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High-power ultrasound pretreatment for efficient extraction of fractions enriched in pectins and antioxidants from discarded carrots (*Daucus carota* L.)

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ABSTRACT

A useful pectin-enriched fraction (PEF) extracted through high-power-ultrasound (HPUS) pretreatment and sodium carbonate was efficiently obtained from discarded carrots. Formerly, the effect of HPUS-power intensity and time (A: 40 min-2.08 W/cm^2; B-E: 5-20 min ≈10W/cm^2) on carrot powder (CP) used for PEF-isolation, was investigated. Reducing carbohydrates-, cell-wall neutral sugars- (NS), uronic acids- (UA) of pectins, and protein releases increased with HPUS-energy. CP had antioxidant capacity, containing α- and β-carotene, lutein, α-tocopherol. Only HPUS B-E treatments (≈10W/cm^2) were capable to produce matrix disruption, promoting polymers' release. CP pretreated through selected E-treatment (20 min; ≈10W/cm^2) followed by 0.1M-Na_2CO_3 (1 h-stirring) extracted the whole pectin content of CP (UA=14.0%). PEFs were orange, with co-extracted antioxidants. More substituted (1.27 NS/UA ratio) three lower molecular weights' components of HPUS-PEF produced higher Newtonian viscosity before shear-thinning, and calcium-crosslinked gels with lower elastic modulus (*G*'=12Pa). Sustainable HPUS/Na_2CO_3 method isolated efficiently an antioxidants-carrying PEF useful as functional food additive.

Keywords: discarded carrots, ultrasound-extracted pectin-fraction, power intensity, carotenes, alpha-tocopherol, calcium-crosslinked gels.

1. Introduction

Food wastes constitute a significant problem for economic, environmental and food security reasons. About one-third of all food produced globally for human consumption (approximately 1.3 billion tons per year) is lost or wasted. Moreover, the major contribution to the food waste comes from vegetables (FAO, 2014). Fruit and vegetable wastes are produced in large quantities in markets, and constitute a big problem in municipal landfills (Varzakas et al., 2016).

Carrot (*Daucus carota* L. var. sativus), an important vegetable of the *Umbelliferae* family, is cultivated throughout the world. It is usually chopped, and eaten raw, cooked, fried or steamed and cooked in soups, stews, salads, cakes, as well as prepared meals for babies and pets (Dansa et al., 2017). Carrot is extensively consumed and considered one of the healthier vegetables for being a rich source of bioactive compounds, dietary fiber, carotenoids, minerals, and vitamins (Idrovo Encalada et al., 2016). In Argentina, between 200,000 and 240,000 tons of carrot roots are produced annually (Gaviola, 2013). The highest percentage of production is destined to fresh consumption, including ready-to-eat salads prepared in small scale food industries and greengrocers. A small proportion is destined mainly to the dehydration industry. These processes generate significant volumes of residues (Dansa et al., 2017). In total, about 25-35% of carrots are usually discarded after harvesting or industrialization because of irregular sizes and forms, being in part used as animal feed, while still contains useful compounds like antioxidants and pectins (Chantaro et al., 2008). Therefore, the use of discarded carrots can be a good alternative for obtaining antioxidant carrying pectins with useful rheological properties.

Pectins are complex polysaccharides that are found in the middle lamella and cell wall of all higher plants and, hence, they are part of the dietary fiber. The structure of pectin is composed mainly by D-galacturonic acid units (GalA) of the homogalacturonan (HG) chains, partly esterified with methanol, and neutral sugars (NS) such as L-rhamnose, L-arabinose, and D- galactose of

the rhamnogalacturonan I (RG-I) core, as well as other 13 different monosaccharides (Hosseini et al., 2016). Pectin composition and structure depends on the origin, developmental stages, and extraction conditions (Petkowicz et al., 2017). They are widely used as a functional ingredient in the food industry as gelling, thickening and stabilizing, and texturizing agent (do Nascimento et al., 2016), as well as in the pharmaceutical industry for their beneficial health properties as soluble dietary fiber, for reducing blood fat, gut processes, and reducing heart disease, among others (Bagherian et al., 2011).

The commercially available pectin is obtained using conventional extraction by means of a mineral acid (hydrochloric, nitric, and sulfuric acid) and it is recovered by precipitation with ethanol (Chan and Choo, 2013). Some innovative pectin extraction techniques such as ultrasound, microwave, and enzymatic extraction, have been developed to improve the yield and the product quality (Marić et al., 2018). Ultrasound-assisted extraction (UAE) uses high-frequency sounds and solvents to enhance the release and diffusion of cell material. The increase of the mass transfer is produced by the acoustic cavitation induced in a liquid medium, which is one of the beneficial effects of this technology (Wang et al., 2015). There are significant advantages of UAE such as the reduced extraction time, low energy consumption, yield increase, and use of lower volumes of solvent when compared to conventional extractive methods (Tao et al., 2014).

By sequential extraction of the polymers from the isolated cell wall material (alcohol insoluble residue), at low temperatures (18-22ºC), it is possible to ascertain the chemical composition and polymer interactions within cell walls (Fry, 1986; Koh & Melton, 2002; Basanta, de Escalada Pla, Stortz, & Rojas, 2013). This sequential scheme begins with isolation for at least 4 h of the loosely (water-soluble) bound pectins, followed by the extraction for 24 h of the CDTA soluble fraction (calcium crosslinked pectins). The third extractive step is performed for 24 h to obtain the 0.1M Na_2CO_3 soluble fraction, composed by the remaining pectins that are anchored in

the cell wall matrix through covalent bonds like diester bridges of ferulate and galacturonate, as reported by Basanta et al. (2013).

The present study proposes HPUS as a pretreatment to facilitate the subsequent extraction of a PEF from misshapen carrots with 0.1M Na_2CO_3 aqueous solution at room temperature, with the aim of using a sustainable method for the valorization of vegetable residues as food additives or ingredients. As a prelude to PEF-isolation, the effect of HPUS-power intensity and sonication time on water soaked CP used for PEF extraction was investigated. Thus, the releases of reducing carbohydrates, UA, NS, and proteins were determined in the water solvent after performing HPUS treatments at 20 kHz, and either 20% of constant amplitude for 40 min (A-treatment) or 80% of amplitude for 5-20 min (B-E treatments). The best conditions for the PEF extraction were then selected.

2. Materials and methods

2.1. Chemicals

Chemicals were of analytical grade. α-carotene, β-carotene, lutein, α-, β- and γ-tocopherols, retinol, bovine serum albumin, and D-galacturonic acid standards were of Sigma-Aldrich, while the rest of the chemicals were of Merck Química (Argentina). Deionized water (Milli-Q™, USA) was used.

