabinofuranosyl residues, is patent in the results and reflected in the model, as temperature, pH and their interaction have a very significant effect on their presence (Table 3, Fig. 3c and d). In fact, Ara was neither detected in pectin obtained at pH 0.5 (Denman & Morris, 2015; Morris, Ralet, Bonnin, Thibault, & Harding, 2010) nor at high temperature and pH 1.0 (Table 4). Gal was the most abundant neutral sugar found in the pectin extracts, ranging between 12.9 and 55.7 $\mu\text{g}/\text{mg}$ (see Table 4), in agreement with previously reported values for persimmon peel (Jiang et al., 2020) or other pectin sources (Müller-Maatsch et al., 2016; Yang, Mu, & Ma, 2018). The reduction of Gal, although not as affected as Ara (Table 4; Fig. 3c), further adds up to the significant effects of temperature and pH on the degree of branching (RB) and linearity (RL) of pectin (Table 5, Fig. 3e and f). On the other hand, less severe extraction conditions result in high RB at the expense in HG purity (Table 5 and Fig. S4). The good fitting of

Table 4

Experimental values for neutral sugar (rhamnose (Rha), arabinose (Ara), galactose (Gal) and xylose (Xyl)) and galacturonic acid (GalA) composition of pectins extracted according to the experimental design.

Runs	Rha (μg/ mg)	Ara (μg/ mg)	Gal (μg/ mg)	Xyl (μg/ mg)	GalA (μg/ mg)
1	19.19 ± 1.46 ^{bc}	4.01 ± 0.48 ^{fg}	36.59 ± 2.72 ^e	3.31 ± 0.32 ^e	501.51 ± 4.03 ^d
2	20.68 ± 4.31 ^b	0 ± 0 ^g	42.85 ± 1.89 ^d	6.86 ± 0.26 ^{bc}	654.9 ± 5.64 ^a
3	8.64 ± 0.38 ^d	0 ± 0 ^g	12.39 ± 0.74 ^{fg}	0 ± 0 ^f	635.98 ± 5.21 ^a
4	19.71 ± 0.67 ^{bc}	23.78 ± 0.71 ^b	46.04 ± 1.64 ^{cd}	3.45 ± 0.25 ^e	409.98 ± 15.25 ^h
5	13.92 ± 9.12 ^{cd}	32.11 ± 15.18 ^a	42.03 ± 4.56 ^d	0 ± 0 ^f	210.15 ± 23.16 ⁱ
6	18.65 ± 5.30 ^{bc}	11.09 ± 3.32 ^{de}	55.43 ± 1.97 ^a	4.51 ± 0.04 ^{cde}	450.59 ± 13.45 ^f
7	19.21 ± 5.86 ^{bc}	15.16 ± 1.78 ^{cde}	55.75 ± 2.63 ^a	4.57 ± 0.26 ^{cde}	460.40 ± 25.5 ^{ef}
8	13.88 ± 6.10 ^{cd}	9.47 ± 0.04 ^{ef}	34.56 ± 0.97 ^e	3.06 ± 0.14 ^e	422.68 ± 1.25 ^{gh}
9	23.56 ± 3.40 ^{ab}	12.6 ± 2.36 ^{cde}	54.39 ± 0.18 ^a	4.50 ± 0.11 ^{cde}	452.58 ± 7.72 ^f
10	21.76 ± 0.22 ^{ab}	13.25 ± 3.07 ^{cde}	55.48 ± 2.33 ^a	7.56 ± 5.31 ^b	461.35 ± 13.29 ^{ef}
11	18.86 ± 0.49 ^{bc}	0 ± 0 ^g	43.47 ± 4.27 ^d	5.91 ± 0.21 ^{bcd}	596.44 ± 20.10 ^b
12	20.79 ± 1.34 ^b	13.96 ± 0.23 ^{cde}	53.10 ± 1.98 ^{ab}	4.37 ± 0.16 ^{de}	444.49 ± 14.16 ^{fg}
13	27.54 ± 0.49 ^a	0 ± 0 ^g	51.82 ± 4.16 ^{ab}	7.89 ± 1.02 ^b	573.22 ± 17.82 ^{bc}
AP	24.19 ± 0.96 ^{ab}	18.47 ± 0.58 ^{bcd}	49.44 ± 1.68 ^{bc}	16.02 ± 0.85 ^a	481.97 ± 17.89 ^{de}
CP	22.27 ± 0.53 ^{ab}	19.46 ± 0.71 ^{bc}	35.18 ± 1.59 ^e	3.56 ± 0.12 ^e	565.91 ± 22.27 ^c

Means of each characteristic followed by different letters in the same column (a–f) are significantly different (Fisher $p \leq 0.05$).

*Traces or zero values were found for Glucose, fructose, mannose, fucose and glucuronic acid.

Table 5

Composition ratios based on the neutral sugars and galacturonic acid content for each enriched-pectin extract.

Run	Branching (RB) ^a	Linearity (RL) ^b	Severity of extraction (RS) ^c	HG % ^d	RG-I % ^e	HG: RG-I
1	2.12	7.95	124.98	48.2	4.1	11.77
2	2.07	9.30	–	63.4	4.3	14.6
3	1.43	30.24	–	62.7	1.2	49.9
4	3.54	4.41	17.24	39.0	7.0	5.5
5	5.32	2.39	6.54	19.6	7.4	2.6
6	3.57	5.02	40.64	43.1	6.6	6.4
7	3.69	4.86	30.38	44.1	7.1	6.1
8	3.17	6.93	44.63	40.8	4.4	9.2
9	2.84	4.76	35.91	42.9	6.7	6.3
10	3.16	5.10	34.83	43.9	6.9	6.3
11	2.31	8.74	–	57.7	4.3	13.1
12	3.23	5.06	31.84	42.3	6.7	6.2
13	1.88	6.57	–	54.5	5.2	10.4
AP	2.81	4.39	26.09	45.7	6.8	6.6
CP	2.45	6.98	29.09	54.3	5.5	9.8

^a RB A larger value is indicative of larger average size of the branching side chains. (Gal + Ara/Rha).

^b RL A larger value suggest of more linear/less branched pectins. (GalA/Xyl + Rha + Ara + Gal).

^c RS larger value or not determined value is indicative of more severe extraction conditions and loss of arabinofuranoside (Araf) residues. (GalA/Ara).

^d (GalA-Rha).

^e (2Rha + Ara + Gal).

the degree of RL, and relative high purity of the pectin extracted evidence the possibility of producing pectin with desired structural features, which in turn govern the functional (texturizing, prebiotic, etc.) properties of the final product. Interestingly, conditions with highest yields and relatively high purity result in slightly higher branching degree compared to reference pectin (AP and CP), while RL values are more similar to apple pectin, citrus pectin being more linear. This is probably explained by the inherent higher Rha to Gal ratio in citrus pectin, pointing towards a lower arabinogalactan contribution in RGI.

