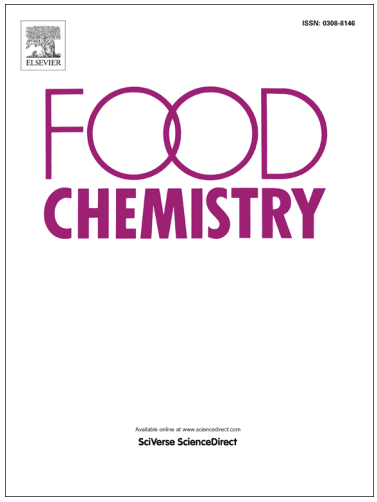

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Antioxidant pectin enriched fractions obtained from discarded carrots (*Daucus carota* L.) by ultrasound-enzyme assisted extraction

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Running title: Ultrasound-enzyme extraction of antioxidant pectins from carrots

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ABSTRACT

Carrot residues were upgraded as pectin-enriched fractions (PEFs) useful for functional food formulation due to co-extracted antioxidants (α- and β-carotenes, lutein, α-tocopherol), and gelling effect. High power ultrasound (US)-enzyme assisted extraction was applied for efficiency and sustainability. Carrot powder (CP) in citrate-buffer (pH 5.20) was submitted to US-pretreatment (12.27 W/cm^2: 20 kHz, 80% amplitude, 20 min) and a subsequent digestion (5h-40ºC) without or with hemicellulase or cellulase. US-hemicellulase led to the highest PEF yield (27.1%), and extracted almost the whole pectin content of CP. US-pretreatment increased the extraction yield of all PEFs, but the existence of an additional positive effect of the following step depended on the enzyme used. PEFs contained 40-47% of UA with low DM (24-49.9%), and co-extracted antioxidants. US decreased the antioxidant contents, DM, and molecular weight, but allowed obtaining calcium crosslinked true gels, also with higher elastic modulus than non-US-extracted PEFs, being promising as food additives.

Keywords: carrot residues, antioxidant pectin enriched fractions, ultrasound-enzyme extraction, cellulase, hemicellulase, carotenoids, gelling.

Chemical compounds studied in this article

α-carotene (PubChem CID: 4369188); α-tocopherol (PubChem CID: 14985); β-carotene (PubChem CID: 5280489); lutein (PubChem CID: 5281243); pectin (PubChem CID: 441476).

1. Introduction

Carrot (*Daucus carota* L.) is a popular vegetable grown worldwide. Its consumption has spread widely and it is available in the markets throughout the year. The consumed part of the carrot is the root of which there are multiple forms and flavors. It stands out for its content in carotene, and is rich in dietary fiber (Idrovo Encalada, Basanta, Fissore, De´Nobili, & Rojas, 2016). Carrot annual production in the world is estimated in 36 millon tons and the main producing country is China with a participation superior to 45%, followed by Russia (4.9%), USA (3.7%), Uzbekistan (3.4%), Poland (2.5%), Ukraine (2.4%), and the United Kingdom (2%). The remaining 36% is distributed among more than 100 countries (FAO, 2016). Although Argentina is not among the main world producers of carrots, it produces significant quantities (240,000 Ton/year) because it is locally considered a vegetable commonly consumed (6 kg per capita per year) in salads, cooked meals and juices, both domestically and industrially (Agro empresario, 2019). In Argentina, carrots are grown in 7.000 to 9.900 hectares, supplying the domestic market and with a surplus that is exported. Mendoza is the main producing province of the country. A small proportion of the production goes to the food industry. After industrial processing, carrot residues such as peels and pomace are usually discarded or used as animal feed (Gaviola, 2013). In the case of carrots destined to fresh consumption, they are graded according to their size, diameter, length, shape. Also carrots that are not bright orange, have a blemish or are broken, are swept off for aesthetic defects. As an example, supermarkets in the United Kingdom insist that all carrots should be straight to facilitate the peeling in the full length by the customers. Therefore, 25-30% of all carrots are out-graded in the farms after being checked by photographic sensor machines searching for defects (Stuart, 2009). Carrot by-products are rich in beneficial substances, especially bioactive compounds with antioxidant activities (Chantaro, Devahastin, & Chiewchan, 2008).

Pectin is a major component of the cell walls of all land plants. Considering that three or four fresh fruits are ingested per day, around 2.0-3.5 g of pectins are then consumed each day in a normal western diet (Dreher, 2018). They are soluble dietary fibers intensively studied due to their properties and complex macromolecular structure. Pectic substances mainly consist of D-galacturonic acid units (GalA) and often contain significant amounts of L-rhamnose, L-arabinose and D-galactose, as well as other 13 different monosaccharides (Pérez, Rodríguez-Carvajal, & Doco, 2003; Vincken et al., 2003). The main pectic polysaccharides are the homogalacturonan (HG), rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II) (Mazzeau & Pérez, 1998). The composition, structure, and physiological properties of pectins are influenced by the conditions of extraction, sources and environmental factors (Fissore et al., 2014). Extracted pectin is widely used as functional food ingredient, being listed as E440 in the EU code. Technological properties such as the ability to thicken and gel allow pectins to be widely used in the food industry. As reported by Ciriminna, Fidalgo, Delisi, Ilharco, & Pagliaro (2016), the pectin market has been growing since 2005 because pectins are replacing other hydrocolloids. In 2015 the average price of pectin exceeded 15 U$D per kilogram and the market, exceeding 60,000 tons, was close to reach U$D 1 billion. Reputed market analysts predict that pectin market will double by 2020, with sales forecasted to exceed U$D 2.4 billion. The traditional usage as stabilizer is being complemented by the emerging utilization of pectin as a fat replacer and health-promoting functional ingredient because it is soluble dietary fiber and prebiotic. The European Food Safety Authority (EFSA) has recognized in 2010 the scientific validity of nutrition and health claims regarding pectin as a nutritional supplement in the reduction of the post-prandial glycemic response, maintenance of normal blood cholesterol levels and the increases in satiety, leading to a reduction in energy intake (Ciriminna et al., 2016). Pectin is a major fruit prebiotic that has been extensively studied and shown to promote a healthy, anti-inflammatory colonic microbiota ecosystem with greater microflora diversity than inulin (Dreher, 2018).

As an environmental friendly alternative to the industrial extraction process, which uses mineral acids at low pH and high temperatures, pectic polysaccharides can be released using cell wall degrading enzymes such as cellulases and hemicellulases, at lower temperatures. Enzyme assisted extraction (EAE) has been widely used for cell wall disintegration, leading to improve the release of target molecules (Fissore et al., 2014; Zhu et al., 2016). The use of ultrasound energy can also facilitate the extraction of plant compounds due to cell disruption caused by cavitation (Zhu et al., 2016).

PEFs were previously extracted from pumpkin (Fissore, Ponce, Stortz, Rojas, & Gerschenson, 2007) and red beet (Fissore et al., 2011) powders through EAE with hemicellulase from *Aspergillus niger* or cellulase from *Trichoderma viride* by stirring in citrate buffer (pH 5.20) for 20 h at 30ºC. In these conditions, PEFs were obtained with lower yields that varied between 3.0 and 7.3% from pumpkin powder and 0.1 and 1.9% from red beet powder. The effect of cellulase was in general more effective but depended on the vegetable source.

The objective of this work was to develop an environmental friendly pectin extraction method to achieve higher yields, consisting of a combination of an US-pretreatment and an enzyme assisted extraction of PEFs from the cell wall enriched powder by which carrots discarded due to size and shape out of standards were stabilized as a powder (CP). Pectin obtained from carrots could be co-extracted with carotenoids and other natural antioxidants, which would add value to the additive for functional food formulation. Chemical composition including the antioxidants' contents, and functional properties of the PEFs such as the rheological performance were subsequently determined in order to evaluate their utility as food additives or ingredients, adding value to the vegetable raw materials.

2. Materials and methods

2.1. Chemicals

Chemicals were of analytical grade, provided by Sigma-Aldrich (Saint Louis, USA) and Merck Química (Argentina's branch). Hemicellulase (H2125) from *Aspergillus niger and c*ellulase (C9422) from *Trichoderma viride*, as well as α-carotene, β-carotene, lutein, α-, β- and γ-tocopherols, retinol, bovine serum albumin, and D-galacturonic acid standards were from Sigma-Aldrich. Deionized water (Milli-Q™, USA) was used.