2.2. Ultrasonic treatments in CP

Carrots (*Daucus carota* L. var. Nantes) harvested in Valle de Uco (Mendoza province, Argentina), discarded after harvesting or industrialization because of irregular sizes and forms, were used in the present work. Carrots were washed with detergent, rinsed carefully with tap water and dried with paper. The petiole was eliminated from the stem, and the rest of the carrot root was cut first in slices and afterward chopped into a food processor (Moulinex FR6001, 700

W, Argentina). The cut tissue was washed three times with deionized water (1g:4mL) by stirring, followed by filtration through a plastic sieve. The last washed residue retained by the filter was then dispersed under stirring in deionized water (1 g:20 mL) at 90ºC for 5 min for blanching, then filtrated, cooled by dispersion in enough volume of cold deionized water/ice, filtrated, and freeze-dried (Christ, Germany; Pfeiffer vacuum pump, Germany). The carrot powder obtained was ground (IKA A10 Basic, A 10.2 Star shaped cutter, Germany) and called CP, packed under vacuum (Multivac C-200, Germany) into small Cryovac bags (Sealed Air, USA), wrapped with aluminum foil for darkness, and maintained at -20ºC until use.

To determine the size distribution, a given weight of CP was sieved through a vibratory sieve shaker provided with a series of six ASTM mesh sizes, as explained by Idrovo Encalada et al. (2016).

An ultrasonic processor (Vibra-Cell VCX 750, net power output: 750 W, Sonics Materials Inc, USA) working at constant 20 kHz and 20% or 80% of wave amplitude and equipped with a 13 mm diameter probe was employed. It was used a glass beaker (Borosilicate glass, IVA, Argentina) of 66-mm internal diameter and 95-mm height containing 200.00 mL of deionized water and 5.0000 g of the CP. The liquid contained into the glass beaker reached a height of 70 mm while the ultrasound probe was 20-mm immersed. Treatments were then performed on CP as indicated in **Table 1**. The temperature was measured with a thermocouple attached to the US device and immersed in the aqueous dispersion next to the glass beaker's wall. The energy and power displayed by the equipment were recorded. Experiments were carried out in triplicate.

To calculate the true power and energy output provided, the temperature was recorded as explained above but maintaining the whole system isolated from the environment, in a calorimetric assay (Mamvura et al., 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:

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

and to the power (P) calculation (in W) by:

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

wherein m is the 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 expressed in W per unit area of the emitting surface or probe (W/cm^2), and W per unit volume of the sonicated sample (W/cm^3), respectively (Santos et al., 2009).

2.3. Water activity

The water activity (a_W) of CP was evaluated in triplicate at 25.0ºC through a Decagon AquaLab (Series 3 Water activity meter, USA) by measurement of the relative humidity (RH) of the equilibrium air (ERH) with the sample (**eq. 3**), as explained by Idrovo Encalada et al. (2016).

$a_W = ERH/100$ (3)

2.4. Extraction of pectin-enriched fractions (PEF)

CP (2.5% w/v) was suspended in 200.00 mL of deionized water. After hydration, the system was sonicated for 20 min using the ultrasonic processor Vibra-Cell (VCX 750, Sonics Materials Inc, USA) at a constant frequency of 20 kHz and an amplitude of 80% with pulses (5 seconds on - 5 seconds off) using a flat tip 13 mm diameter titanium probe. After sonication, 100 mL of a 0.3 M Na_2CO_3 solution were added to the sample in order to reach a final concentration of 0.1 M. The system was stirred for 1 hour on a magnetic stirrer (IKA, Germany) at room temperature (22ºC) and filtered under vacuum. Subsequently, the supernatant (containing the PEF) was neutralized, and then precipitated with ethanol 96% (1:2 v/v - supernatant to ethanol),

leaving the system for 12 h at 4ºC for complete precipitation. Finally, the PEF was collected through filtration under vacuum using a glass fiber filter (Schleicher & Schuell, Germany), washed three times with ethanol 96% by re-suspension and filtration, freeze-dried and milled. A PEF was separately obtained without HPUS pretreatment for comparison. The yield was calculated as grams of PEF per 100g of CP. The experiment was performed in triplicate.

2.5. Color

The values of the *L**, *a** and *b** parameters of the CIELab space were measured on CP and PEFs through a colorimeter (Minolta CM-600D, Tokyo, Japan), using D-65 sodium illuminant and a 2º observer, according to Idrovo Encalada et al. (2016).

2.6. Chemical characterization

CP and PEFs were chemically analyzed through the spectrophotometric methods reported by Fissore et al. (2007). In this way, reducing carbohydrates were determined through the Somogyi-Nelson method using D-glucose as standard, in the supernatant obtained after extraction with water for 24 h (23ºC) under magnetic stirring, and centrifugation (10,000×g; Eppendorf 5804R, Germany). Cellulose, lignin, uronic acids (UA) of pectins and non-cellulosic carbohydrates (pectins and hemicelluloses) were separately determined in the CP by selective extraction from 0.0100 g of CP with different concentrations of sulfuric acid (1 M or 72% w/w), as reported by Ng et al. (1998) and Basanta et al. (2014). Cellulose and lignin were separately quantified by weighing the respective insoluble residues obtained after extraction with either 1M-sulfuric acid aqueous solution (2.5 h), which dissolves only the non-cellulosic polysaccharides (pectins and hemicelluloses), or 72% w/w sulfuric acid, which dissolves cellulose but not lignin. In the supernatants of the 1M sulfuric acid treatments, UA of pectins were determined through the colorimetric method of Filisetti-Cozzi and Carpita, while non-cellulosic carbohydrates were

288 determined by the phenol-sulfuric acid spectrophotometric method, in both cases using D-
289 galacturonic acid as standard. Through these colorimetric methods, the UA and total
290 carbohydrates-content were respectively determined in each isolated PEF after its dissolution in
291 water. The NS content was calculated as the arithmetical difference between the non-cellulosic
292 polysaccharides (called total carbohydrates in the PEFs) and the UA contents. The protein
293 content was determined through the spectrophotometric method of Lowry et al. (1951) using
294 bovine serum albumin as standard. Starch was evaluated through an enzymatic method involving
295 amylase, amyloglucosidase and o- dianisidine. The methanol content and acetyl groups were
296 evaluated spectrophotometrically as reported by Fissore et al. (2007). The DM of each PEF was
297 then calculated as the percent ratio between the moles of methanol and the moles of UA
298 previously determined, whereas the degree of acetylation (DA) was calculated as the percent
299 ratio between moles of acetyl group and moles of non-cellulosic polysaccharides in the sample.

300 When evaluating the effect of HPUS on the CP suspended in water, the dispersion was
301 filtered under vacuum after sonication. Reducing carbohydrates-, UA, and protein contents were
302 determined in the filtrated supernatant as above indicated. The NS content was calculated as
303 previously mentioned.

304 Antioxidants' (carotenoids, xanthophylls, tocopherols, retinol) contents were determined
305 according to the procedure described by Rossetti et al. (2010) which involves a saponification
306 with 12 N KOH for 30 min at 70ºC. Quantification was carried out through a quaternary gradient
307 pump (P4000, Thermo Scientific, USA), with a membrane vacuum degasser connected to an
308 auto sampler AS2000 (Thermo Separation Products) with an injection loop (10 to 100 µL), and a
309 C18 column (250×4.6mm i.d., Alltima, 5 µm particle size; Alltech, USA) fitted with a guard column
310 (Security GuardAlltima C18, Alltech, USA). The mobile phase was ethanol:methanol (60:40 v/v)
311 used at 1.0 mL/min. The technique was optimized to determine tocopherols, carotenoids and
312 retinol within the same elution time of 25 min. For tocopherols, a fluorescent detector (FL3000;

Thermo Separation Products, USA) was set at 296-330 nm, k_{exc} and k_{em}, respectively. A diode array detector (UV6000; Thermo Separation Products, USA) was set at 445 nm and 325 nm for the detection of carotenoids and retinol, respectively. Chromatograms were recorded using a Chromquest 4.0 Software platform. Calibration curves were performed with the corresponding external standards freshly prepared in absolute ethanol. All chemical analyses were carried out in triplicate.