3.2.4. TPC content and phenolic profile of persimmon pectin

The visual appearance of the pectin extracts is shown in Fig. S6, where a dark brown coloration is evidenced most likely due to the presence of polyphenols. During pectin extraction, the sequential ethanol and acetone washings to precipitate pectin were expected to remove the major part of polyphenols from pectin extracts (Mamet et al., 2018; Taira et al., 1997). Nevertheless, the detection of high amounts of polyphenols on the pectin extracts suggests the existence of pectin-polyphenol interactions. The TPC of the pectin enriched extracts was therefore analyzed and could also be properly fitted (R^2 96.6) using the polynomial model described by Equation (12) (Table 3). The effect of pH and temperature on the TPC can also clearly be observed in the response surface plot (Fig. 1c). As expected, TPC decreased with decreasing pH, while temperature mostly affected TPC if extreme acid pH was used for extraction. Specifically, the TPC ranged between 53.26 and 111.71 mg GAE/g pectin, pointing out the very high polyphenol content of persimmon. In fact, extraction conditions with high pectin yields also showed higher TPC values (e.g. yield 4.32% and 101.96 ± 1.78 mg GAE/g pectin, Table 1) than those reported for other pectin sources with high TPC values and antioxidant activity (Hosseini, Parastouei, & Khodaiyan, 2020; Rodsamran & Sothornvit, 2019b; Sanchez-Aldana, NOE Aguilar, Nevarez-Moorillon, & Contreras Esquivel, 2013).

Although the TPC value is a good indicator for overall phenolic content and potential beneficial effects as food ingredients, TPC values usually overestimate the real phenolic content, as the Folin reagent readily reacts with other components, such as ascorbic acid, soluble protein, aromatic amines, sulfur dioxide, organic acids, and Fe (II) (Prior, Wu, & Schaich, 2005).

The identification of specific phenolic compounds and their interaction with other constituents is therefore a better approach to describe the potential bioactive effects of the extracts and their stability upon processing, storage or bioavailability after digestion. In order to identify the specific phenolic compounds present, their potential type of interaction, and relate compositional changes in the phenolic profile to the different extraction treatments, a quantification of conjugated, esterified and free phenolic compounds of the initial raw material (CI) and 3 representative pectin extracts after extreme, averagely severe and less severe extraction conditions was performed. Although the release of phenolics upon alkali or acid treatment is not a direct evidence of their covalent bonding, this approach serves for a rapid estimation of putatively esterified or conjugated phenolic compounds (Dvořáková et al., 2008; Krygier, Sosulski, & Hogge, 1982; Mattila & Kumpulainen, 2002; Russell, Labat, Scobbie, Duncan, & Duthie, 2009). Results on the main individual phenolic compounds present in persimmon fruit and in the pectin extracts are summarized in Table 6. For detailed extended information of all phenolic compounds identified and analyzed in these and one additional pectin extract refer to Table S1 (supplementary material).

For persimmon fruit, the sum of all identified phenolic compounds (as the sum of free phenolics, alkali fraction and acid fraction) was 11847 mg/kg (Table 6). In agreement with the present results, Senica, Veberic, Grabnar, Stampar, & Jakopic, (2016) and Lucas-González, Viuda-Martos, Pérez-Álvarez, & Fernández-Lopez, (2017) reported a concentration of total phenolic between 10000 and 17500 mg/kg in different cultivars of persimmon flours. To the best of our knowledge,

Table 6

Concentration (mg/kg sample standard deviation ()) of individual phenolic compounds in lyophilized persimmon fruit (CI) and persimmon fruit extracts at less severe (L, run 5), averagely severe (M, run 11), extreme acidic conditions (H, run 2).

Phenolic compounds	Free Phenolics				Alkali fraction				Acid fraction			
	CI	M (run 11)	H (run 2)	L (run 5)	CI	M (run 11)	H (run 2)	L (run 5)	CI	M (run 11)	H (run 2)	L (run 5)
Benzoic acids												
Vanillin	22.9 (1.55)	123 (29.70)	157 (3.67)	126(8)	14.6 (17.90)	31.4 (5.56)	35.8 (0.33)	19.5 (5.22)	21.8 (1.59)	30.6 (0.89)	18.3 (1.21)	16(0.11)
Gallic acid	161 (25.20)	1560 (190)	781 (128)	1618 (158)	9646 (25.20)	258 (205)	27 (2.32)	3874 (1955)	762 (57.40)	484(16)	41.3 (30.3)	1072 (143)
Gallic acid glucoside	298 (34.30)	n.d.	n.d.	n.d.	1.57 (0.04)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Protocatechuic acid	n.d.	192 (49.7)	75.8 (8.78)	69.4 (24.70)	211 (39.10)	22.8 (9.60)	21.9 (9.20)	561(268)	44.5 (7.27)	184 (7.01)	68 (15.3)	126 (26.50)
Protocatechuic acid glucoside	4.8(1.30)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Vanillic acid	n.d.	n.d.	n.d.	n.d.	11(0.20)	n.d.	n.d.	26.2(0)	15.7 (1.59)	n.d.	n.d.	n.d.
Vanillic acid glucoside	278 (43.40)	n.d.	n.d.	n.d.	19(0.46)	4.29 (0.71)	2.83 (1.41)	n.d.	4.53 (2.38)	n.d.	n.d.	27.9 (14.9)
Total Benzoic acids^a	4.33 (0.86)	n.d.	n.d.	n.d.	6.71 (1.27)	19.36 (8.98)	17.35 (3.82)	21.78 (2.64)	3.39 (0.23)	n.d.	n.d.	2.76 (0.84)
Hydroxycinnamic acids^a	2.62 (0.17)	n.d.	n.d.	n.d.	68.73 (6.71)	9.79 (2.29)	4.7 (0.28)	31.96 (3.45)	10.41 (1.09)	n.d.	n.d.	n.d.
Total Phenolic acids	772	1874	1013	1814	9978	346	110	4534	862	698	128	1245
Flavan-3-ols												
Epigallocatechin	15.5 ± 3.19	n.d.	n.d.	n.d.	3.33 ± 0.12	n.d.	n.d.	4.75 ± 0.24	n.d.	n.d.	n.d.	n.d.
Epigallocatechin gallate	1.87 ± 0.76	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Other Flavan-3-ols^a	29.11 (3.77)	n.d.	n.d.	n.d.	3.05 (0.54)	n.d.	n.d.	32.8 (18.10)	n.d.	n.d.	n.d.	11.03 (3.53)
Total Flavan-3-ols	46.48 (7.72)	n.d.	n.d.	n.d.	6.38 (0.66)	n.d.	n.d.	37.55 (18.34)	n.d.	n.d.	n.d.	11.03 (3.53)
Flavonol												
Quercetin ^a	18.9 (0.68)	3.88 (1.78)	n.d.	n.d.	10.57 (2.22)	n.d.	n.d.	14.6 (8.16)	2.23 (0.37)	n.d.	n.d.	1.39 (0.47)
Other Flavonol^a	73.47 (20.71)	10.4 (6.12)	n.d.	n.d.	11.51 (4.06)	n.d.	1.26 (0.11)	20.3 (13.80)	1.7(0.31)	n.d.	n.d.	7.05 (1.18)
Total Flavonol	92.37 (21.39)	14.28 (6.12)	n.d.	n.d.	22.08 (6.22)	n.d.	1.26 (0.11)	34.9 (13.80)	3.93 (0.35)	n.d.	n.d.	8.44 (1.18)
Total Flavanone^a	1.61 (0.38)	n.d.	n.d.	n.d.	0.42 (0.02)	n.d.	n.d.	0.15 (0.21)	n.d.	n.d.	n.d.	n.d.
TOTAL PHENOLICS	970	1889	1013	1814	10011	346	111	4607	866	698	128	1264