2.2. Sample preparation - Carrot powder (CP)

Carrot roots (*Daucus carota* L. var. Nantes) harvested in Uco Valley (Mendoza province, Argentina), discarded after harvesting because of their irregular shapes and/or sizes were used in this study. After washing the roots and removing the ends, carrots were peeled, sliced and reduced to 1-2 mm particles (Moulinex FR6001, 700W, Argentina). The processed carrot was suspended in deionized water (1:4 w/v) at room temperature, stirred at 600 rpm and filtered. The solid residue was recovered and the aqueous phase was discarded. The washing process was repeated other three times. The solid residue was finally stirred in deionized water at 90ºC for 5 min for blanching, decanted, dehydrated by lyophilization (Christ, Germany; Pfeiffer vacuum pump, Germany), and reduced to a powder with a cutting mill (Wemir, Argentina). The CP obtained was sealed under vacuum (Multivac C-200, Germany) into small Cryovac bags (Sealed Air, USA), and stored in darkness at −18ºC until usage.

For characterization, a given weight of CP was sieved through a vibratory sieve shaker (Retsch, Germany) according to Idrovo Encalada et al. (2016), and the yield of powder corresponding to each average particle size was calculated.

Swelling capacity (SC) of the CP was determined as explained by Basanta, de Escalada Pla, Stortz, and Rojas (2013). SC was calculated as:

$$SC\left(mL/gCP\right) = \frac{(V_{swelled})}{m_{CP}}$$

where $V_{swelled}$ is the volume occupied by the swelled CP sample, and m_{CP} is the dry CP sample weight.

2.3. Scanning electron microscopy (SEM)

After 20 min of magnetic stirring or 20 min of US-treatment of a CP dispersion in water (1g:100 mL), each suspension was filtrated, the separated residue was then frozen with liquid nitrogen and lyophilized (Christ, Germany; Pfeiffer vacuum pump, Germany). For morphological characterization, samples of each treated powder were mounted on the stationary support, then gold-coated in a chamber under high vacuum, and afterwards placed into the FEI Quanta 250 FEG Scanning Electron Microscope (Thermo Fischer Scientific, USA) chamber. Images were acquired under high vacuum, at an accelerating voltage of 3kV and using an ETD detector for secondary electrons.

2.4. Extraction of pectin enriched fractions (PEFs)

CP (particle size ≤ 840μm) was subjected to the extraction of the PEFs with different methods: a) stirring in citrate buffer either without (control system) or with an enzyme (hemicellulase or cellulase); b) US pretreatment followed by one of the treatments mentioned in the step (a).

2.4.1. Buffer citrate- or enzyme-assisted extraction

The enzyme extraction of the PEFs from CP was performed using hemicellulase with side cellulase activity as informed by the supplier (Activity: One unit will produce a relative fluidity change of 1 per 5 minutes using locust bean gum as substrate at pH 4.5 at 40°C), or cellulase (Activity: One unit will liberate 1.0 micromole of glucose from cellulose in one hour at pH 5.0 and 37°C). The conditions for enzymatic extraction were performed in general according to Fissore et al. (2007).

The process was carried out by stirring a dispersion of 5.00 g of CP in 500 mL of 0.5 M sodium citrate buffer (pH 5.20) for 5 h at 40ºC with no enzyme added (control system). For systems with enzyme addition, an aliquot of hemicellulase (0,125 g/g CP) or cellulase (0,025 g/g CP) was added to the respective dispersion of CP in the citrate buffer (5.00g:500mL) and continuously stirred for 5 h at 40ºC. After buffer (control system) or buffer/enzymatic treatment, each suspension was filtered, the supernatant recovered in a glass beaker and then precipitated by addition of 96% v/v ethanol (1:2 v/v). It was left overnight at 8ºC, and the PEF was recovered by filtration under vacuum. Finally, the PEF was lyophilized, sealed under vacuum into Cryovac bags and stored in darkness at -18 °C.

2.4.2. Ultrasound-pretreatment

It was performed using an ultrasonic processor (Vibracell®, net power output: 750 Watt, Sonics Materials Inc, USA) which operates at 20 kHz of constant frequency. An 80% of wave amplitude was selected with pulses (5 seconds on - 5 seconds off). A flat tip 13-mm diameter titanium probe was used. CP (5.00 g) was mixed with 500 mL of 0.5 M sodium citrate buffer (pH 5.20). A 90-mm internal diameter and 125-mm height glass beaker (Borosilicate glass, IVA, Argentina) was used to contain the dispersion. The liquid contained into the glass beaker reached a height of 90 mm while the ultrasound probe was 20-mm immersed. The suspension was sonicated for 20 min. During the ultrasonic treatment, the temperature was measured with a thermocouple attached to the US processor as a function of processing time. The energy and power displayed by the equipment were recorded. The suspension was then left under magnetic stirring for 5 h at 40ºC according to the complete procedure explained in **section 2.4.1**. The extraction yield of each PEF was calculated on CP basis. Experiments were carried out in triplicate.

To calculate the true energy and power provided by the equipment, the temperature was recorded as explained above but in a separated experiment where the whole system was

maintained isolated, in a calorimetric assay (Mamvura, Iyuke, & Paterson, 2018). Since the ultrasonic radiation of a liquid produces heat, recording the temperature as a function of time into an isolated system leads to the energy (*E*) estimation (in J) by the equation:

$$E = m \cdot c_P \cdot dT \quad (1)$$

as well as to the power (*P*) calculation (in W):

$$P = m \cdot c_P \cdot dT/dt \quad (2)$$

where *m* is the total sonicated mass (g), c_P is the heat capacity of water ($J \cdot g^{-1} \cdot C^{-1}$), *T* is the temperature (C) recorded at time *t* (s) of effective sonication, and *dT*/*dt* is the rate of temperature change. The assay was performed in triplicate.

Power intensity and power density were respectively indicated in W per unit area of the emitting surface or probe (W/cm^2), and W per unit volume of the whole sonicated sample (W/cm^3). Efficiency was calculated as the ratio between the calculated power and the power displayed by the ultrasonic equipment.

2.5. Color

Lightness (*L**=0% black, 100% white), *a** (-*a** greenness; +*a** redness) and *b** (-*b** blueness; +*b** yellowness) color parameters of the CIELab space were measured for CP and each PEF with a colorimeter (Minolta CM-600D, Tokyo, Japan), as explained by Idrovo Encalada et al. (2016).

2.6. Fast Fourier Transformed Infrared Spectroscopy (FTIR)

Transmission spectra were recorded according to Idrovo Encalada et al. (2016) from KBr pellets of CP and PEFs extracted (1% w/w), using a Nicolet 8700 (Thermo Scientific Nicolet, MA, USA) spectrometer.

2.7. Molecular weights by gel permeation chromatography (GPC)

The molecular weight (Mw) of the PEFs was estimated using GPC as explained by Santo Domingo, Rojas, Fissore and Gerschenson (2019). Aliquots of each PEF were dissolved in 0.1 M $NaNO_3$ aqueous solution (0.25% w/w). Dextrans (PSS kit, Waters, Germany) of 5,200-668,000 Da of Mw range were used as standards for calibration. Runs were carried out in triplicate.

2.8. Chemical analyses of CP and PEFs

All chemical analyses were performed in triplicate. Protein, total carbohydrates' and UA contents, DM (%, molar) and degree of acetylation (DA, % molar) were determined on the PEFs extracted according to the methods reported by Fissore et al. (2007). The NS content was calculated as the arithmetical difference between the total carbohydrates' (polysaccharides) and the UA contents. The NS/UA is the ratio between the moles of NS and moles of UA.

The same chemical determinations were also performed on the CP. In addition, reducing carbohydrates were determined through the Somogyi-Nelson method using D-glucose as standard. Cellulose, lignin and non-cellulosic total carbohydrates-content were determined as explained by Basanta et al. (2013).

Antioxidants' (carotenoids, xanthophylls, tocopherols, retinol) contents were determined according to the methodology described by Rossetti et al. (2010). Briefly, it includes a first step of saponification for 30 min at 70ºC with 12 N KOH, and a quantification through high performance liquid chromatography (HPLC) using a C18 column (Alltima 250 x 4.6 mm, 5 µm particle size; Alltech, USA). Calibration curves were performed with the corresponding external standards freshly prepared in absolute ethanol.

2.9. Oscillatory assays

Oscillatory shear rheometry was used to obtain the mechanical spectra at 20.0ºC of each PEF, in triplicate. Each PEF was dissolved by heating (70ºC) in deionized water (2.00% w/v) and enough amount of a $CaCl_2$ solution was added (30mg Ca^{2+}/gUA). The storage or elastic (*G*') and loss or viscous (*G*'') moduli were recorded against the angular frequency (ω, 0.1-100 rad/s) at a constant strain value selected from the linear viscoelastic range of the amplitude sweeps previously performed.