2.7. Antioxidant capacity

The antioxidant capacity of the CP was evaluated in triplicate through the free-radical scavenging activity (DPPH, 2,2-diphenyl- 1-picrylhydrazyl, assay), and the ferric reducing antioxidant power (FRAP assay), as reported by Idrovo Encalada et al. (2016). Samples were extracted with methanol and results were expressed as L-(+)-ascorbic acid (AA). The AA standard was also dissolved in methanol.

2.8. GPC

The molecular weight (Mw) of the PEFs was estimated using gel permeation chromatography (GPC) according to the method described by Munarin et al. (2013) with some modification. PEF samples (0.25% w/w) were dissolved in aqueous 0.1 M $NaNO_3$. The solutions were left in agitation overnight at room temperature. The equipment used was a GPC chromatograph (Waters System, MA, USA), equipped with a heater (TCM 5 CH, Singapore), a pump, a refractive index detector (Waters 2414), a DAD diode array detector (Waters 2998), a 100µL loop connected in series to a Ultrahydrogel pre-column (60x40 mm) and two Ultrahydrogel (500 and 1000 mm) columns. The mobile phase was 0.1 M $NaNO_3$ solution (0.6 mL/min) thermostatized at 40 °C. Dextrans (PSS kit, Waters, Germany) of 5,200-668,000 Da of Mw range were used as standards for calibration. All samples were filtered through a 0.22 µm nylon filter

prior to injection to the GPC system. The data were processed using the software Breeze, Empower 2, Sweden. Determinations were performed in triplicate.

2.9. Fast Fourier-transform infrared spectroscopy (FTIR)

Transmission spectra of the CP and PEFs were recorded from KBr pellets with a Nicolet 8700 (Thermo Scientific Nicolet, MA, USA) spectrometer, as described by Idrovo Encalada et al. (2016).

2.10. Rheological characterization

The 2.00% w/v pectin systems were prepared by dissolving 0.1000 g of each PEF in 4000 μL of deionized water. These systems were heated into a thermostatic water bath at 70ºC (Julabo, Germany), alternating with continuous magnetic stirring and vortexing for dissolution. After 24h of hydration, for the flow assay, enough volume of deionized water was added to make 5000 μL of solution, and then homogenized by vortexing. For dynamic assays, 500 μL of an aqueous calcium solution was added (30 mg Ca^{2+}/g UA) at 70ºC and then homogenized by vortexing. The volume was then made up to 5000 μL through the addition of enough deionized water (70ºC) followed by homogenization and heating. Rheological characterization through rotational experiments (flow assays) and oscillating experiments (dynamic assays) were performed using 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 points were recorded at steady-state.

2.10.1. Flow assays

The flow behaviour was evaluated in the aqueous PEF solutions by recording the viscosity (η) in the 0.01-100 s^{-1} shear rate ($\dot{\gamma}$) range, for 50 min. The data were fitted according to the Cross model (**eq. 4**):

$$\eta(\dot{\gamma}) = \eta_{\infty} + \frac{(\eta_O - \eta_{\infty})}{1 + (\tau \cdot \dot{\gamma})^m} \qquad (4)$$

where η_0 represents the zero-shear rate viscosity or Newtonian viscosity, η_{∞} represents the viscosity at time $t \rightarrow \infty$, τ is the time constant corresponding to the Cross model, and m is a dimensionless constant.

2.10.2. Dynamic assays

The dynamic assays were performed in the PEF solutions containing calcium ions. Amplitude (stress *versus* strain) sweeps were first performed at a constant frequency of 1 Hz in order to determine the linear viscoelastic range of each sample solution, from which the value of strain was chosen for the subsequent record of the mechanical spectra (frequency sweeps).

For mechanical spectra, the storage or elastic (*G*') and loss or viscous (*G*'') shear moduli were recorded against the angular frequency (ω), at a constant strain value selected from the linear viscoelastic region determined previously in the amplitude sweep.

2.11. 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 for statistical analyses and nonlinear regression fitting.

3. Results and discussion

3.1. HPUS energy and power

385 **Table 1** summarizes the amplitude and treatment times, as well as the energy and power
386 values displayed by the HPUS equipment (20 kHz constant frequency) while performing the
387 assays in open systems constituted by the dispersion of CP in a volume of water contained into a
388 glass beaker of determined dimensions. On the other hand, adiabatic experiments were
389 performed only to calculate the acoustic energy (**eq. 1**) and power (**eq. 2**) actually provided by the
390 ultrasound equipment through the 13-mm-diameter tip probe into the glass beaker used
391 containing the CP dispersed in water (**section 2.2**). The temperature range recorded from them,
392 as well as the energy and power calculated are reported in **Table 1**. There was no difference
393 between the temperature profiles recorded from dispersions of CP. The energy calculated
394 increased with the time of HPUS treatment (B-E) due to the increment in temperature, whereas
395 the power decreased with increasing time as expectable from **eq. 2** (**Table 1**). The efficiency
396 determined as the ratio between the power calculated and the power displayed by the HPUS
397 equipment was 84.9% for treatment A, and decreased from 100% to 82.5% as the treatment (B to
398 E) time increased. The power density and power intensity determined from the corresponding
399 calculated power were low for the A treatment (20% amplitude), while increased 5 and 10 times
400 for the B treatment at 80% of amplitude, decreasing with the increase in time of processing
401 (**Table 1**). The temperature range recorded during the real experiments performed in open
402 systems constituted by the CP dispersed in water is also reported in **Table 1**.
403
404 *3.2. Ultrasound effect on freeze-dried carrot powder (CP)*
405 Carrot roots discarded because of irregular sizes and forms have to be stabilized prior to
406 their utilization as a source of compounds useful as food additives or ingredients. Therefore, a
407 sugar-exhausted blanched carrot tissue was produced, freeze-dried and finally milled into a
408 powder (CP) with the average particle size distribution displayed in **Fig. 1a** (mainly 60.6% w/w of
409 420 μm, 19% of 210 μm). Water activity of CP was 0.300 (**Table 2**), which is sufficiently low to

avoid microbial growth, chemical hydrolysis, enzymatic activities, and browning reactions during storage at room temperature (Labuza et al., 1972). The CIE-Lab color parameters pointed to a powder with a high lightness (L^* = 78.3%), accompanied by redness (a^* > 0) and yellowness (b^* = +26.6), corresponding to its orange color (**Table 2**).

Chemical composition of the CP is informed in **Table 2**. CP was obtained with a yield of 6.9% w/w with respect to the raw carrot tissue, and was mainly constituted by non-cellulosic carbohydrates (60.5 g/100 g CP), which included the UA (GalA) of pectins (14.0%) with a DM of 61.9%, and the NS (46.4%) of pectins and hemicelluloses. According to this composition, a NS/UA molar ratio of 3.55 was calculated. Cellulose (10.1%) and lignin (4.2%) were the other cell wall polymers found, together with 5.9% of proteins (**Table 2**). A very low content of residual starch (0.72%) was determined.