^a Not included phenolic compounds can be consulted in [Supplementary Material Table S1](#). The values in brackets represent standard deviation.

analysis of the polyphenolic profile in persimmon fruit pectin extracts has not been reported. Phenolic acids, and specifically gallic acid, account by far for the highest part (11613 mg/kg), followed by flavonols (120 mg/kg), flavan-3-ols (112 mg/kg), and minor amounts of flavanones (2.03 mg/kg). In agreement with our results, [Fu, Lu, and Zhou \(2016\)](#) reported that phenolic acids, flavan-3-ols and flavonols are the most abundant phenolic group detected in persimmon fruits ([Fu et al., 2016](#)). The abundant gallic acid contents in the immature fruit (CI) was mainly found in the alkali fraction (9646 mg/kg), pointing towards ester bonding to pectin constituents. Protocatechuic acid and p-coumaric acid were also found in significant amounts as esters while the glucosides of gallic acid and vanillic acid, quercetin and its derivatives, and epigallocatechin gallate were found as non-covalently bound in the free fraction. A small fraction of most phenolics found as esters in the alkali fraction could also be detected in the acid extracted fraction.

Although the range of relatively severe acid treatments (pH 0.5–1.5) at high temperatures could be expected to degrade most phenolic compounds, a significant amount is retained in the pectin extracts after acid treatment, coagulation and washing. In fact, at less severe (L) conditions, >7500 mg/kg identified phenolics were found in the pectin extracts (as the sum of identified free phenolics, or in the alkali and acid fractions), whereas lower but still very significant quantities (2933 mg/kg and 1252 mg/kg) were identified at averagely severe (M) and extreme acidic conditions (H), respectively ([Table 6](#)). The presence of

strong pectin-polyphenol interactions via ester and glycosidic linkages has already been suggested and associated to the ripening process, where the formation of complexes between soluble tannins and pectin is concomitant to the partial depolymeration of pectin, resulting in fruits with decreased astringency and firmness through ripening ([Mamet et al., 2018; Taira et al., 1997](#)). The use of increasingly severe acidic conditions and increase temperature results, however, in the cleavage of these bonds (and loss of the free polyphenols) or their degradation ([Saura-Calixto & Pérez-Jiménez, 2018; Zhang & Mu, 2011](#)) together with the pectin backbone (as commented before). Interestingly, significant amount of “free” phenolics were also detected in all pectin extracts, the proportion of which increases with extraction severity. As an example, averagely severe conditions (M) display a very significant reduction in esterified phenolics, while non-covalently bound, “free” phenolics significantly increase compared to less severe conditions (L). This might be explained by the formation of electrostatic and hydrogen bonding between pectin constituents and tannins. This interaction would then be broken when applying the liquid-liquid extraction procedure to extract free phenolics. ([Bermúdez-Oria, Rodríguez-Gutiérrez, Fernández-Prior, Vioque, & Fernández-Bolaños, 2019](#)).

In agreement with the model for TPC, pH is the determinant factor for polyphenol recovery ([Fig. 2c](#)), with gallic acid as the main constituent in all extractions, but the detailed profile provides further unreported insights on qualitative aspects. The glucoside forms of phenolic

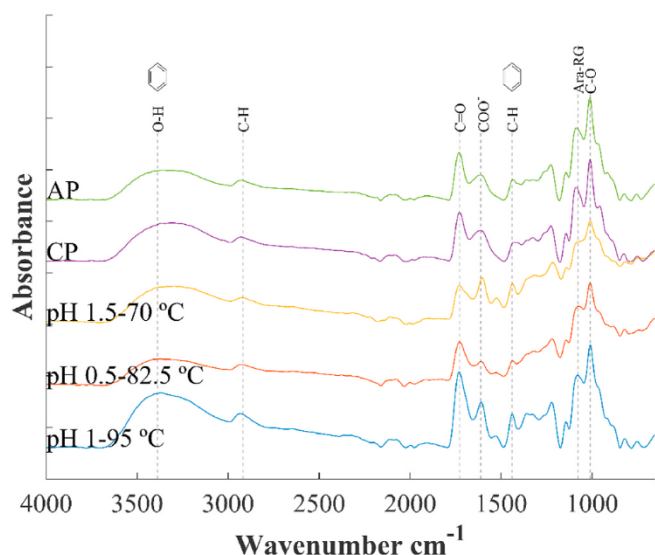


Fig. 2. FT-IR spectra comparing harsh, medium and softer pectin extraction conditions from persimmon “Rojo brillante”, apple pectin (AP) and citrus pectin (CP).

compounds were not detected in the pectin extracts, most probably as a result of the acid treatment (Tsao, 2010). Flavan-3-ols and flavanones were only detected at pH > 1 and in minor amounts as compared to the starting material. Although it is generally accepted that flavan-3-ols are stable in acidic pH environments (Guyot, Cheynier, Souquet, & Moutounet, 1995), extremely low pH conditions might enhance losses of these phenolic compounds or facilitate the conversion of the *trans*-configured form into the *cis*-isomers. Li, Taylor, Ferruzzi, and Mauer (2012) suggested that the *cis*-configured catechins such as epigallocatechin gallate, epicatechin gallate and epicatechin could be easily transformed to the *trans*-configured catechins such as gallic catechin gallate and catechin gallate as isomerization products produced under high temperatures.

On the other hand, an increase in the presence of protocatechic acid, vanillin, quercetin and myricetin is observed. In all cases, the increase might be the result of the enrichment in a polyphenol rich pectin, especially at less severe extraction conditions. The partial degradation of gallic acid (3,4,5-trihydroxybenzoic acid) into protocatechic acid (3,4-dihydroxybenzoic acid) or *trans*-esterification to yield vanillin are also plausible. However, knowledge of the mechanism involved in degradation of hydroxybenzoic acids is rather limited, particularly with regard to fruits and vegetables. The results demonstrate the high relative resistance of polyphenols to pectin acid extraction and shows that the susceptibility of plant phenolic compounds to extraction conditions depends on the phenol structure.