Amplitude sweeps were formerly carried out to determine the linear viscoelastic range where a linear relationship between the stress and strain exists. The storage or elastic modulus (*G*') and stress were then recorded against the strain at a constant frequency of 1 Hz.

All the above assays described were carried out in an MCR300 Paar Physica shear rheometer (Anton Paar, Austria) equipped with a 25-mm-diameter serrated parallel plate (PP25/S) geometry. The temperature (20.0ºC) was maintained constant through a Peltier system. A gap size of 1000 μm was set. Data were recorded at steady-state.

2.10. Statistical analyses

The results are reported as the average and standard deviation (SD) for *n* replicates. Statistical analyses of results were performed through ANOVA (α: 0.05), followed by Tukey's significance difference test. The GraphPad Prism software (version 5.00, 2007, USA) was used.

3. Results and discussion

3.1. Ultrasound effect on the sugar-exhausted blanched lyophilized carrot powder (CP)

PEF extractions were performed in an open system by applying an US pretreatment with a 20kHz-ultrasonic equipment working at 80% constant amplitude for 20 min. The temperature collected versus time into the glass beaker containing the CP dispersion in aqueous citrate buffer varied between 20 and 60ºC (**Table 1**). The actual energy provided through a 13-mm diameter tip

probe to the CP dispersed in citrate buffer (pH 5.20), while contained into the glass beaker described in **section 2.4.2**, was calculated through **eq. 1** using the temperature-time data recorded from this system in adiabatic conditions. The power was also calculated, using **eq. 2**. Results are reported in **Table 1**. An efficiency of 82.5% was calculated because the energy determined from the temperature-time record was lower than the energy displayed by the ultrasonic device. The same trend was observed for the respective power values. The calculated power density and intensity were 0.13 W/cm^3 and 12.27 W/cm^2, respectively (**Table 1**). As indicated by Santos, Lodeiro, and Capelo-Martinez (2009), transient cavitation occurs when bubbles are formed using power intensities above 10 W/cm^2, which expand enough through a few acoustic cycles, before collapsing violently on compression. Collapse of transient bubbles is considered as the main source of the chemical and mechanical effects of ultrasonic energy. Additionally, the presence of particulate matter like CP is important because it increases the number of bubble nucleation sites for acoustic cavitation. Therefore, the experimental conditions used in the present work for PEF extraction were selected in order to achieve a minimal power intensity of above 10 W/cm^2 without higher increase in the temperature recorded at the end of the pretreatment step (60ºC; **Table 1**).

Carrots discarded at harvesting because of irregular size and shapes were stabilized as a sugar-exhausted blanched lyophilized carrot powder (CP), which was used as the source for the extraction of the PEFs. As expected, CP contained low amounts of residual reducing sugars (1.62% w/w), and also remaining starch (0.72% w/w) and proteins (5.9% w/w), while it was mainly constituted by non-cellulosic carbohydrates of polysaccharides (60.5% w/w). The latter included UA (14.0% w/w) with high DM (61.9%), and a calculated NS content of 46.4% (w/w). Cellulose (10.1% w/w) and lignin (4.2% w/w) were also determined in the CP, which was enriched in cell wall polymers.

As determined through sifting, CP was mainly constituted by average particle sizes of 420 and 210 μm, in a 60.6 and 19% w/w, respectively, with low proportions of 810 (10.32%) and 105 μm (7.9%), and a residual amount of 53 μm. The effect of the US-pretreatment (12.27 W/cm^2) on the CP dispersed in water (**1**g:**100** mL) was evaluated through SEM. As observed in the image displayed in **Fig. 1a,** the ultrastructure of non-US treated CP shows particles of different sizes. They show a porous surface with many holes originally occupied by the cell contents, and protrusion of flakes constituted by the cell walls. Instead, **Fig. 1b** shows particles with non-porous, continuous surfaces that seem formed by superimposed layers, which can be ascribed to opened and extended cell walls disrupted. The US particles seem crushed. **Figs. 1c-d** can confirm through higher magnification the respective surface characteristics described. **Fig. 1e** shows an isolated structure of tubes that can correspond to colenchyma cells wrapped by the cell walls, where the cell wall thickness (gray arrow) can be clearly observed in the non-US treated CP. On the contrary, ultrasounded particles show the remaining skeleton of colenchyma cells in the form of isolated springs (black arrow) which lost the surrounding cell walls (**Fig. 1f**).

3.2. Ultrasound-enzyme assisted extraction of pectin-enriched fiber fractions (PEFs) from CP

As a consequence of the CP matrix alteration generated by the US-pretreatment, the following step performed by stirring for 5h at 40ºC either in the citrate buffer (pH 5.20) or in citrate buffer with hemicellulase or cellulase allowed extracting, after ethanolic precipitation, PEFs with significant (p<0.05) higher yield than in the absence of the US-pretreatment (**Table 2**). PEFs contained proteins (4.5-11% w/w) and mainly total carbohydrates (83-99.4% w/w), which included the UA of pectins (39-47% w/w) and the calculated proportion of NS (41.9-54.8% w/w). Pectins (UA) had a low DM (< 50%), especially after applying the US pretreatment (**Table 2**). The DA was very low for all pectins extracted. The NS/UA molar ratios of the PEFs were between 1.06 and 1.27, values expected for pectins (Basanta et al., 2013), excepting for the citrate-buffer extracted

PEF without US pretreatment, which showed some higher value (1.43) of NS/UA molar ratio. As observed in **Table 2**, the molecular weight profiles of the corresponding PEFs extracted without and with US pretreatment, either through citrate-buffer or citrate-buffer with hemicellulase, were different. All US-pretreated PEFs lacked of macromolecular components with molecular weights higher than ≈150,000 Da.

Cellulase was the only enzyme that *per se* produced a significant increase in the yield of PEF extraction (12.4%) when compared to the citrate-buffer extracted PEF without US pretreatment (6.9%) (**Table 2**). It was determined that additional increment in the yield by cellulase activity was almost not observed after US-pretreatment (22.6%; **Table 2**), since the same yield value was obtained by extraction with citrate buffer (20.6%). A positive effect of the combined action of both US-pretreatment and enzymatic step was only observed for the extraction with hemicellulase, which led to obtain the highest PEF yield (27.1%; **Table 2**). Evidently, degradation of cellulose and not of hemicelluloses in the cellulose-hemicellulose elastic network of the cell walls should be of primary importance for extraction of pectins in the case of carrot roots. Also, hemicellulase activity probably could need a previous mechanical disorganization of the cellulose-hemicellulose network by US in this case to have accessibility to their substrates, which was not necessary for cellulase attack.

Considering the 27.1% yield value (**Table 2**) and the UA content of this PEF (45%), a 12.2% of UA or pectins was extracted by this combined treatment of US and hemicellulase, which was almost the whole UA content found in the CP above reported (14.0%). On the contrary, only 2.91, 3.85 and 5.47% of the UA were respectively extracted with citrate buffer, hemicellulase and cellulase without US pretreatment, values that were well below the UA content of the CP. Therefore, the US pretreatment was necessary to increase the yield of extraction of all PEFs in relatively short times of processing (20 min US + 5h stirring), but the existence of an additional positive effect due to the following enzymatic effect depended on the enzyme used. High

efficiency in the pectin extraction by using hemicellulase was not the only advantage arising from the US pretreatment. Low volume of extractive solvent (citrate buffer) with respect to CP (**1**g:**100** mL) was also necessary. Volumes of solvent below 100 mL per 1g of CP were not possible because CP showed a high swelling capacity (SC = 39±2 mL/g CP) and, hence, significant volumes of buffer are needed to maintain the system fluid enough for homogeneous enzyme activity into the CP matrix. For the isolation of PEFs without US-pretreatment from pumpkin (Fissore et al., 2007) or beet root (Fissore et al., 2011) powders, longer times of extraction (20 h at 30°C) were necessary, and PEFs were obtained with lower yields from pumpkin (4.3-7.0%) and red beet (8.0-20%) after treatment with different concentrations of hemicellulase or cellulase, as previously mentioned. Wikiera, Mika, & Grabacka (2015) described the optimization of pectin extraction from apple pomace using three different commercial cellulases (Celluclast, Econase, Viscoferm). The 4.0-5.0 pH values were tested and the more efficient extractive pH was 4.5. Solid to extractive solution ratios were 1:10 to 1:15 g/mL. The optimal temperature values were between 50 and 60ºC, depending on the enzyme used, and the optimal time of extraction was 18 h, leading to yields of ≈ 18%. Dominiak, Søndergaard, Wichmann, Vidal-Melgosa, Willats, Meyer et al. (2014) used commercial cellulases (GC880, LaminexC2K, ValidaseTRL, MultifectB) in 0.05 M citrate buffer to recover pectin from dried lime peel by treatment for 4 h at 50ºC, with a solid to liquid proportion of 100g to 3 L. Acidic extractions with nitric acid (pH 1.7 for 8h or pH 2.0 for 4h) at 70°C were also performed for comparison. These authors found that by changing the process pH (3.0-4.8) the yield and length of the pectin polymer can be manipulated. The highest pH used allowed obtaining the highest yield but with loss of pectin molecular weight. In addition, all fractions isolated by enzymatic digestion presented gelling capacity and viscosity similar to the acid-extracted pectins. On the other hand, Li, He, Lv, & He (2014) extracted water soluble dietary fiber from apple pomace using cellulase and US assisted methods (pH 4.5 acetate buffer, 3 h, 50ºC; 4g:100 mL solid:liquid ratio), and compared this process with a conventional acid method