The HPUS treatments applied (**Table 1)** led to the release profiles that can be observed in **Fig. 1b-c**. As above mentioned, the acoustic field acted on the particles suspended in water (1g:40 mL water). As expectable, very low proportions of reducing carbohydrates (1.3 to 1.6 g/100g dry powder; **Fig. 1b**) were determined in the water in contact with the CP. This result permitted to corroborate that the intracellular content was released because of the disruption of cell membrane and vacuoles during the washing steps and thermal blanching of cut carrot tissue by water immersion (**section 2.2**). Reducing carbohydrates increased significantly (p<0.05) with the energy levels provided by the B-E treatments. Low levels of NS were released from CP after HPUS (1.3 to 1.7%), and increased significantly (p<0.05) for C-E treatments (**Fig. 1b**). The protein release (0.64-0.90%) also increased significantly (p<0.05) from A to B-E treated powders (**Fig. 1b**). The UA (pectins) were released at levels of 0.59-1.36% (**Fig. 1c**), and increased significantly (p<0.05) with the time (**Fig. 1c**) and energy (**Fig. 1d**) of the 80% amplitude-HPUS treatment. The CP submitted to 20%-amplitude HPUS (A treatment) extracted again the lowest amount of UA in the largest time of contact (40 min), due to the low power intensity (2.0 W/cm^2),

as reported in **Table 1**. Samples of the CP left in water under high-rpm magnetic stirring for the same time involved in treatment A (**Table 1**) did not release significant (p<0.05) amounts of the components reported in **Fig. 1** (**b-c**), including non-detectable proportions of UA or pectins. Consequently, only through the application of HPUS energy it was possible to produce enough disruption in the dehydrated matrix of CP for promoting the extraction of polymeric components in a short time of treatment.

The antioxidant capacity of CP was evaluated as the radical (DPPH) scavenging and FRAP activities, which were of 21.6 and 41.3 mg of AA per 100 g of CP, respectively (**Table 2**). As can be observed in the chromatograms shown in **Fig. 2**, α-carotene, β-carotene and lutein (**Fig. 2a**) as well as α-tocopherol (**Fig. 2b**) were the antioxidants identified in the CP, and in amounts of 52, 80, 6.4 and 7.1 mg/100 g of CP, respectively (**Table 2**).

3.3. Ultrasound extraction of pectins from the freeze-dried carrot powder (CP)

After considering the results above described, the E-treatment (**Table 1**) was selected as the HPUS pre-treatment for the extraction of the PEFs from CP. This processing was performed with the CP dispersed in water (1g:40 mL water) as above mentioned. After that, enough Na_2CO_3 was added under stirring to reach 0.1 M concentration in the solution (5g:300 mL), and extraction was performed at room temperature (22.0ºC) by stirring for 1 h or 24 h. Since the same yields were obtained for both extraction times, only the 1-h extraction was herein considered. After insolubilization in 65% v/v ethanol a fraction was obtained with 35.4% of yield and the composition reported in **Table 3**. Simultaneously, a control system was produced without the HPUS pre-treatment, which was replaced by the stirring in water for the same time (**Table 1**). In this case, the fiber fraction was extracted with a yield of 23%, significantly lower than that obtained through the HPUS assisted extraction (**Table 3**). The UA content (≈ 40%) was the same for both fiber fractions extracted with 0.1 M Na_2CO_3, with low DM due to the alkaline treatment

460 (pH = 11.2) (**Table 3**). The DA was low for both PEFs, and lower for the HPUS extracted pectins
461 (**Table 3**).

462 Both PEFs were mainly constituted by total carbohydrates, especially the ultrasound
463 extracted fraction that contained an 85% of them, which included 40% of demethylated UA and
464 the calculated 45.0% NS (**Table 3**). By considering a weighted average monosaccharide molar
465 mass of 166.73 g/mol coming from the typical monosaccharide composition of pectins (Basanta
466 et al., 2013), a NS to UA molar ratio of 1.27 was calculated for the fiber fraction obtained through
467 HPUS treatment, and of 1.0 for the control system (**Table 3**), both expectable for pectins. The
468 HPUS extracted PEF also presented lower protein content (**Table 3**).

469 Taking into account that per 100 g of CP, 35.4 g corresponded to the fiber fraction
470 isolated with the HPUS-E pretreatment, from which 85% (30.1 g) were total carbohydrates and
471 40% (14.1 g) were UA (**Table 3**), hence, this procedure permitted to extract the whole UA (pectin)
472 content found in the CP source through the following 0.1M Na_2CO_3 treatment (**Table 2**). This can
473 be inferred because the UA content in the CP was 14.0% (14.0 g/100 g CP; **Table 2**). However,
474 when the HPUS-E pretreatment was not used, only ≈ 9 g of pectins were recovered per 100 g of
475 CP (**Table 3**). Evidently, the power intensity of cavitation produced by ultrasound waves and
476 transient bubbles (≈10 W/cm^2; **Table 1**) led to the disruption of CP matrix, favoring the following
477 extraction of polysaccharides from the cell walls in an actually short time (1 h). Transient bubbles
478 function as micro-reactors, being responsible for the chemical and mechanical effect of HPUS
479 (Santos et al., 2009). As reported in **Table 3**, these pectins were characterized by a different
480 molecular weight profile (119,240Da; 45,266 Da and 35,940 Da) to that of pectins extracted
481 without HPUS pretreatment (130,013 Da and 52,623 Da).

482 In the sequential extraction of polymers from the cell walls of vegetable tissues, habitually
483 performed to determine the cell wall composition and crosslinks, the extraction of pectins with 0.1
484 M Na_2CO_3 involves 24 h of stirring at room temperature and uses a high proportion of solvent

485 solution in relation to the cell wall powder (1g powder:1000 mL 0.1 M Na_2CO_3) (Basanta et al.,
486 2013). However, only 1 h of extraction with 0.1 M Na_2CO_3 solution was applied in the present
487 work, together with an actual low proportion of aqueous solution (1g CP:60 mL solution).

488 Interestingly, the pectins were characterized by a strong orange color whose *L**, *a** and
489 *b** parameters are summarized in **Table 3**, which can be ascribed to co-extracted carotenes. As
490 also reported in the CP composition, α-carotene and β-carotene, lutein and α-tocopherol were
491 found in the isolated PEFs, and the HPUS pre-treatment permitted to obtain a product "more
492 purified" from these co-extracted antioxidants (**Table 2**). As determined by Waldron et al. (2003),
493 crystals of β-carotene are poorly digested from carrot tissues if the cell walls are not ruptured.
494 This is due to the inaccessibility of the crystals to bile salts, fats, and lipases in the gut. Therefore,
495 carotenes co-extracted with pectins from CP can be bioavailable.