3.2.5. Antioxidant capacity (TEAC, β -carotene/linoleic acid bleaching)

To confirm the antioxidant properties of the functional persimmon pectins and investigate how it is affected by the extraction parameters, the ABTS^{•+} radical scavenging and the β -carotene/linoleic acid bleaching assay were carried out on all pectin extracts. TEAC and AA% values could also be modeled with a good adjustment (R^2 89.3 and 86.0 respectively; Table 3, Eqs. (13) and (14)). TEAC values varied between 0.29 and 2.77 μ mol trolox/mg between samples and was significantly affected by the two factors ($p < 0.05$). Although previous reports attribute some antioxidant capacity to pectins, related to the GalA content (Hu et al., 2016; Sun, Chen, & Zhu, 2020), it is the TPC content which significantly affects the antioxidant activity (Jiang et al., 2020). The antioxidant capacity based on TEAC values was highly correlated with TPC values (Fig. 1c and d), and very low pH ($p = 0.001$) and high temperatures ($p = 0.001$) adversely affected the antioxidant activity.

AA% values for the natural pectin extracts based on the β -carotene/linoleic acid bleaching assay showed very good results compared to the artificial BHT positive control (79.21%), with values ranging between 4.42 and 45.86%. Interestingly, unlike for TPC and TEAC, extraction temperature also had a very significant impact ($p < 0.05$) on AA%. Higher AA% capacity was achieved when severe acid or temperature conditions were not combined (e.g. pH 1.5 and 95 °C or pH 0.5 and 70 °C; Fig. 1e). Commercial pectins (AP, CP) showed no detectable antioxidant activity, which is probably ascribed to the lower quantities of polyphenols in the raw cultivars (Wikiera, Grabacka, Byczyński, Stodolak, & Mika, 2021) and purification steps used at industrial scale contributing to eliminate proteins and polyphenols (Venkatanagaraju, Bharathi, Hema Sindhuja, Roy Chowdhury, & Sreelekha, 2020). Differences between results from both ABTS and β -carotene/linoleic acid bleaching methods are expected due to their very different approach of measuring antioxidant activity. Different compounds or conjugated polyphenols could, for example, not readily interact with ABTS and nevertheless prevent beta-carotene bleaching. In fact, some authors suggested that the antioxidant activity of pectin-polyphenolic complexes was significantly higher than that reported for free-polyphenols, based on a “protective effect” (Mercado-Mercado, de la Rosa, & Alvarez-Parrilla, 2020), which could be related to with the quantities reached of conjugated and esterified polyphenols of some of the functional pectins. The reduced solubility and different supramolecular conformation of pectin in the more hydrophobic β -carotene/linoleic acid bleaching method might also play a role in the differences found. The results point out the great potential to be used as bioactive ingredients due their relatively high TPC and antioxidant capacity. Although enriched-pectin extracts could have a high concentration of non-extractable polyphenols which probably affect some physico-chemical properties of the neat pectin extracts, the benefit associated to the non-extractable polyphenols in health-promoting properties (Yaqub et al., 2016), like gastrointestinal health and cardiovascular risk reduction, substantially expands the scope of potential applications.

3.2.6. Fourier transformed infrared analysis

Differences in the composition and molecular organization of the pectin enriched extracts derived from the different treatment aggressive could also be qualitatively examined by FTIR. Fig. 2 displays the spectra of two different enriched pectin extracts obtained at two different pH. The spectra of commercial AP and CP samples were also included for comparative purposes. The vibrational band located at 1722 cm^{-1} is usually assigned to esterified carbonyl groups C=O and the peak centered at 1612 cm^{-1} is related to free carboxylic groups COO⁻ (Edison & Sethuraman, 2013; Pasandide, Khodaiyan, Mousavi, & Hosseini, 2018), the ratio of which is usually used for DE measurement. The band at 1722 cm^{-1} was more intense in enriched pectin extracts from persimmon than in AP and CP samples, indicating a higher esterification degree. Furthermore, the vibrational band around 1438 cm^{-1} suggests the presence of aliphatic or aromatic (C-H) plane deformation vibrations of methyl, methylene and methoxy groups (Edison & Sethuraman, 2013; Grassino et al., 2016). The band observed at 1011 cm^{-1} is ascribed to glycosidic linkages (C-O) and is typical for backbone vibrations of pectin samples (Koštalova et al., 2013). In addition, the band at 1077 cm^{-1} could be attributed of neutral arabinose-based glycans, possibly from RG (Koštalova et al., 2013).

It is worth noting that when the extraction severity was higher, the intensity of the peak corresponding to glycosidic linkages (1011 cm^{-1}) increased, confirming higher pectin content at the expense of decreased phenolics. Although, the peak at 1722 cm^{-1} (esterified carboxyl group) was more prominent for the sample obtained at pH 1 and 95 °C, further lowering the pH to 0.5 produced the decrease of this specific band, along with a decrease in the intensity of the bands located at 1438 and 3362 cm^{-1} and associated to the presence of aromatic compounds and -OH groups, respectively (Bulut & Özacar, 2009; Edison & Sethuraman, 2013), which would confirm the partial degradation of pectin and