(20g apple pomace powder in 400 mL of pH 2.0 sulfuric acid solution, 4h, 80°C in a water bath). For US extraction the same method was applied but instead of using a water bath, they used an ultrasonic cleaning bath. The highest yield (16.4%) was obtained after 40 min of sonication at 400 W, while for conventional acid extraction the yield was 10.3%. For EAE, they used cellulase from *Aspergillus niger* and obtained a yield of 18.7%, which was higher than the yields obtained by acid and US methods. The water retention capacity (WRC) and SC of the dietary fibers extracted from apple pomace were all improved by US and cellulase extraction in comparison with the conventional acid method.

The mechanism of cellulase hydrolysis may involve physical disruption of insoluble cellulose in addition to endo- and exo-hydrolyzing enzymes. The *T. reese*i (formerly *T. viride*) cellulase mixture is constituted by at least two exo-cellobiohydrolases (I, II), five endo-1,4-β-D-glucanases, as well as β-glucosidases which act at the last β-1,4-D-glucosyl bond of the cellulose chain and cellotetraose, releasing cellobiose from the non-reducing ends (Zhang & Lynd, 2004). Cellobiose disaccharides removed from the hydrolysed cellulose network are expected to remain soluble into the buffer solution, facilitating the extraction of pectins from the cell walls' structure. Some pectins can persist in part insolubilized in the fiber residue due to non-disrupted covalent bonds and, in part, solubilized in the buffer solution, which then precipitated after ethanol addition. Enzymic dissolution of crystalline and amorphous cellulose appears to require the concerted action of endo-1,4-β-glucanase and exo-cellobiohydrolases (McCleary, McKie, & Draga, 2012).

Hemicellulases constitutes an enzymes' group defined and classified according to their hemicellulose substrate. It is an array of endo- and exo-enzymes that includes xylanases, mannases, arabinases, and their corresponding glycosidases (Ghose & Bisaria, 1987). Most hemicelluloses are quite water soluble due, in part, to the side chains attached to the backbone polymer. Debranching enzymes remove these substituents, decreasing the substrate solubility and, in turn, lowering the polysaccharide's susceptibility to endoxylanases (Brigham, Adney, &

Himmel, 1996). It is important to note that according to the suppliers, the hemicellulase used in the present work has side activity of cellulase and, hence, certain degree of cellulose network disruption can be produced.

3.3. Functionality of the pectin-enriched fractions isolated from CP

3.3.1. Color and Antioxidant composition

CP contained antioxidant compounds like α-carotene (52 mg/100g CP), β-carotene (80 mg/100g CP), lutein (6.4 mg/100g CP), and α-tocopherol (7.1 mg/100g CP).

All PEFs were orange, in correspondence with the positive values obtained for the *a** (16.2 to 34.2) and *b** (21.5 to 31.8) color parameters, with 40.6 to 66.7% of lightness, *L** ( ). The color can be associated to the presence of α-carotene and β-carotene, whose contents were ≈ 12 and ≈ 18 mg/100g PEF, respectively, whereas these contents tended to decrease but not significantly in the US-pretreated fractions (**Fig. 2)**.

Lutein and α-tocopherol were also co-extracted with the isolated PEFs in levels that varied between 0.2 and 0.7 mg of lutein, and 0.9 and 1.3 mg of α-tocopherol per 100 g of PEF (**Fig. 2**). The US-pretreatment produced PEFs with significantly (p<0.05) lower contents of lutein. A lower content of α-tocopherol was determined only for US-cellulase extracted PEF.

Consequently, the PEFs extracted in the present work are also enriched in antioxidant compounds.

3.3.2. Rheological properties

The mechanical spectra of the respective 2.00% w/v aqueous solution of the PEF were obtained through oscillatory assays performed at 20.0°C by recording the elastic (*G*') and viscous (*G*") moduli as a function of the angular frequency (ω), at linear viscoelastic conditions (**Fig. 3**). Since all PEFs contained UA of low DM (**Table 2**), calcium was added to the aqueous solution

(30 mg Ca^{2+}/g UA). By observation of the mechanical spectra, it can be concluded that the use of the US pretreatment permitted to extract PEFs not only with higher elastic modulus *G*' but also with the mechanical spectrum of a true gel. For fractions treated with enzymes but without ultrasound, the true gel behavior was only observed for the one extracted with cellulase (**Fig. 3c**). Although a non significant difference between the *G*' values (≈ 200Pa) is seen in the latter case, a noticeable lower dependence of *G*'' on the angular frequency can be observed for the US-cellulase extracted PEF solution and, hence, a more structured or less transient gel was produced in the calcium presence. Two macromolecular components of very similar molecular weights characterized the PEFs obtained with cellulase, either without or with US pretreatment (**Table 2**). The UA contents were the same, and similar NS/UA molar ratios were obtained, but the DM of the US-cellulase PEF was a half (27%) of that shown by the cellulase extracted PEF (49.9%) (**Table 2**), which can produce a more structured gel (**Fig. 3c**) due to longer or more numerous demethylated HG blocks.

On the other hand, the presence of at least one macromolecular component of highest molecular weight like those of 787,345 Da for the citrate buffer extracted PEF, and of 299,548 Da for the hemicellulase extracted PEF (**Table 2**) seemed to contribute to the development of a less strong (initial G' value ≈ 20 Pa) and less structured gel (more frequency-dependent viscous modulus *G*'') from the 2.00% w/v citrate buffer extracted PEF solution (**Fig. 3a**), and of a dilute solution from the 2.00% w/v hemicellulase extracted PEF solution (**Fig. 3b**). In the latter case, this mechanical performance where the *G*'' was always above the elastic modulus *G*' in the three logarithmic decades swept, being also both moduli highly dependent on frequency, corresponded to the PEF only constituted by one predominant macromolecular component, which also showed higher average molecular weight (299,548 Da; **Table 2**). This fact seemed to be detrimental for the development of a gel, being then the hemicellulase extracted PEF with 48.7% DM (**Table 2**) no sensible to calcium ions. The formation of calcium bridges between neighbor demethylated

homogalacturonan blocks was affected. Conversely, the US-hemicellulase extracted PEF in a 2.00% w/v aqueous solution showed the mechanical spectrum of a true gel, with *G*' values of ≈ 200 Pa (**Fig. 3b**). Two macromolecular components, each one of lower molecular weight (109,414 and 42,405 Da) together with a lower DM (24%) were the differential characteristics of this fiber fraction (**Table 2**). The same can be mentioned for the US-citrate extracted PEF (**Table 2**), which also permitted to this fraction to develop a true gel at 2.00% w/v concentration, with *G*' values of ≈ 200 Pa (**Fig. 3a**). Therefore, the US pretreatment permitted to obtain PEFs with the best rheological functionality.

3.4. FTIR analysis of the pectin-enriched fractions

The FTIR spectra obtained from the PEFs are displayed in **Fig. 4**. They corresponded essentially to a typical polygalacturonic acid enriched fraction, similar to that determined by Lee, Hung, Cheng, and Wang (2005) for polygalacturonic acid. As observed in **Fig. 4**, the characteristic bands corresponding to the carboxylate group of the D-galacturonic acid monomers can be observed at 1735-1764 cm^{-1} and 1585-1604 cm^{-1}. The former corresponded to the stretching of the C=O group of the methyl-esterified carboxylate, while the strong and intense band that varied between 1585 and 1604 cm^{-1} corresponded to the C=O group of the unesterified and ionized carboxylate group. Purcell and Fishman (1987) determined that the 1604 cm^{-1} band is attributable to the antisymmetric stretching vibration of ionized carboxylate groups, and the increase in area was ascribed to dissociation of pectin counter-ions simultaneous with the dissociation of pectin aggregates. The relative intensity or height of these bands at 1735-1764 cm^{-1} and 1585-1604 cm^{-1} coincides with pectins of low DM (<50%; **Table 2**). In the CDTA and Na_2CO_3 extracted pectins from carrots, Szymanska-Chargot and Zdunek (2013) determined the unesterified carboxylate band at 1580-1605 cm^{-1} as in the PEFs isolated from carrots in the present work (**Fig. 4**).