496

497 *3.4. FTIR spectra of the pectin-enriched fractions isolated from CP*

498 The FTIR spectra recorded from the PEFs are shown in **Fig. 2c**. They corresponded to
499 the typical polygalacturonic acid backbone of pectins. The broad absorption band at ≈ 3400 cm^{-1}
500 is characteristic of the –OH groups, and the 2910 cm^{-1} signal corresponded to the C-H
501 (saturated) stretching of $–CH_2$ groups of the pectin backbone. The 1604 cm^{-1} strong band of
502 symmetrical and asymmetrical oscillations is characteristic of ionized carboxyl groups, while the
503 band at ≈ 1730 cm^{-1} of the esterified carboxylate groups is almost absent (**Fig. 2c**), which is
504 coherent with the non-detectable DM (**Table 3**). The signals at 1404, 1319 and 1220 cm^{-1} of the
505 fingerprint zone corresponding to the –C–O–C– groups, as well as the bands at 1122, 1093,
506 1010 and 946 cm^{-1}, are all typical of the polygalacturonic acid chain, according to that determined
507 by Lee et al. (2005).

508

509 *3.5. Rheological properties of the pectin-enriched fractions isolated from CP*

The PEFs separately dissolved in deionized water at 2.00% w/v concentration had a pH of 5.8. At resting, these solutions showed a constant initial or Newtonian viscosity (η_0), while performing the rotational flow assay (20.00ºC) at the lowest values of shear rate (**Fig. 3a**). This value was higher for the solution containing the HPUS-E extracted PEF (2.0 Pa·s) than for the other solution (1.0 Pa·s) (**Fig. 3a**). When the shear rates increased above 0.06 s^{-1} for the HPUS-extracted pectin solution and above 0.3 s^{-1} for the control pectin-fraction solution, the viscosity decreased down to 0.037 and 0.01 Pa·s, respectively, values that corresponded to the limit or infinite viscosity (η_∞) (**Fig. 3a**). These viscosity profiles indicated a pseudoplastic behavior for both solutions, which corresponds to hydrated polysaccharides that form physical entanglements, constituting structured solutions in the *at rest* condition at the lowest shear rates (η_0). When a critical value of shear rate is surpassed, the hydrated macromolecules disentangle and, just relaxed, they begin to flow in the same direction of the bulk solvent flux lines.

The mechanical spectra (20.00ºC) obtained from the 2.00% w/v aqueous solution of each PEF in the presence of calcium ions (30 mg/g UA) corresponded to "true gel" structures (**Fig. 3b**). These spectra showed the typical behavior of solvated physical networks between 0.1 and 100 rad/s of angular frequency, where the elastic (*G*') modulus is slightly dependent on the angular frequency in the three log-decades swept. Higher frequency-dependence was observed for the viscous modulus (*G*''), which was always bellow *G*' and in almost one log-cycle between 0.1 and 100 rad/s, especially for the gel developed by the non-ultrasounded PEF (**Fig. 3b**). At the beginning of the respective mechanical spectrum, the *G*' value was of ≈225 Pa for the aqueous solution of the PEF obtained without HPUS pretreatment, whereas *G*' was ≈ 12 Pa for the aqueous solution of the HPUS-extracted PEF.

The molecular weight profiles of these PEFs were different, as reported in **Table 3**. The PEF obtained without HPUS-E pretreatment was constituted by macromolecules of two average molecular weights, 130 kDa and 52,623 Da, while the HPUS-E-extracted pectins were

535 characterized by three lower average molecular weights (119,240; 45,266 and 35,940 Da) with
536 higher degree of substitution at the rhamnogalacturonan I (RG-I) core (NS/UA molar ratio = 1.27;
537 **Table 3**). The latter can be responsible for a higher probability of physical entanglements when
538 dissolved in water (higher η_0; **Fig. 3a**) but can hinder the calcium-crosslinking at the
539 homogalacturonan blocks between the RG-I cores (lower values of *G'*; **Fig. 3b**). By studying
540 through rheology the formation kinetics and properties of alginate fluid gels produced by in-situ
541 calcium release, Fernández Farrés and Norton (2014) determined that longer linear polymer
542 chains allow a higher number of feasible sites for calcium crosslinking per chain, which enhances
543 the formation of a percolating network and increases the number of rheologically-effective
544 network crosslinks. Consequently, high molecular weight alginate fluid gels exhibited faster
545 gelation kinetics and greater viscosities than those of low molecular alginate fluid gels.

546 Szymańska-Chargot et al. (2017) isolated cellulose, hemicellulose and pectins from
547 carrot, tomato, cucumber and apple pomaces. A total content of pectins of 18.6% expressed as
548 D-galacturonic acid was found in the cell wall or alcohol insoluble residue. Functionality of the
549 fiber fractions obtained was not analyzed. For studying the effect of pH (0.5-2.5), temperature
550 (50-90ºC) and heating time (30-150 min), as well as of the liquid/solid ratio (10-50 v/w) on the
551 yield and degree of esterification, Jafari et al. (2017) optimized the process conditions for acidic
552 extraction of pectins from carrot pomace with citric acid. Pectins of low DM (22-52%) were
553 obtained. The optimal extractive conditions corresponded to a pH of 1.3, at 90ºC for 79.8 min,
554 and with a liquid/solid ratio of 23.3 v/w, which led to a maximum yield of 15.6% and to a pectin
555 fraction with a 75.5% of UA content. This high proportion of UA is expectable for pectins obtained
556 at low pH and high temperatures, where considerable peeling of the RG-I hairy regions occurred,
557 with loss of NS. Depending on the molecular weight, this effect can derive in lower rheological
558 functionality and distribution of demethylated blocks in the HG regions. The 1% w/v solution of the
559 isolated pectin in water at 25ºC showed a pseudoplastic behavior in a rotational viscometer, while

its mechanical spectrum corresponded to a concentrated solution. The pectin fraction extracted from carrot pomace at optimized conditions showed important emulsifying properties. Antioxidants were not mentioned in these works.

4. Conclusions

Smaller, twisted, and misshapen carrots discarded at harvesting were used to produce a sugar-exhausted blanched freeze-dried powder (CP; water activity=0.300) enriched in cell wall polymers, whose whole pectin content (UA: 14.0% w/w) was successfully extracted at room temperature in a short period (1h) by stirring in 0.1M Na_2CO_3, when CP was HPUS pretreated in water (1g:40 mL) for 20 min net time (E treatment: power intensity of ≈10 W/cm^2). This PEF had low DM and was co-extracted with antioxidants (α- and β-carotene, lutein and α-tocopherol), but in a proportion that was the half of that contained in the PEF isolated without HPUS pretreatment. Carotenes were also responsible for the orange color of the isolated PEFs. The efficient HPUS-E process was selected after investigation of the effect of different HPUS-energy levels or power intensities (A-E treatments) on the structure of CP dispersed in water (1g:40 mL). Through chemical analyses of the supernatants, it was determined that only HPUS-power intensities of ≈10 W/cm^2 were capable to produce enough matrix disruption for promoting polymers' extraction from CP in a short period (5-20 min). As a consequence of the disruption of the polymeric matrix of the CP source by the HPUS-E-energy, the following 0.1M Na_2CO_3 treatment was then able to extract the whole pectin content of CP (14.0%) in only 1 h, and with a low volume of solution (1g CP:60 mL). When compared to the non-HPUS pretreated fraction, the HPUS-PEF with demethoxylated polygalacturonic acid showed a 1.27 NS/UA molar ratio, and three macromolecular components of lower molecular weights (119,240; 45,266; 35,940 Da), which showed higher Newtonian viscosity (η_O) in water before shear-thinning, and developed calcium-crosslinked true gels with lower elastic modulus (*G'* =12 Pa). The HPUS/0.1M-Na_2CO_3 treatment