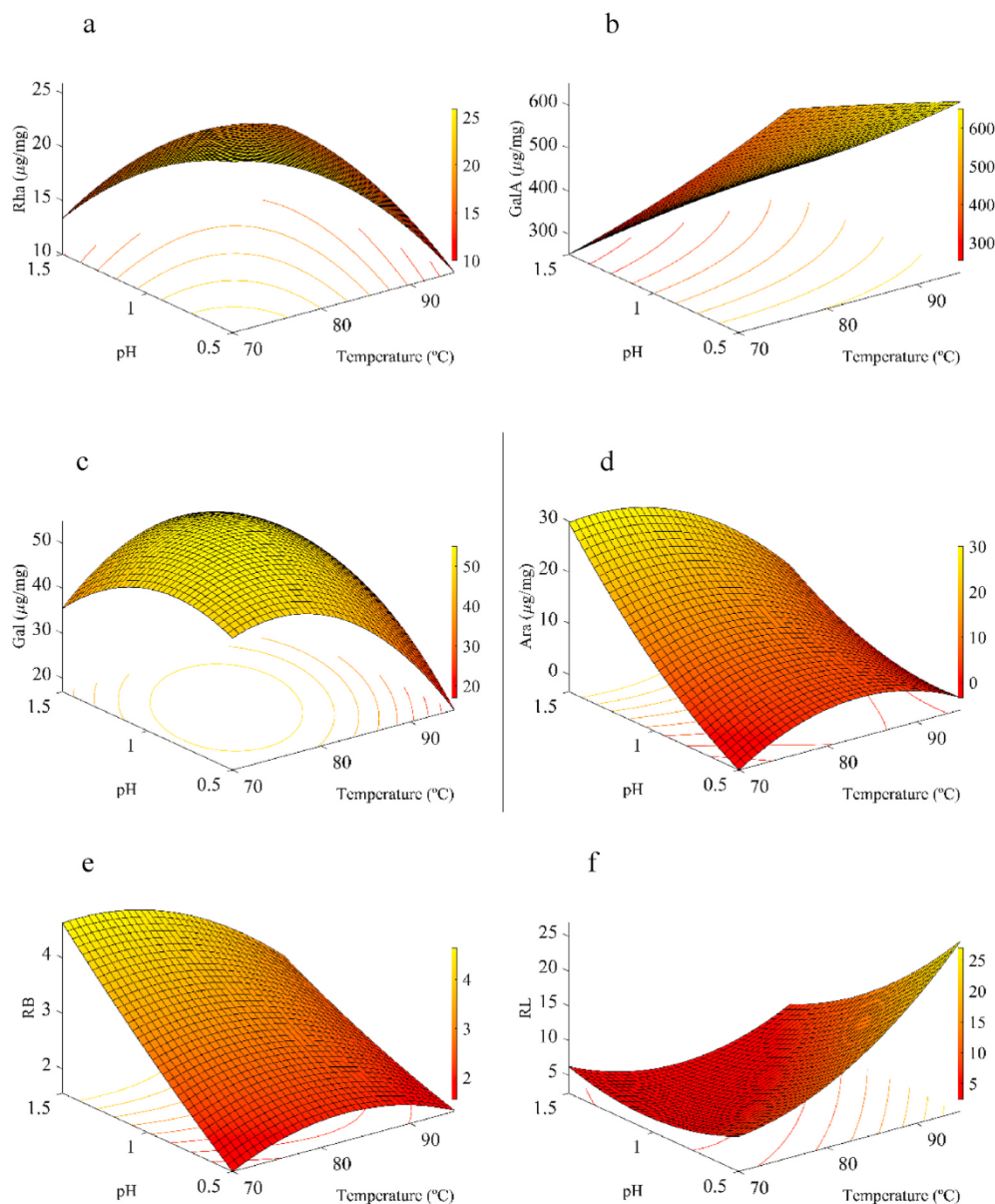


Fig. 3. Surface response for (a) GalA, (b) Rha, (c) Gal and (d) Ara content from persimmon pectin. (e) RL and (f) RB as ratios for linearity and branching respectively with temperature and pH as main factors.

polyphenols under the harshest acidic conditions.

4. Conclusions

The potential use of discarded persimmon fruit as a source for a functional pectin-polyphenol based ingredient was studied. Immature non-treated rejected fruits were selected due to their higher pectin and polyphenol content. A full factorial design could accurately model how extraction temperature and pH affected process efficiency, as well as the composition and functional properties of pectin. Persimmon pectin was comparable in terms of composition, purity and esterification degree but was found to be more acid resistant compared to reference pectin or other alternative pectin sources. The presence of strong pectin-polyphenol interactions also enabled a high acid resistance of the very abundant polyphenolic fraction, with gallic acid as the main constituent. Only extreme acid conditions (pH 0.5) were able to degrade both the pectin structure and polyphenolic profile, and only the combination of both low pH and temperature produced a decrease in antioxidant activity. Averagely severe conditions (pH 1) produced the highest pectin

and polyphenol yields, with abundant non-covalent interaction, while less severe conditions (pH 1.5) produced a pectin with intact pectin-polyphenol ester and O-glycosyl bonds (indirectly inferred by the released polyphenols after alkali or acid treatment). The very high TPC and antioxidant activity together with their acid resistance points out the great potential of persimmon waste as a potential source of functional food pectin based ingredients enhancing the quality and shelf-life of foods. The potential enhanced bioavailability of the pectin-polyphenol complexes and their health benefits is the subject of future investigations.

CRediT authorship contribution statement

D.A. Méndez: Methodology, Investigation, data acquisition and processing, Writing – original draft. **M.J. Fabra:** Conceptualization, Writing – review & editing. **I. Odriozola-Serrano:** Investigation, Writing – original draft. **O. Martín-Belloso:** Funding acquisition, Project administration, Resources. **L. Salvia-Trujillo:** data acquisition and processing, Writing – review & editing. **A. López-Rubio:** Methodology,

Funding acquisition, Project administration, Resources, Writing – review & editing. **A. Martínez-Abad:** Conceptualization, Supervision, Methodology, Writing - 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodhyd.2021.107066>.