The bands in the fingerprint zone between 1419 and 1225 cm^{-1}, ascribed to the C–O–H bending and C–O tensile vibrations, as well as the wide band containing some typical peaks of the polygalacturonic acid backbone of pectins such as those at 1126, 1097, 987, and 945 cm^{-1} can be observed (**Fig. 4**).

The broad and intense band at ≈ 3412 cm^{-1} is assigned to the –OH groups of pectin, interacting through hydrogen bonds, while the small band at ≈ 2915-2846 cm^{-1} is ascribed to the C-H (saturated) bond vibration.

4. Conclusions

By using the environmentally friendly US and enzymes, discarded carrots were upgraded to low-methoxylated (24-49.9% DM) PEFs (40-47% UA; NS/UA=1.06-1.27) with gelling capacity (2.00% w/v in water; 30 $mgCa^{2+}$/gUA), and co-extracted antioxidants (α- and β-carotenes, lutein and α-tocopherol) that could be useful for applications as food additives or ingredients. US-citrate buffer or US-enzyme (cellulase or hemicellulase) assisted extractions of PEFs produced the highest yields (20.6-27.1% on CP dry basis). US-pretreatment decreased the DM, the antioxidant contents and molecular weight of the PEFs. It disrupted the network of cell walls that constituted the CP particles, permitting noticeable short times of extraction (20 min US + 5h stirring against 20 h) in a low citrate buffer to CP ratio (100 mL/g) as permitted by the SC of CP. It was then determined that the US pretreatment was necessary to increase the yield of extraction, but the existence of an additional positive effect due to the subsequent enzymatic step depended on the enzyme used. Cellulase was the enzyme that per se produced a significant increase in the yield of extraction (12.4%), while the US-hemicellulase assisted extraction produced PEFs with the highest yield (27.1%). The US-pretreatment allowed obtaining functional PEFs with co-extracted antioxidants and the best rheological performance.

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Conflict of interest

Authors report no conflict of interest.

References

Agro empresario, 2019. La zanahoria, una hortaliza de gran consumo en Argentina. URL https://www.agroempresario.com.ar/nota-2593.html. Accessed 08.03.19.

Basanta, M. F., de Escalada Pla, M. F., Stortz, C. A., & Rojas, A. M. (2013). Chemical and functional properties of cell wall polymers from two cherry varieties at two developmental stages. *Carbohydrate Polymers, 92*, 830-841.

Brigham, J., Adney, W., & Himmel, M. (1996). Hemicellulases: diversity and applications. In C.E. Wyman (Ed.), *Handbook of Bioethanol: Production and Utilization* (pp. 119-142). Washington DC: Taylor and Francis.

Chantaro P., Devahastin S., & Chiewchan N. (2008). Production of antioxidant high dietary fiber powder from carrot peels. *LWT - Food Science and Technology, 41*, 1987-1994.

Ciriminna, R., Fidalgo, A., Delisi, R., Ilharco, L.M., & Pagliaro, M. (2016). Pectin production and global market. *Agro Food Industry Hi Tech, 27(5).* URL https://www.teknoscienze.com/tks_article/pectin-production-and-global-market/ Accessed 22.09.18.

Dominiak, M., Søndergaard, K.M., Wichmann, J., Vidal-Melgosa, S., Willats, W.G.T., Meyer, A.S., Mikkelsen, J.D. (2014). Application of enzymes for efficient extraction, modification, and development of functional properties of lime pectin. *Food Hydrocolloids, 40*, 273-282.

Dreher, M.L. (2018). Whole fruits and fruit fiber emerging health effects. *Nutrients, 10*, 1833; doi:10.3390/nu10121833

FAO (2016). FAOSTAT, URL http://www.fao.org/faostat/en/#data/QC/ Accessed 18.09.18.

Fissore, E. N., Ponce, N. M., Stortz, C. A., Rojas, A. M., & Gerschenson, L. N. (2007). Characterisation of fiber obtained from pumpkin (*Cucumis moschata* Duch.) mesocarp through enzymatic treatment. *Food Science and Technology International, 13,* 141-152.

Fissore, E. N., Ponce, N. M. A., Matkovic, L., Stortz, C. A., Rojas, A. M., & Gerschenson, L. N. (2011). Isolation of pectin enriched products from red beet (*Beta vulgaris* L. var. conditiva) wastes: composition and functional properties. *Food Science and Technology International, 17(6)*, 517-527.

Fissore, E., Santo Domingo, C., Pujol, C., Damonte, E., Rojas, A. M., & Gerschenson L. (2014). Upgrading of residues of bracts, stems and hearts of *Cynara cardunculus* L. var. *scolymus* to functional fractions enriched in soluble fiber. *Food and Function, 5*, 463–470.

Gaviola, J. C. (2013). El Cultivo de la Zanahoria, In J. C. Gaviola (Ed.), *Manual de producción de Zanahoria, Publicaciones Regionales INTA, La Consulta, Mendoza, Argentina*. URL https://inta.gob.ar/sites/default/files/script-tmp-inta_-_cap_1__el_cultivo_de_la_zanahoria.pdf, pp. 1-26. Accessed 09.09.18.

Ghose, T. K., & Bisaria, V.S. (1987). Measurement of hemicellulase activities. Part 1: Xylanases. *Pure and Applied Chemistry, 59(12),* 1739-1752.

Idrovo Encalada, A., Basanta, M. F., Fissore, E., De'Nobili, M. D., & Rojas, A. M. (2016). Carrot fiber (CF) composite films for antioxidant preservation: Particle size effect. *Carbohydrate Polymers, 136,* 1041-1051.

Lee, M. W., Hung, C. L., Cheng, J. C., & Wang, Y. J. (2005). A new anti-adhesion film synthesized from polygalacturonic acid with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide crosslinker. *Biomaterials, 26*, 3793-3799.

Li, X., He, X., Lv, Y., & He, Q. (2014). Extraction and functional properties of water-soluble dietary fiber from apple pomace. *Journal of Food Process Engineering, 37*, 293-298.

McCleary, B. V., McKie, V., & Draga, A. (2012). Measurement of endo-1,4-β-Glucanase. Chapter 1. Methods in Enzymology, 510, 1-17.

Mamvura, T. A., Iyuke, S. E., & Paterson, A. E. (2018). Energy changes during use of high-power ultrasound on food grade surfaces. *South African Journal of Chemical Engineering, 25*, 62-73.

Mazeau, K., & Pérez, S. (1998). The preferred conformations of the four oligomeric fragments of Rhamnogalacturonan II. *Carbohydrate Research, 311*, 203-217.

Pérez, S., Rodríguez-Carvajal, M. A., & Doco, T. (2003). A complex plant cell wall polysaccharide: rhamnogalacturonan II. A structure in quest of a function. *Biochimie, 85,* 109–121.

Purcell, J. M., & Fishman, M. L. (1987). Dissociation of dissolved pectins: Fourier-transform infrared spectroscopy. *Carbohydrate Research, 159*, 185-190.

Rossetti, L., Langman, L., Grigioni, G. M., Biolatto, A., Sancho, A. M., Comerón, E., & Descalzo, A. M. (2010). Antioxidant status and odor profile in milk from silage or alfalfa-fed cows. *Australian Journal of Dairy Technology,* 65, 3-9.

Santo Domingo, C., Rojas, A.M., Fissore, E.N., & Gerschenson, L.N. (2019). Rheological behavior of soluble dietary fiber fractions isolated from artichoke residues. *European Food Research and Technology*, 1-11, DOI 10.1007/s00217-019-03242-y .

Santos, H. M., Lodeiro, C., & Capelo-Martínez, J. L. (2009). The Power of Ultrasound. In J. L. Capelo-Martínez (Ed.), *Ultrasound in Chemistry: Analytical Applications* (pp. 1-16). Weinheim: Wiley VCH.