585 is a sustainable method to extract efficiently the antioxidants-carrying PEF from carrot roots'
586 powder (CP), which can be useful as an additive or ingredient for functional foods.
587
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709

710 **Figure captions**

711 **Fig. 1.** Average particle size composition of the sugar-exhausted 85ºC-blanched freeze-dried
712 carrot powder (CP) (**a**). Levels of reducing carbohydrates (CH) expressed as D-glucose (Glc), of
713 neutral sugars (NS), and protein (**b**) in the water of CP sonication through A-E treatments,
714 expressed per 100 g of CP. Concentration of uronic acids in the water of CP sonication
715 (expressed per 100 g of CP) plotted as a function of time (**c**) or calculated energy (**d**) of
716 sonication: A (◆) and B-E (●) treatments. Error bars indicate the standard deviation (n=3).

717

718 **Fig. 2.** Chromatograms obtained within the same elution time of 25 min for a given sample:
719 carotenoids (α-carotene, β-carotene, lutein) with the diode array detector (**a**), α-tocopherol with
720 the fluorescent detector (**b**). FTIR spectra of the pectin-enriched fractions (PEFs) extracted from
721 carrot powder (CP) either directly through 0.1 M sodium carbonate (black line) or with a previous
722 HPUS E-treatment (gray line) (**c**).

723

724 **Fig. 3.** (**a**) Flow curves of viscosity against shear rate recorded at 20.0ºC for 50 min from the
725 2.00% w/v aqueous solutions of HPUS E-pretreated PEF (△) and non-sonicated PEF (○).
726 Continuous lines corresponded to the Carreau model fitted. (**b**) Mechanical spectra recorded at
727 20.0ºC from 2.00% w/v aqueous solutions (30 mg Ca^{2+}/g PEF) of HPUS E-pretreated PEF (*G*'▲;
728 *G*"△) and non-sonicated PEF (*G*'●; *G*"○).

729

Table 1

Energy, power, and efficiency calculated from the temperature range recorded during high power ultrasound (HPUS) treatments performed in adiabatic conditions on sugar exhausted freeze-dried carrot powder (CP)[a] dispersed in water. The actual temperature range recorded during treatment under HPUS in the open system is also summarized.

Treatment	Amplitude (%)	HPUS time (min)	Energy displayed by the device (J)	Power displayed by the device (W)	Temperature range recorded adiabatically[b] (C)	Energy[a,b] calculated for the treatment (J)	Power[a,c] calculated for the treatment (W)	Efficiency calculated (%)	Power density calculated (W/cm^3)	Power intensity calculated (W/cm^2)	Temperature range recorded during open assays[c] (C)
A	20	40	22333	13	19 – 47	26499	11.00	84.9	0.05	2.08	20 - 39
B	80	5	26337	65	20 – 41	19854	66.18	100	0.29	12.47	19 - 40
C	80	10	51832	64	20 – 61	38256	63.76	99.6	0.28	12.01	19 - 57
D	80	15	73811	63	20 – 75	52300	58.11	92.2	0.25	10.95	19 - 67
E	80	20	94215	60	20 - 83	59378	49.48	82.5	0.22	9.32	19 - 72

[a]Dispersion of 5.0000 g of CP in 200.00 mL of water.
[b]Calculated according to **equation (1)** for a 13mm-diameter ultrasound tip probe and the sample container dimensions (66-mm internal diam. x 95-mm height glass beaker), using the temperatures recorded at each ultrasonication time of HPUS treatment in adiabatic conditions.
[b]Calculated according to **equation (2).**

Table 2

Yield[a], chemical composition[a], water activity[a] and CIE-Lab color parameters[a] of the freeze-dried carrot powder (CP).

	Values
Water activity (a_W°)	0.300±0.002
*L** (%)	78.3±0.6
*a**	+17.3±0.2
*b**	+26.6±0.5
	% (w/w)[a]
Yield[a]	6.9±0.4
Proteins	5.9±0.4
Non-cellulosic carbohydrates	60.5±0.8
Uronic acids (UA)	14.0±0.2
DM (%, molar ratio)	61.9±0.4
Neutral sugars (NS)[b]	46.4
NS/UA[c] (molar ratio)	3.55
NS ascribed to pectins[d]	16.8
NS/UA[e] (molar ratio) for pectins	1.27
Hemicelluloses[e]	30
Starch	0.72±0.05
Cellulose	10.1±0.4
Lignin	4.2±0.6
DPPH (mg AA/100g powder)	41±3
FRAP (mg AA/100g powder)	22±4
α-carotene (mg/100g powder)	52±4
β-carotene (mg/100g powder)	80±7
Lutein (mg/100g powder)	6.4±0.4
γ-tocopherol (mg/100g powder)	7.1±0.8

[a]Mean and standard deviations for n = 3 or n = 10 for color parameters are reported.
Yield was calculated as % w/w with respect to the fresh raw carrot root tissue, while the rest of the chemical components are expressed as % w/w with respect to CP.

[b]Neutral sugars were calculated as the arithmetical difference between Non-cellulosic carbohydrates- and Uronic acids-contents.

[c]Moles of NS were calculated with a weighted average monosaccharide molar mass of 164 g/mol by supposing the existence of pectins as well as of xyloglucans as the main hemicelluloses in primary cell walls of the dicot walls of carrot roots (Scheller and Ulvskov, 2010).

[d]Neutral sugar content ascribed to pectins was calculated according to the following equation, where Yield is that corresponding to the 0.1M-Na_2CO_3+US treatment (35.4%; Table 1), TC% and UA% are respectively the total carbohydrate (%) and UA (%) contents of the extracted pectins (Table 1), while the figures correspond to the weighted average molar mass of monosaccharides typical of pectins (rhamnose, arabinose, galactose, low contents of xylose) (166.735), and to the molar mass of D-galacturonic acid (194) minus the molar mass of water (176):

$$NS\left(\%\frac{w}{w}\right) = \left(\frac{\left(Yield \cdot \frac{TC\%}{100}\right)}{166.735} - \frac{\left(Yield \cdot \frac{UA\%}{100}\right)}{176}\right) \cdot 166.735$$

[e]Hemicelluloses were calculated as the arithmetical difference between the NS content (46.4%) and the NS content ascribed to pectins (16.8%).

AA: L-(+)-ascorbic acid.