References

- Adetunji, L. R., Adegkunle, A., Orsat, V., & Raghavan, V. (2017). Advances in the pectin production process using novel extraction techniques: A review. *Food Hydrocolloids*, 62, 239–250. <https://doi.org/10.1016/j.foodhyd.2016.08.015>
- Aina, V. O., Barau, M. M., Mamman, O. A., Zakari, A., Haruna, H., Umar, H., et al. (2012). Extraction and characterization of pectin from peels of lemon (*Citrus limon*), grape fruit (*Citrus paradisi*) and sweet orange (*Citrus sinensis*). *British Journal of Pharmacology and Toxicology*, 3(6), 259–262.
- Alba, K., Bingham, R. J., Gunning, P. A., Wilde, P. J., & Kontogiorgos, V. (2018). Pectin conformation in solution. *Journal of Physical Chemistry B*, 122(29), 7286–7294. <https://doi.org/10.1021/acs.jpbc.8b04790>. research-article.
- AOAC. (1996). *Official methods of analysis* (Maryland: AOAC International) 16th ed.
- Berbel, J., & Posadillo, A. (2018). Review and analysis of alternatives for the valorisation of agro-industrial olive oil by-products. *Sustainability*, 10(1), 237. <https://doi.org/10.3390/su10010237>
- Bermúdez-Oria, A., Rodríguez-Gutiérrez, G., Fernández-Prior, Á., Vioque, B., & Fernández-Bolaños, J. (2019). Strawberry dietary fiber functionalized with phenolic antioxidants from olives. Interactions between polysaccharides and phenolic compounds. *Food Chemistry*, 280, 310–320. <https://doi.org/10.1016/j.foodchem.2018.12.057>
- Bulut, E., & Özacar, M. (2009). Rapid, facile synthesis of silver nanostructure using hydrolyzable tannin. *Industrial & Engineering Chemistry Research*, 48(12), 5686–5690. <https://doi.org/10.1021/ie801779f>
- Butt, M. S., Sultan, M. T., Aziz, M., Naz, A., Ahmed, W., Kumar, N., et al. (2015). Persimmon (*Diospyros kaki*) fruit: Hidden phytochemicals and health claims. *EXCLI Journal*, 14, 542–561. <https://doi.org/10.17179/excli2015-159>
- Campbell, M. (2006). *Extraction of pectin from watermelon rind*. Oklahoma State University.
- Carocho, M., Morales, P., & Ferreira, I. C. F. R. (2018). Antioxidants: Reviewing the chemistry, food applications, legislation and role as preservatives. *Trends in Food Science & Technology*, 71, 107–120. <https://doi.org/10.1016/j.tifs.2017.11.008>
- Chen, J., Du, J., Ge, Z., Zhen, Zhu, W., Nie, R., et al. (2016). Comparison of sensory and compositions of five selected persimmon cultivars (*Diospyros kaki* L.) and correlations between chemical components and processing characteristics. *Journal of Food Science & Technology*, 53(3), 1597–1607. <https://doi.org/10.1007/s13197-015-2102-y>
- Chen, X. N., Fan, J. F., Yue, X., Wu, X. R., & Li, L. T. (2008). Radical scavenging activity and phenolic compounds in persimmon (*Diospyros kaki* L. cv. Mopan). *Journal of Food Science*, 73(1). <https://doi.org/10.1111/j.1750-3841.2007.00587.x>
- Denman, L. J., & Morris, G. A. (2015). An experimental design approach to the chemical characterisation of pectin polysaccharides extracted from *Cucumis melo* Inodorus. *Carbohydrate Polymers*, 117, 364–369. <https://doi.org/10.1016/j.carbpol.2014.09.081>
- Dranca, F., & Oroian, M. (2018). Extraction, purification and characterization of pectin from alternative sources with potential technological applications. *Food Research International*, 113(February), 327–350. <https://doi.org/10.1016/j.foodres.2018.06.065>
- Dvořáková, M., Guido, L. F., Dostál, P., Skulílová, Z., Moreira, M. M., & Barros, A. A. (2008). Antioxidant properties of free, soluble ester and insoluble-bound phenolic compounds in different barley varieties and corresponding malts. *Journal of the Institute of Brewing*, 114.
- Edison, T. J. I., & Sethuraman, M. G. (2013). Biogenic robust synthesis of silver nanoparticles using *Punica granatum* peel and its application as a green catalyst for the reduction of an anthropogenic pollutant 4-nitrophenol. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 104, 262–264. <https://doi.org/10.1016/j.saa.2012.11.084>
- FAO. (2016). Food wastage: Key facts and figures. Retrieved May 23, 2019, from <http://www.fao.org/news/story/pt/item/196402/icode/>
- FAOSTAT. (2021). Crops. Retrieved May 27, 2021, from <http://www.fao.org/faostat/en/#data/QC/visualize>
- Fontes-Candia, C., Erboz, E., Martínez-Abad, A., López-Rubio, A., & Martínez-Sanz, M. (2019). Superabsorbent food packaging bioactive cellulose-based aerogels from *Arundo donax* waste biomass. *Food Hydrocolloids*, 96, 151–160. <https://doi.org/10.1016/j.foodhyd.2019.05.011>
- Fujihara, S., Kasuga, A., & Aoyagi, Y. (2001). Nitrogen-to-Protein conversion factors for common vegetables in Japan. *Journal of Food Science*, 66(3), 412–415. <https://doi.org/10.1111/j.1365-2621.2001.tb16119.x>
- Fu, L., Lu, W. Q., & Zhou, X. M. (2016). Phenolic compounds and in vitro antibacterial and antioxidant activities of three tropic fruits: Persimmon, guava, and sweetsop. *BioMed Research International*, 2016. <https://doi.org/10.1155/2016/4287461>
- Grassino, A. N., Halambek, J., Djaković, S., Rimac Brčić, S., Dent, M., & Grabarić, Z. (2016). Utilization of tomato peel waste from canning factory as a potential source for pectin production and application as tin corrosion inhibitor. *Food Hydrocolloids*, 52, 265–274. <https://doi.org/10.1016/j.foodhyd.2015.06.020>
- Guyot, S., Cheynier, V., Souquet, J.-M., & Moutounet, M. (1995). Influence of pH on the enzymatic oxidation of (+)-Catechin in model systems. *Journal of Agricultural and Food Chemistry*, 43(9), 2458–2462. <https://doi.org/10.1021/jf00057a027>
- Hamauz, Y. (2018). Non-extractable polyphenols in fruit: Distribution, changes, and potential health effects. In , Vol. 2018. *Food chemistry, function and analysis* (pp. 284–306). Royal Society of Chemistry. <https://doi.org/10.1039/9781788013208-00284>
- Hamauz, Y., & Suwannachot, J. (2019). Non-extractable polyphenols and in vitro bile acid-binding capacity of dried persimmon (*Diospyros kaki*) fruit. *Food Chemistry*, 293, 127–133. <https://doi.org/10.1016/j.foodchem.2019.04.092>
- Han, Y., Zhu, Q., Zhang, Z., Meng, K., Hou, Y., Ban, Q., ... Rao, J. (2015). Analysis of xyloglucan endotransglycosylase/hydrolase (XTH) genes and diverse roles of isoenzymes during persimmon fruit development and postharvest softening. <https://doi.org/10.1371/journal.pone.0123668>
- Hosseini, S., Parastouei, K., & Khodaiyan, F. (2020). Simultaneous extraction optimization and characterization of pectin and phenolics from sour cherry pomace. *International Journal of Biological Macromolecules*, 158, 911–921. <https://doi.org/10.1016/j.ijbiomac.2020.04.241>
- Houben, K., Jolie, R. P., Fraeye, I., Loey, Van, & Hendrickx, M. E. (2011). Comparative study of the cell wall composition of broccoli, carrot, and tomato: Structural characterization of the extractable pectins and hemicelluloses. *Carbohydrate Research*, 346(9), 1105–1111. <https://doi.org/10.1016/j.carres.2011.04.014>
- Hu, S., Yin, J., Nie, S., Wang, J., Phillips, G. O., Xie, M., et al. (2016). In vitro evaluation of the antioxidant activities of carbohydrates. *Bioactive Carbohydrates and Dietary Fibre*, 7(2), 19–27. <https://doi.org/10.1016/j.bcdf.2016.04.001>
- Jafari, F., Khodaiyan, F., Kiani, H., & Hosseini, S. S. (2017). Pectin from carrot pomace: Optimization of extraction and physicochemical properties. *Carbohydrate Polymers*, 157, 1315–1322. <https://doi.org/10.1016/j.carbpol.2016.11.013>
- Jiang, Y., Xu, Y., Li, F., Li, D., & Huang, Q. (2020). Pectin extracted from persimmon peel: A physicochemical characterization and emulsifying properties evaluation. *Food Hydrocolloids*, 101. <https://doi.org/10.1016/j.foodhyd.2019.105561>, 105561.
- Koštalova, Z., Hromádková, Z., Ebringerová, A., Polovka, M., Michaelsen, T. E., & Paulsen, B. S. (2013). Polysaccharides from the Styrian oil-pumpkin with antioxidant and complement-fixing activity. *Industrial Crops and Products*, 41(1), 127–133. <https://doi.org/10.1016/j.indcrop.2012.04.029>
- Krygier, K., Sosulski, F., & Hogge, L. (1982). Free, esterified, and insoluble-bound phenolic acids. 1. Extraction and purification procedure. *Journal of Agricultural and Food Chemistry*, 30(2), 330–334. <https://doi.org/10.1021/jf00110a028>
- Kulkarni, S. G., & Vijayanand, P. (2010). Effect of extraction conditions on the quality characteristics of pectin from passion fruit peel (*Passiflora edulis* f. *flavicarpa* L.). *LWT - Food Science and Technology*, 43(7), 1026–1031. <https://doi.org/10.1016/j.lwt.2009.11.006>
- Lara-Espinoza, C., Carvajal-Millán, E., Balandrán-Quintana, R., López-Franco, Y., & Rascón-Chu, A. (2018). Pectin and pectin-based composite materials: Beyond food texture. *Molecules*. <https://doi.org/10.3390/molecules23040942>. MDPI AG.
- Li, N., Taylor, L. S., Ferruzzi, M. G., & Mauer, L. J. (2012). Kinetic study of catechin stability: Effects of pH, concentration, and temperature. *Journal of Agricultural and Food Chemistry*, 60(51), 12531–12539. <https://doi.org/10.1021/jf304116s>
- Lucas-González, R., Viuda-Martos, M., Pérez-Álvarez, J. Á., & Fernández-López, J. (2017). Evaluation of Particle Size Influence on Proximate Composition, Physicochemical, Techno-Functional and Physio-Functional Properties of Flours Obtained from Persimmon (*Diospyros kaki* Trumb.) Coproducts. *Plant Foods for Human Nutrition*, 72(1). <https://doi.org/10.1007/s1130-016-0592-z>, 67–63.
- Mamet, T., Ge, Z., Zhen, Zhang, Y., & Li, C. mei (2018). Interactions between highly galloylated persimmon tannins and pectins. *International Journal of Biological Macromolecules*, 106(2018), 410–417. <https://doi.org/10.1016/j.ijbiomac.2017.08.039>
- Martínez-Abad, A., Giummarella, N., Lawoko, M., & Vilaplana, F. (2018). Differences in extractability under subcritical water reveal interconnected hemicellulose and lignin recalcitrance in birch hardwoods. *Green Chemistry*, 20(11), 2534–2546. <https://doi.org/10.1039/c8gc00385h>
- Martínez-Las Heras, R., Amigo-Sánchez, J. C., Heredia, A., Castelló, M. L., & Andrés, A. (2016). Influence of preharvest treatments to reduce the seasonality of persimmon production on color, texture and antioxidant properties during storage. *CYTA - Journal of Food*, 14(2), 333–339. <https://doi.org/10.1080/19476337.2015.1113204>