Stuart, T. (2009). Part II. Squandered Harvests. Farming: Potatoes have Eyes. In T. Stuart (Ed.), *Waste: Uncovering the Global Food Scandal* (pp. 104). New York-London: W.W. Norton & Company.

Szymanska-Chargot, M., & Zdunek, A. (2013). Use of FT-IR spectra and PCA to the bulk characterization of cell wall residues of fruits and vegetables along a fraction process. *Food Biophysics, 8,* 29-42.

Vincken, J. P., Schols, H. A., Oomen, R. J. F. J., McCann, M. C., Ulvskov, P., Voragen A. G. J., & Visser, R. G. F. (2003). If homogalacturonan were a side chain of rhamnogalacturonan I. Implications for cell wall architecture. *Plant Physiology, 132,* 1781-1789.

Wikiera, A., Mika, M., Grabacka, M. (2015). Multicatalytic enzyme preparations as effective alternative to acid in pectin extraction. *Food Hydrocolloids, 44*, 151-161.

Zhang, Y. H. P., & Lynd, L. R. (2004). Toward an aggregated understanding of enzymatic hydrolysis of cellulose: noncomplexed cellulose systems. *Biotechnology and Bioengineering 88(7),* 797-824.

Zhu, Z., He, J., Liu, G., Barba, F., Koubaa, M., Ding, L., Bals, O., Grimi, N., & Vorobiev, E. (2016). Recent insights for the green recovery of inulin from plant food materials using non-conventional extraction technologies: A review. *Innovative Food Science and Emerging Technologies, 33,* 1-9.

Figure captions

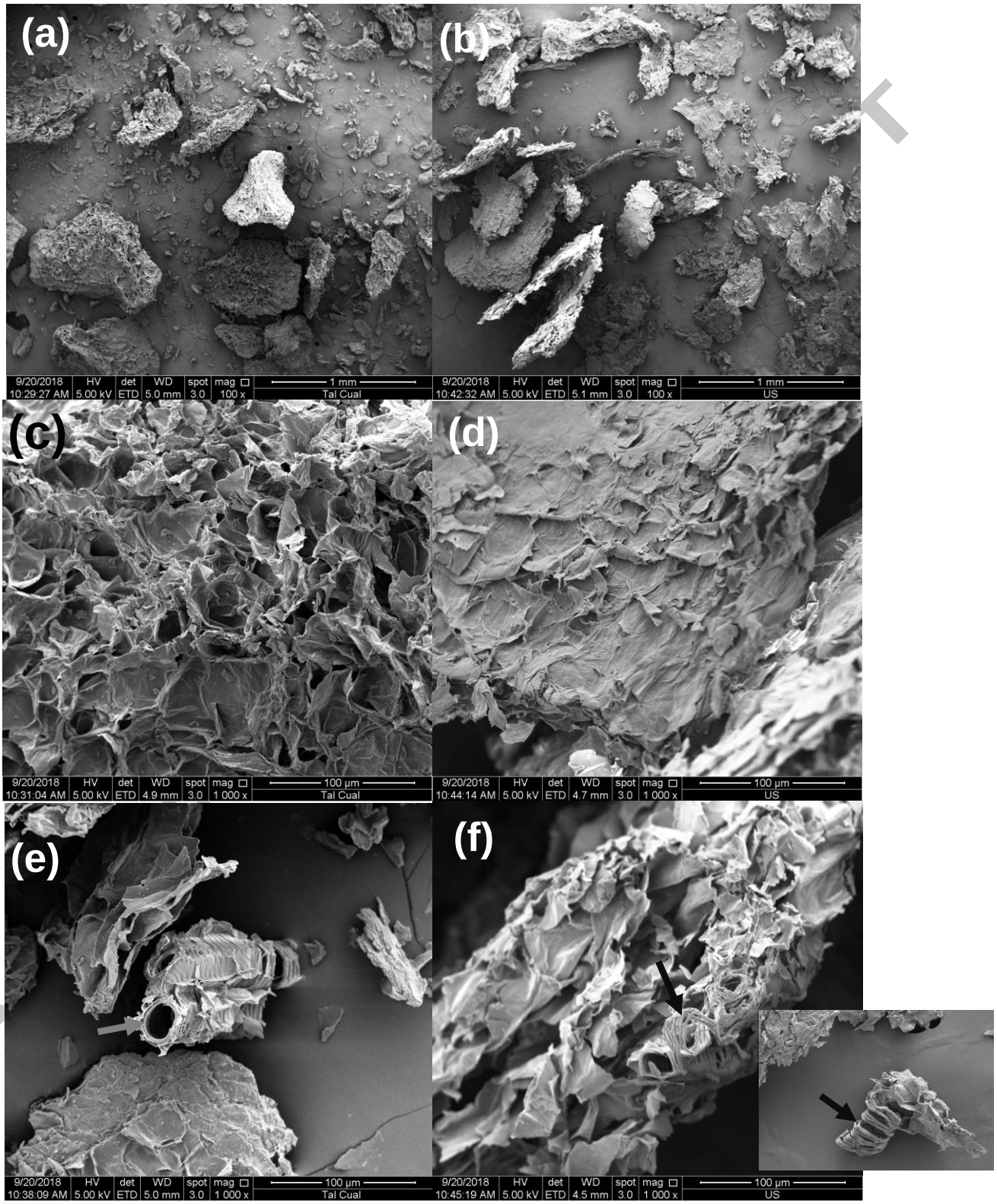
Fig. 1. SEM images obtained from non-US treated (**a**, **c**, **e**) and US-treated (**b**, **d**, **f**) carrot powder (CP).

Fig. 2. Antioxidants present in the PEFs isolated either with citrate buffer (1), citrate buffer and hemicellulase (2), or citrate buffer and cellulase (3) without or with HPUS pretreatment (4,5,6, respectively). Error bars are the standard deviations for n=3. The same lower case or capital letters for a given antioxidant indicate non-significant differences ($p<0.05$).

Fig. 3. Mechanical spectra obtained at 20.0ºC from each 2.00% w/v aqueous solution of the PEF isolated either by citrate buffer (**a**), citrate buffer and hemicellulase (**b**) or citrate buffer and cellulase (**c**) with (△) or without (○) ultrasound (US) pretreatment. Fill symbols *G*' and empty symbols *G*'' moduli.

Fig. 4. FTIR spectra recorded from the pectin-enriched fractions obtained either through citrate buffer (black line; a), citrate buffer with hemicellulase (red line; b), or citrate buffer with cellulase (blue line; c). The three inferior spectra are those of the respective pectin-fractions obtained also with high-power ultrasound pretreatment (a'; b'; c'). The spectrum at the top of the plot (orange line) is that of the carrot powder (CP).

Fig. 1.


(a)
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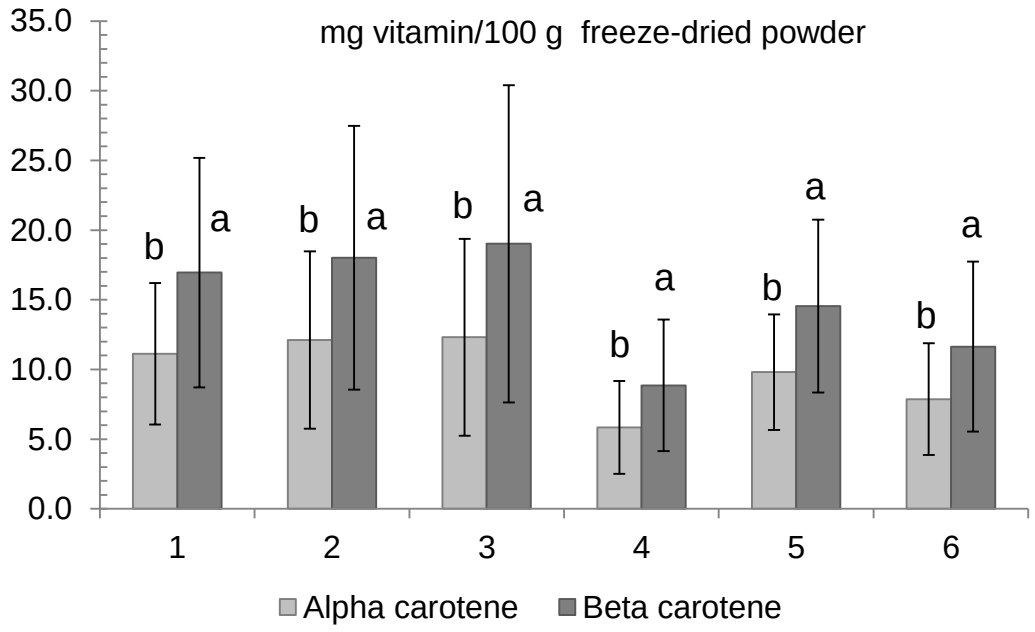
(b)
9/20/2018 10:42:32 AM HV 5.00 kV det ETD WD 5.1 mm spot 3.0 mag 100 x 1 mm US
(c)
9/20/2018 10:31:04 AM HV 5.00 kV det ETD WD 4.9 mm spot 3.0 mag 1 000 x 100 µm Tal Cual
(d)
9/20/2018 10:44:14 AM HV 5.00 kV det ETD WD 4.7 mm spot 3.0 mag 1 000 x 100 µm US
(e)
9/20/2018 10:38:09 AM HV 5.00 kV det ETD WD 5.0 mm spot 3.0 mag 1 000 x 100 µm Tal Cual
(f)
9/20/2018 10:45:19 AM HV 5.00 kV det ETD WD 4.5 mm spot 3.0 mag 1 000 x 100 µm US

Fig. 2.