DM: degree of methylation

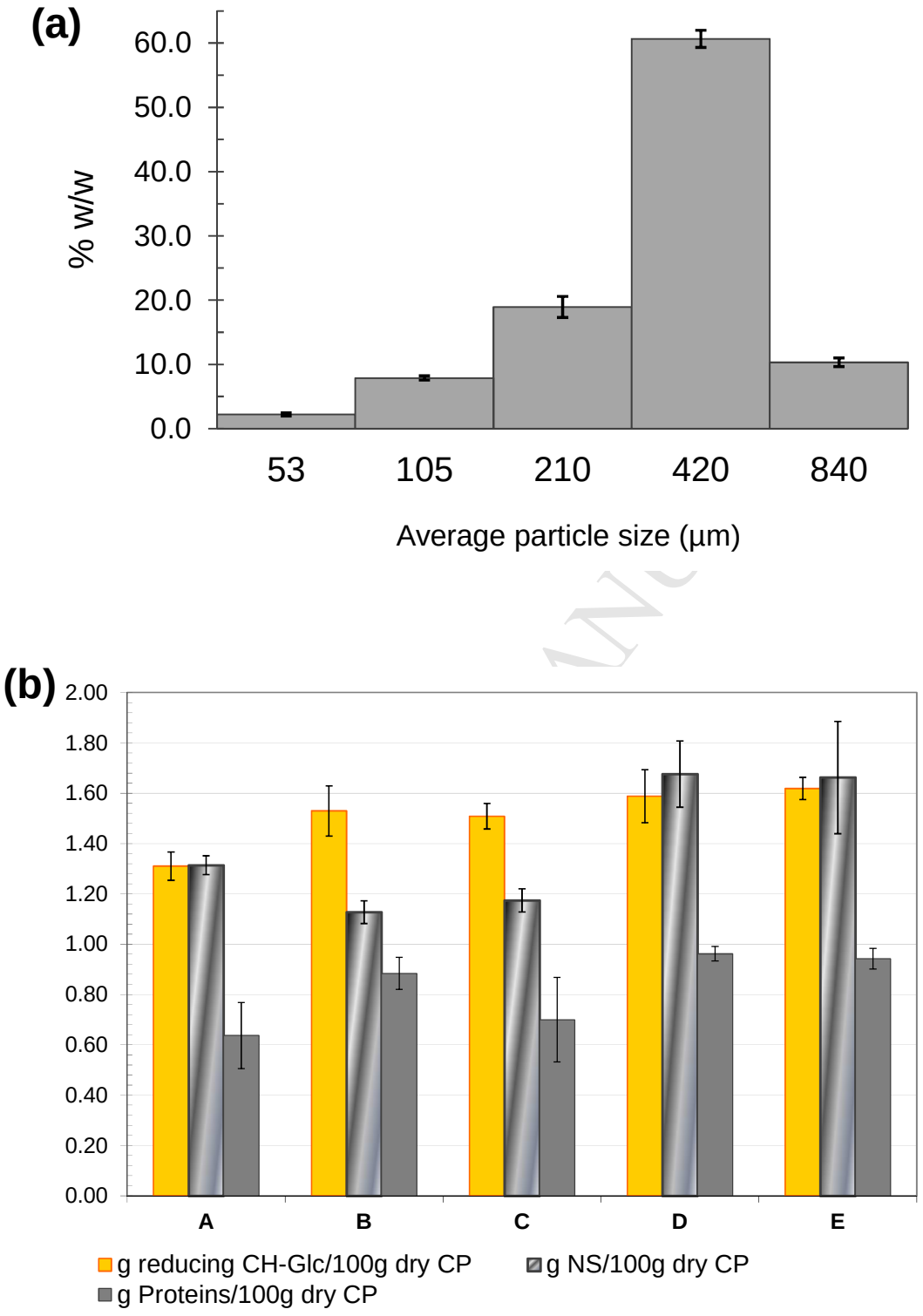
Table 3

Yield[a,b], chemical composition[a], molecular weight, and CIE-Lab color parameters[a] of the pectin enriched fractions (PEFs) extracted from CP.

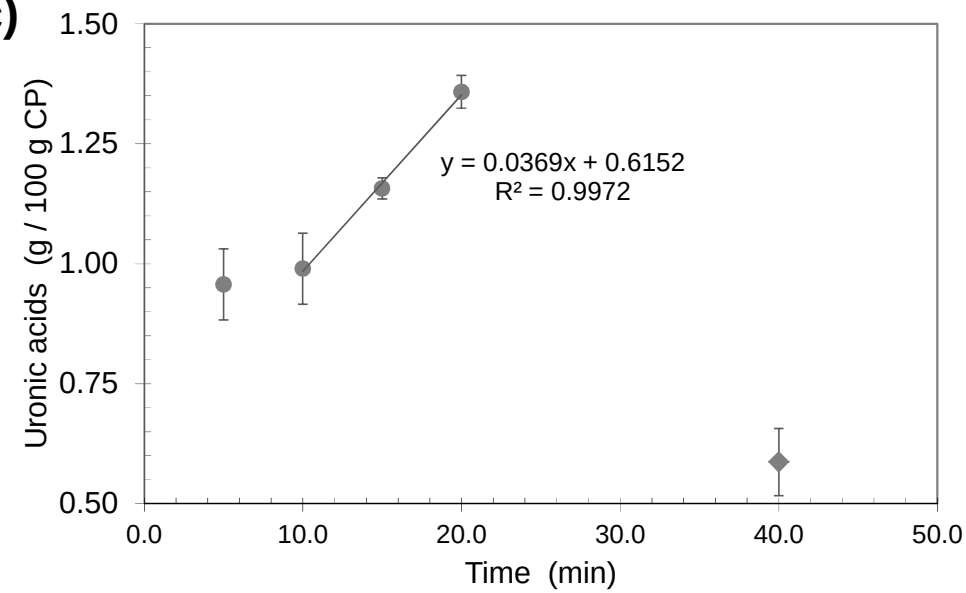
	% (w/w)[b]	
	0.1M Na_2CO_3 (1 h)	HPUS + 0.1M Na_2CO_3 (1 h)
Yield[a,b]	23±2[A]	35.4±0.9[B]
Proteins	5.4±0.2[A]	2.0±0.5[B]
Total carbohydrates	75±2[A]	85±3[B]
Uronic acids (UA)	39±2[A]	40±2[A]
DM (%, molar)	ND	ND
DA (%, molar)	5.8±0.6[A]	0.9±0.1[B]
Neutral sugars (NS)[c]	36.0	45.0
NS/UA[d] (molar ratio)	1.0	1.27
Molecular weight (Da)	130,013 52,623	119,240 45,266 35,940
*L** (%)	54.5±0.6[A]	45.7±0.4[B]
*a**	+34.2±0.2[A]	+36.9±0.3[B]
*b**	+36.8±0.3[A]	+39.5±0.7[B]
α-carotene (mg/100g powder)	15±3[A]	8±4[B]
β-carotene (mg/100g powder)	22±4[A]	12±7[A]
Lutein (mg/100g powder)	0.88±0.03[A]	0.42±0.02[B]
γ-tocopherol (mg/100g powder)	1.2±0.2[A]	0.7±0.1[B]

[a]Mean and standard deviations for n = 3 or n = 10 for color parameters are reported. The same capital letters as superscripts for results in a given row mean non-significant differences (p<0.05).
[b]Yield was calculated as g/100 g of carrot powder, while the rest of the chemical components are expressed as % w/w with respect to the corresponding PEF.
[c]Neutral sugars are calculated as the arithmetical difference between Total carbohydrates and Uronic acids-content.
[d]Moles of NS were calculated with a weighted average monosaccharide molar mass of 166.73 g/mol considering the typical monosaccharide composition of pectins (Basanta et al., 2013).
ND: non-detectable. DM: Degree of methylation. DA: Degree of acetylation.