- Mattila, P., & Kumpulainen, J. (2002). Determination of free and total phenolic acids in plant-derived foods by HPLC with diode-array detection. *Journal of Agricultural and Food Chemistry*, 50(13), 3660–3667. <https://doi.org/10.1021/jf020028p>
- Méndez, D. A., Fabra, M. J., Gómez-Mascaraque, L., López-Rubio, A., & Martínez-Abad, A. (2021). Modelling the extraction of pectin towards the valorisation of watermelon rind waste. *Foods*, 10(4), 738. <https://doi.org/10.3390/foods10040738>
- Mercado-Mercado, G., de la Rosa, L. A., & Alvarez-Parrilla, E. (2020). Effect of pectin on the interactions among phenolic compounds determined by antioxidant capacity. *Journal of Molecular Structure*, 1199. <https://doi.org/10.1016/j.molstruc.2019.126967>, 126967.
- Morris, G. A., Ralet, M. C., Bonnini, E., Thibault, J. F., & Harding, S. E. (2010). Physical characterisation of the rhamnogalacturonan and homogalacturonan fractions of sugar beet (*Beta vulgaris*) pectin. *Carbohydrate Polymers*, 82(4), 1161–1167. <https://doi.org/10.1016/j.carbpol.2010.06.049>
- Müller-Maatsch, J., Bencivenni, M., Caligiani, A., Tedeschi, T., Bruggeman, G., Bosch, M., ... Sforza, S. (2016). Pectin content and composition from different food waste streams. *Food Chemistry*, 201, 37–45. <https://doi.org/10.1016/j.foodchem.2016.01.012>
- Munera, S., Aleixos, N., Besada, C., Gómez-Sanchis, J., Salvador, A., Cubero, S., ... Blasco, J. (2019). Discrimination of astringent and deastringed hard 'Rojo Brillante' persimmon fruit using a sensory threshold by means of hyperspectral imaging. *Journal of Food Engineering*, 263, 173–180. <https://doi.org/10.1016/j.jfoodeng.2019.06.008>
- Nguyễn, H. V., & Savage, G. P. (2013). The effects of temperature and pH on the extraction of oxalate and pectin from green kiwifruit (*Actinidia deliciosa* L.), golden kiwifruit (*Actinidia chinensis* L.), kiwiberry (*Actinidia arguta*) and persimmon (*Diospyros kaki*). *International Journal of Food Science and Technology*, 48(4), 794–800. <https://doi.org/10.1111/jifs.12029>
- Novillo, P., Salvador, A., Crisosto, C., & Besada, C. (2016). Influence of persimmon astringency type on physico-chemical changes from the green stage to commercial harvest. *Scientia Horticulturae*, 206, 7–14. <https://doi.org/10.1016/j.scienta.2016.04.030>
- Ognyanov, M., & Kussovski, V. (2013). Isolation, characterization and modification of citrus pectins Isolation. *J. BioSci. Biotech*, 1(October), 223–233. Retrieved from <http://www.jbb.uni-plovdiv.bg>
- Pasandide, B., Khodaiyan, F., Mousavi, Z., & Hosseini, S. S. (2018). Pectin extraction from citron peel: Optimization by box-behnken response surface design. *Food Science and Biotechnology*, 27(4), 997–1005. <https://doi.org/10.1007/s10068-018-0365-6>
- Pellegrini, M., Lucas-Gonzalez, R., Fernández-López, J., Ricci, A., Pérez-Álvarez, J. A., Sterzo, C., et al. (2017). Bioaccessibility of polyphenolic compounds of six quinoa seeds during in vitro gastrointestinal digestion. *Journal of Functional Foods*, 38, 77–88. <https://doi.org/10.1016/j.jff.2017.08.042>
- Pérez-Burillo, S., Oliveras, M. J., Quesada, J., & Rufán-Henares, J. A. (2018, March 1). Relationship between composition and bioactivity of persimmon and kiwifruit. *Food Research International*. Elsevier Ltd.. <https://doi.org/10.1016/j.foodres.2017.11.022>
- Petkowicz, C. L. O., Vriesmann, L. C., & Williams, P. A. (2017). Pectins from food waste: Extraction, characterization and properties of watermelon rind pectin. *Food Hydrocolloids*, 65, 57–67. <https://doi.org/10.1016/j.foodhyd.2016.10.040>
- Pettolino, F. A., Walsh, C., Fincher, G. B., & Bacic, A. (2012). Determining the polysaccharide composition of plant cell walls. *Nature Protocols*, 7(9), 1590–1607. <https://doi.org/10.1038/nprot.2012.081>
- Phytochemicals, H., Claims, H., Butt, M. S., Sultan, T., Aziz, M., Naz, A., ... Claims, H. (2015). Review article :: Persimmon (DIOSPYROS kaki) FRUIT. *EXCLI Journal*, 14, 542–561. <https://doi.org/10.17179/excli2015-159>
- Posé, S., Paniagua, C., Matas, A. J., Gunning, A. P., Morris, V. J., Quesada, M. A., et al. (2019). A nanostructural view of the cell wall disassembly process during fruit ripening and postharvest storage by atomic force microscopy. *Trends in Food Science & Technology*, 87(August 2017), 47–58. <https://doi.org/10.1016/j.tifs.2018.02.011>
- Prakash Maran, J., Sivakumar, V., Thirugnanasambandham, K., & Sridhar, R. (2014). Microwave assisted extraction of pectin from waste Citrullus lanatus fruit rinds. *Carbohydrate Polymers*, 101(1), 786–791. <https://doi.org/10.1016/j.carbpol.2013.09.062>
- Prasanna, V., Prabha, T. N., & Tharanathan, R. N. (2007). Fruit ripening phenomena-an overview. *Critical Reviews in Food Science and Nutrition*, 47(1), 1–19. <https://doi.org/10.1080/10408390600976841>
- Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, 53(10), 4290–4302. <https://doi.org/10.1021/jf0502698>
- Raji, Z., Khodaiyan, F., Rezaei, K., Kiani, H., & Hosseini, S. S. (2017). Extraction optimization and physicochemical properties of pectin from melon peel. *International Journal of Biological Macromolecules*, 98, 709–716. <https://doi.org/10.1016/j.ijbiomac.2017.01.146>