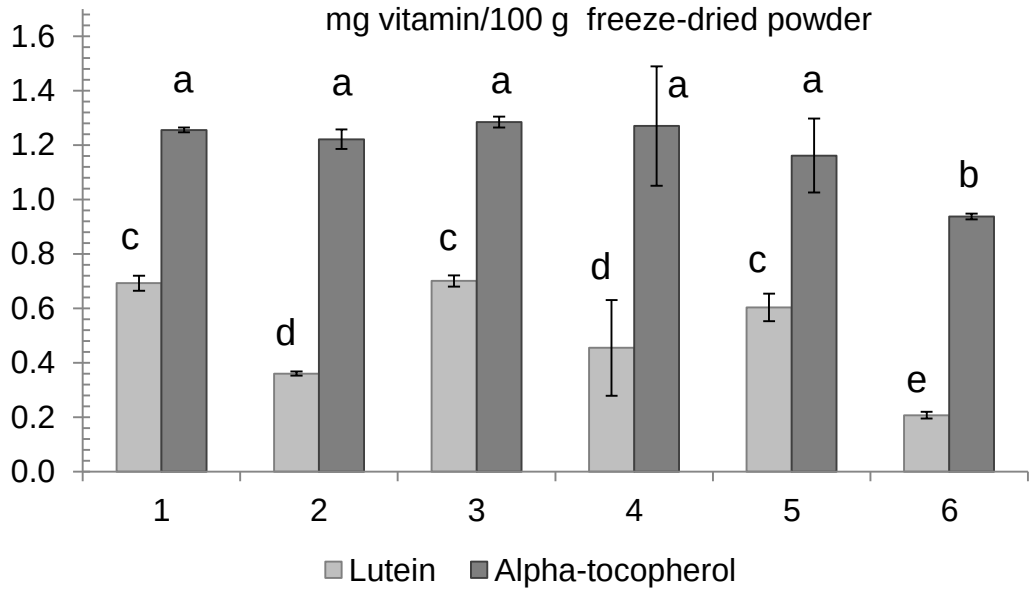
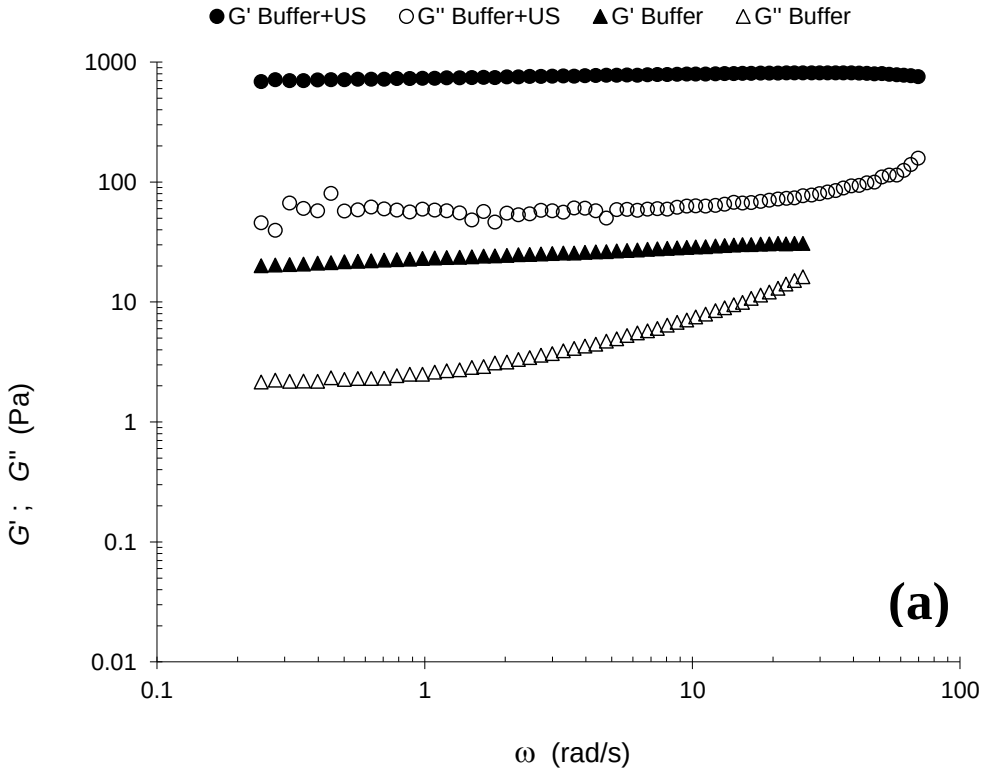


Fig. 3.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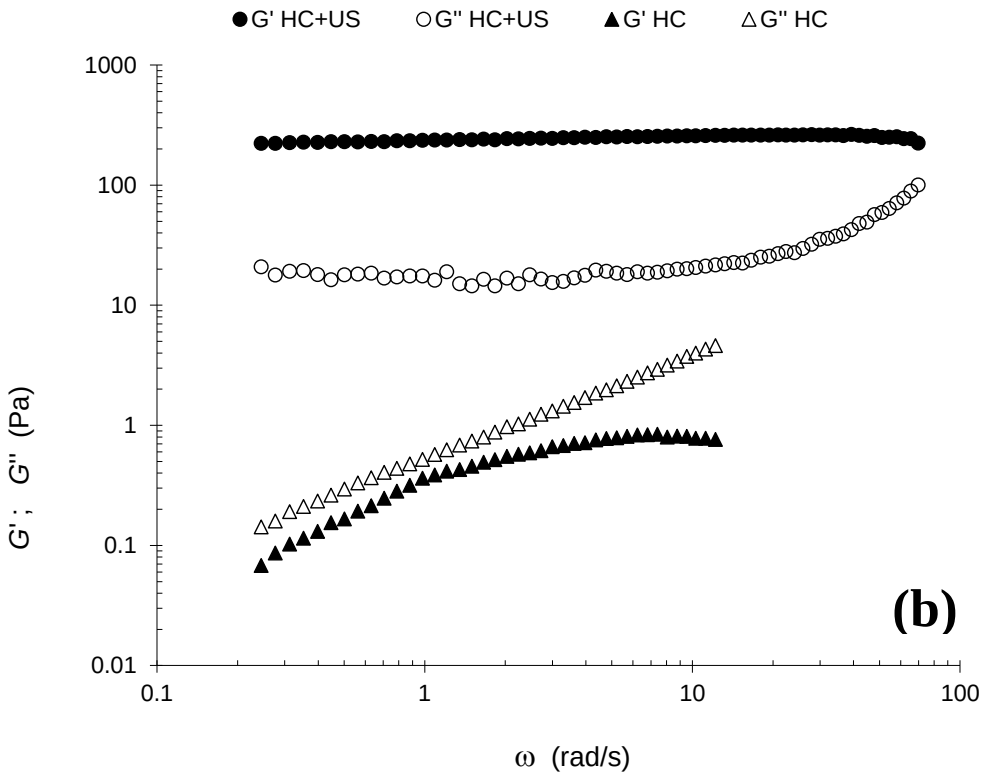
1000
100
10
1
0.1
0.01
G' ; G'' (Pa)
0.1
1
10
100
ω (rad/s)
(a)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ω (rad/s)
(b)

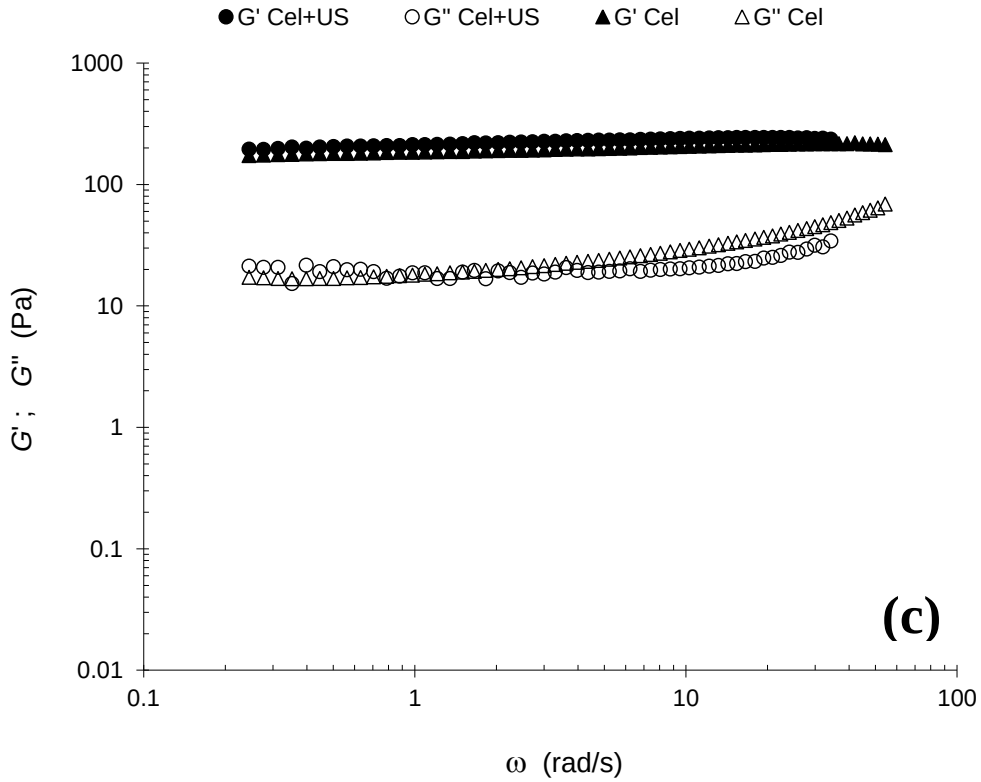

Fig. 4.

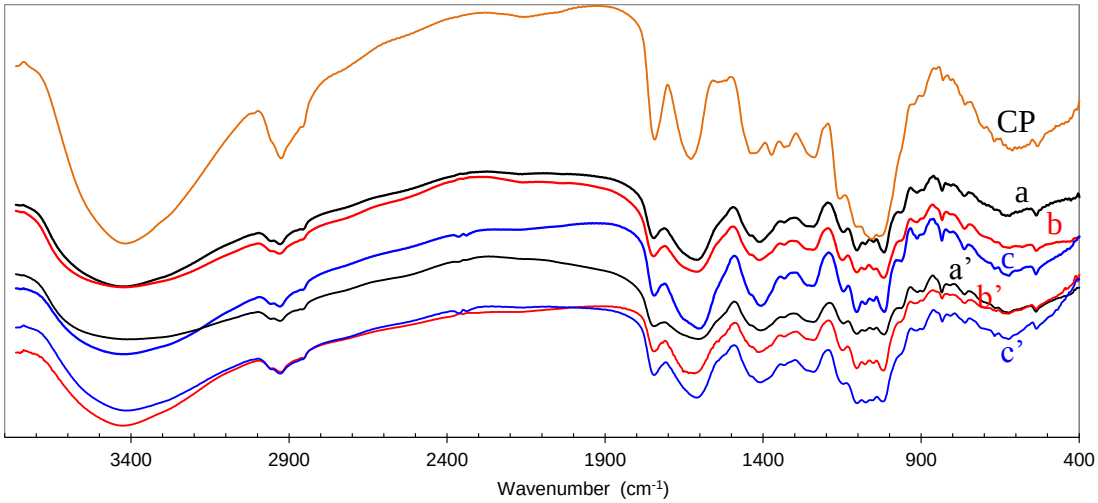

Table 1

Energy and power values displayed by the ultrasonic equipment during processing. Energy value provided during ultrasonication as calculated from the temperature-time continuous record determined in the CP dispersions[a] when adiabatically isolated. From this calculated energy, power, efficiency, power density and power intensity are also evaluated.

20 kHz, 80% amplitude, 20 min	
Temperature range recorded during open assays performed for the extraction of each PEF (C)	20 - 60
Energy displayed by the device (J)	63901
Power displayed by the device (W)	86
Temperature range recorded adiabatically (C)	20 - 61
Energy[b] calculated for the treatment (J)	78178
Power[c] calculated for the treatment (W)	65.15
Efficiency calculated (%)	75.8
Power density calculated (W/cm^3)	0.13
Power intensity calculated (W/cm^2)	12.27

[a]Dispersions of 5.0000 g CP/500.00 mL citrate buffer (pH 5.2).
[b]Calculated according to equation (1) for a 13mm-diameter ultrasound tip probe, and the sample container dimensions (90-mm internal diam. x 125-mm height glass beaker), using the temperatures recorded up to 20 min of HPUS treatment in adiabatic conditions.
[c]Calculated according to equation (2).

Table 2

Yield[a], chemical composition[a], molecular weight, and CIE-Lab color parameters[a] of the PEFs extracted from the CP obtained from carrot roots-residues.

	% (w/w)					
	Buffer pH 5.2	Buffer pH 5.2 + US	Hemicellulase	Hemicellulase + US	Cellulase	Cellulase + US
Yield	6.9±0.4^{A}	20.6±0.7^{B}	8.2±0.4^{A}	27.1±0.8^{C}	12.4±0.9^{D}	22.6±0.9^{B}
Proteins	11.0±2.0^{A,B}	11.0±0.1^{A}	4.5±0.2^{C}	10.0±1.0^{A}	7.9±0.4^{B}	11.0±2.0^{A}
Total carbo-hydrates	97.0±2.0^{A}	83.0±3.0^{B}	99.4±0.6^{A}	97.0±3.0^{A}	86.0±3.0^{B}	87.0±3.0^{B}
Uronic acids (UA)	42.2±0.9^{A,B}	39.0±3.0^{B}	47.0±4.0^{A}	45.0±5.0^{A,B}	44.1±0.2^{A}	43.0±2.0^{A,B}
DM (%, molar)	48.0±1.0^{A}	37.2±0.5^{B}	48.7±0.7^{A}	24.0±1.0^{C}	49.9±0.6^{A}	27.0±3.0^{C}
DA (%, molar)	2.1±0.2^{A}	1.8±0.1^{A}	2.0±0.2^{A}	1.2±0.2^{B}	1.8±0.2^{A}	1.3±0.1^{B}
Neutral sugars (NS)[b]	54.8	44.0	52.4	52	41.9	44.0
NS/UA[c] (molar ratio)	1.4	1.2	1.2	1.3	1.1	1.1
Molecular weight (Da)	787,345 130,236 43,901	136,887 48,175	299,548	109,414 42,405	127,397 34,714	123,717 29,460
*L** (%)	50.0±1.0^{A}	48.9±0.3^{B}	66.7±0.9^{C}	40.6±0.8^{D}	47.2±0.6^{B}	47.2±0.8^{B}
*a**	29.5±0.7^{A}	29.9±0.4^{A}	25.7±0.4^{B}	16.2±0.2^{C}	34.2±0.1^{D}	25.6±0.2^{B}
*b**	27.6±0.8^{A}	31.7±0.3^{B}	26.3±0.6^{A}	21.5±0.3^{C}	31.8±0.1^{B}	31.8±0.6^{B}

[a]Mean and standard deviations for n = 3 or n = 10 for color parameters are reported. Different letters in the same line show significant differences (p<0.05).

[b]Yield was calculated as g/100 g of CP.

[c]Neutral sugars (NS) are calculated as the arithmetical difference between Total carbohydrates and Uronic acids-content.

[d]Moles of NS were calculated with a mean monosaccharide molar mass of 166.73 g/mol considering the typical monosaccharide composition of pectins as well as a NS/UA ratio of 1.2 (Basanta et al., 2013).

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

HIGHLIGHTS

Misshapen carrots were upgraded to Ca^{2+} gelling pectin enriched fractions (PEFs)

PEFs were orange colored because of carotenes, lutein and α-tocopherol antioxidants

Sustainable ultrasound-pretreatment (US) and enzyme assisted extraction was used

US-hemicellulase led to the highest PEF yield (27.1%), extracting the whole pectins

US fell methylation degree and molecular weight, but gave the best yields and gelling