- Re, R., Pellegrini, N., Progettente, a., Pannala, a., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved abts radical cation decolorization assay. *Free Radical Biology and Medicine*, 26(9), 1231–1237. [https://doi.org/10.1016/S0891-5849\(98\)00315-3](https://doi.org/10.1016/S0891-5849(98)00315-3)
- Rodsamran, P., & Sothornvit, R. (2019a). Microwave heating extraction of pectin from lime peel: Characterization and properties compared with the conventional heating method. *Food Chemistry*, 278(November 2018), 364–372. <https://doi.org/10.1016/j.foodchem.2018.11.067>
- Rodsamran, P., & Sothornvit, R. (2019b). Preparation and characterization of pectin fraction from pineapple peel as a natural plasticizer and material for biopolymer film. *Food and Bioproducts Processing*, 118, 198–206. <https://doi.org/10.1016/j.fbp.2019.09.010>
- Russell, W. R., Labat, A., Scobbie, L., Duncan, G. J., & Duthie, G. G. (2009). Phenolic acid content of fruits commonly consumed and locally produced in Scotland. *Food Chemistry*, 115(1), 100–104. <https://doi.org/10.1016/j.foodchem.2008.11.086>
- Sanchez-Aldana, D., Aguilar, C. N., Nevarez-Moorillon, G. V., & Esquivel, J. C. C. (2013). Comparative extraction of pectic and polyphenols from Mexican lime pomace and bagasse. *American Journal of Agricultural and Biological Sciences*, 8(4), 309–322. <https://doi.org/10.3844/ajabssp.2013.309.322>
- Saura-Calixto, F., & Pérez-Jiménez, J. (2018). Non-extractable polyphenols and carotenoids. *The royal society of chemistry*. <https://doi.org/10.1039/9781788013208>, 2018.
- Senica, M., Verberic, R., Grabnar, J. J., Stampar, F., & Jakopic, J. (2016). Selected chemical compounds in firm and mellow persimmon fruit before and after the drying process. *Journal of the Science of Food and Agriculture*, 96(9), 3140–3147. <https://doi.org/10.1002/jsfa.7492>
- Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology*, 299, 152–178. [https://doi.org/10.1016/S0076-6879\(99\)99017-1](https://doi.org/10.1016/S0076-6879(99)99017-1)
- Somashekar, D., Venkateshwaran, G., Srividya, C., Krishnanand, Sambaiah, K., & Lokesh, B. R. (2001). Efficacy of extraction methods for lipid and fatty acid composition from fungal cultures. *World Journal of Microbiology and Biotechnology*, 17(3), 317–320. <https://doi.org/10.1023/A:1016792311744>
- Sun, D., Chen, X., & Zhu, C. (2020). Physicochemical properties and antioxidant activity of pectin from hawthorn wine pomace: A comparison of different extraction methods. *International Journal of Biological Macromolecules*, 158, 1239–1247. <https://doi.org/10.1016/j.ijbiomac.2020.05.052>
- Taira, S., Ono, M., & Matsumoto, N. (1997). Reduction of persimmon astringency by complex formation between pectin and tannins. *Postharvest Biology and Technology*, 12(3), 265–271. [https://doi.org/10.1016/S0925-5214\(97\)00064-1](https://doi.org/10.1016/S0925-5214(97)00064-1)
- Tsao, R. (2010). Chemistry and biochemistry of dietary polyphenols. *Nutrients*, 2(12), 1231–1246. <https://doi.org/10.3390/nu2121231>
- Tsuchida, Y., Sakurai, N., Morinaga, K., Koshita, Y., & Asakura, T. (2004). Effects of water loss of "Fuyu" persimmon fruit on molecular weights of mesocarp cell wall polysaccharides and fruit softening. *Journal of the Japanese Society for Horticultural Science*, 73(5), 460–468. <https://doi.org/10.2503/jjshs.73.460>
- Venkatanagaraju, E., Bharathi, N., Hema Sindhuja, R., Roy Chowdhury, R., & Sreelekha, Y. (2020). Extraction and purification of pectin from agro-industrial wastes. In pectins - extraction, purification, characterization and applications. IntechOpen. <https://doi.org/10.5772/intechopen.85585>
- Virk, B. S., & Sogi, D. S. (2004). Extraction and characterization of pectin from apple (*Malus Pumila*. Cv Amri) peel waste. *International Journal of Food Properties*, 7(3), 683–703. <https://doi.org/10.1081/JFP-200033095>
- Wai, W. W., Alkarkhi, A. F. M., & Easa, A. M. (2010). Effect of extraction conditions on yield and degree of esterification of durian rind pectin: An experimental design. *Food and Bioproducts Processing*, 88(2–3), 209–214. <https://doi.org/10.1016/j.fbp.2010.01.010>
- Wikiera, A., Grabacka, M., Byczyński, L., Stodolak, B., & Mika, M. (2021). Enzymatically extracted apple pectin possesses antioxidant and antitumor activity. *Molecules (basel, Switzerland)*, 26(5). <https://doi.org/10.3390/molecules26051434>
- Yang, J. S., Mu, T. H., & Ma, M. M. (2018). Extraction, structure, and emulsifying properties of pectin from potato pulp. *Food Chemistry*, 244(October 2017), 197–205. <https://doi.org/10.1016/j.foodchem.2017.10.059>
- Yaqub, S., Farooq, U., Shafi, A., Akram, K., Murtaza, A., Kausar, T., et al. (2016). Chemistry and functionality of bioactive compounds present in persimmon. <https://doi.org/10.1155/2016/3424025>
- Yuste, S., Macià, A., Ludwig, I. A., Romero, M.-P., Fernández-Castillejo, S., Catalán, Ú., ... Rubiò, L. (2018). Validation of dried blood spot cards to determine apple phenolic metabolites in human blood and plasma after an acute intake of red-fleshed apple snack. *Molecular Nutrition & Food Research*, 62(23). <https://doi.org/10.1002/mnfr.201800623>
- Zhang, C., & Mu, T. (2011). Optimisation of pectin extraction from sweet potato (*Ipomoea batatas*, Convolvulaceae) residues with disodium phosphate solution by response surface method. *International Journal of Food Science and Technology*, 46(11), 2274–2280. <https://doi.org/10.1111/j.1365-2621.2011.02746.x>