Fig. 1.


(a)
60.0
50.0
40.0
30.0
20.0
10.0
0.0
% w/w
53
105
210
420
840
Average particle size (µm)
(b)
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
A
B
C
D
E
g reducing CH-Glc/100g dry CP
g NS/100g dry CP
g Proteins/100g dry CP

(c)


1.50
1.25
1.00
0.75
0.50
Uronic acids (g / 100 g CP)
y = 0.0369x + 0.6152
R² = 0.9972
0.0
10.0
20.0
30.0
40.0
50.0
Time (min)

(d)

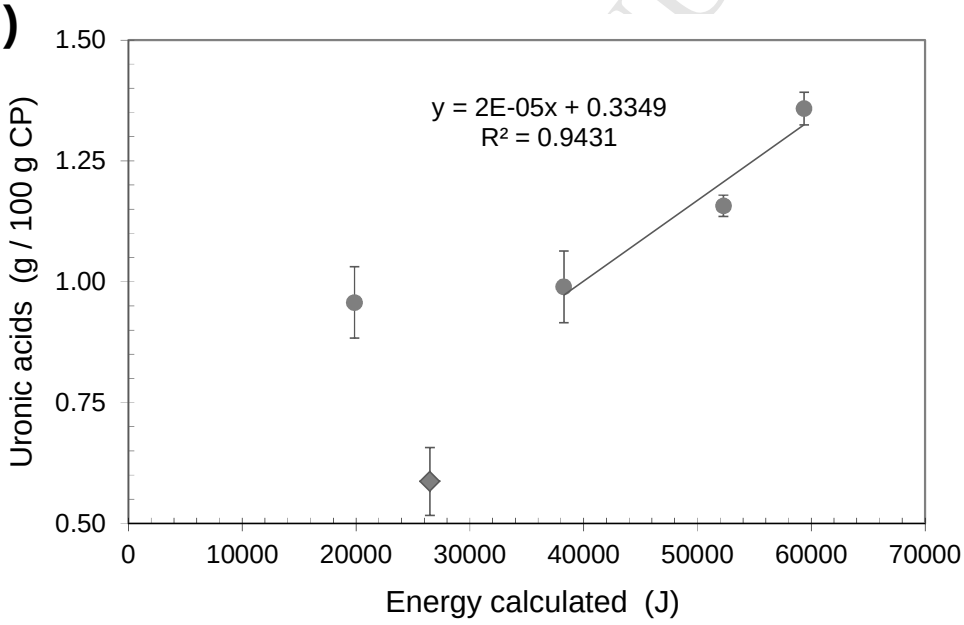
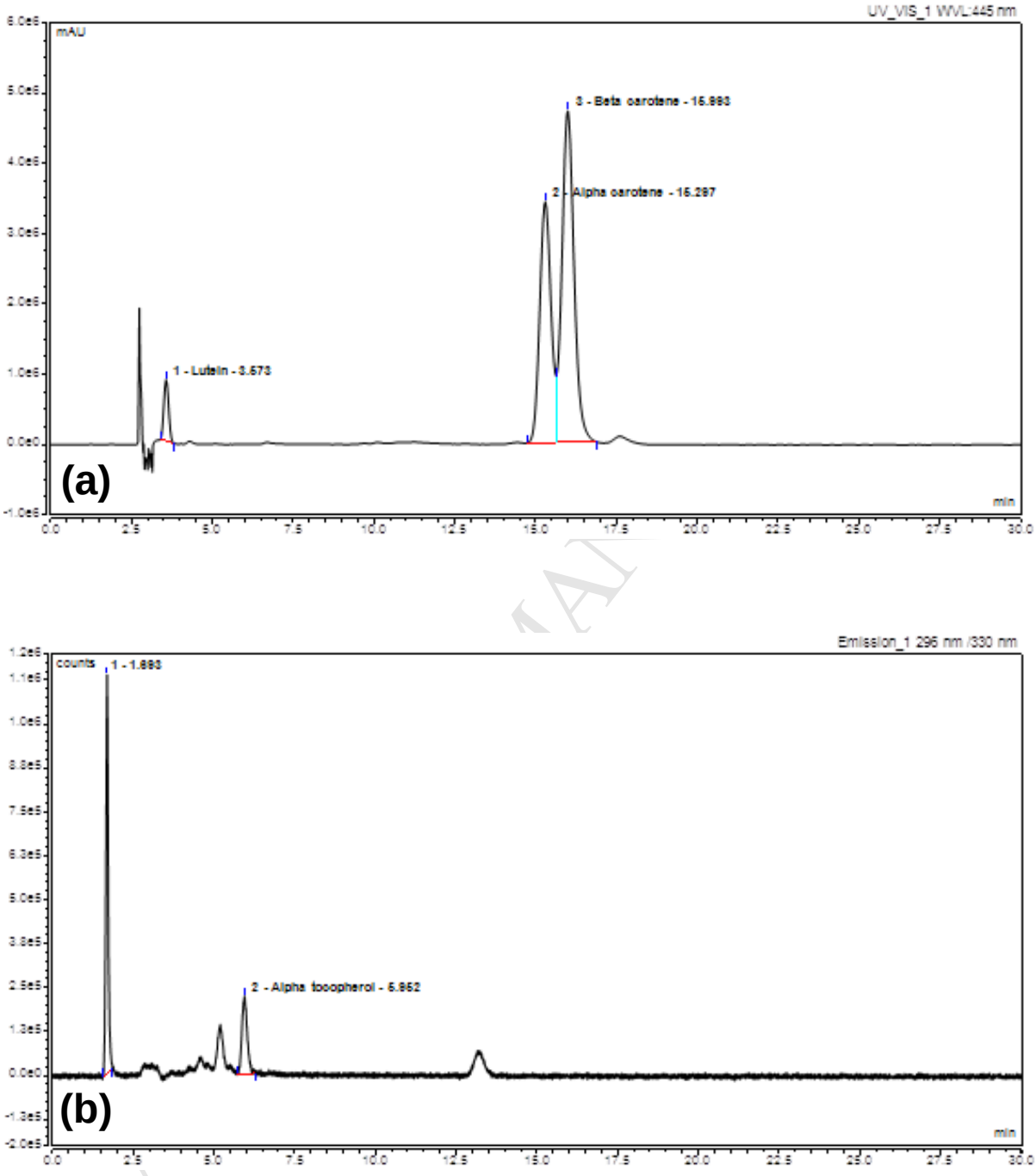

1.50
1.25
1.00
0.75
0.50
Uronic acids (g / 100 g CP)
y = 2E-05x + 0.3349
R² = 0.9431
0
10000
20000
30000
40000
50000
60000
70000
Energy calculated (J)

Fig. 2.


UV_VIS_1 WVL:445 nm
mAU
3 - Beta carotene - 16.883
2 - Alpha carotene - 16.287
1 - Lutein - 3.573
(a)
min
Emission_1 296 nm /330 nm
counts
1 - 1.883
2 - Alpha tocopherol - 5.952
(b)
min

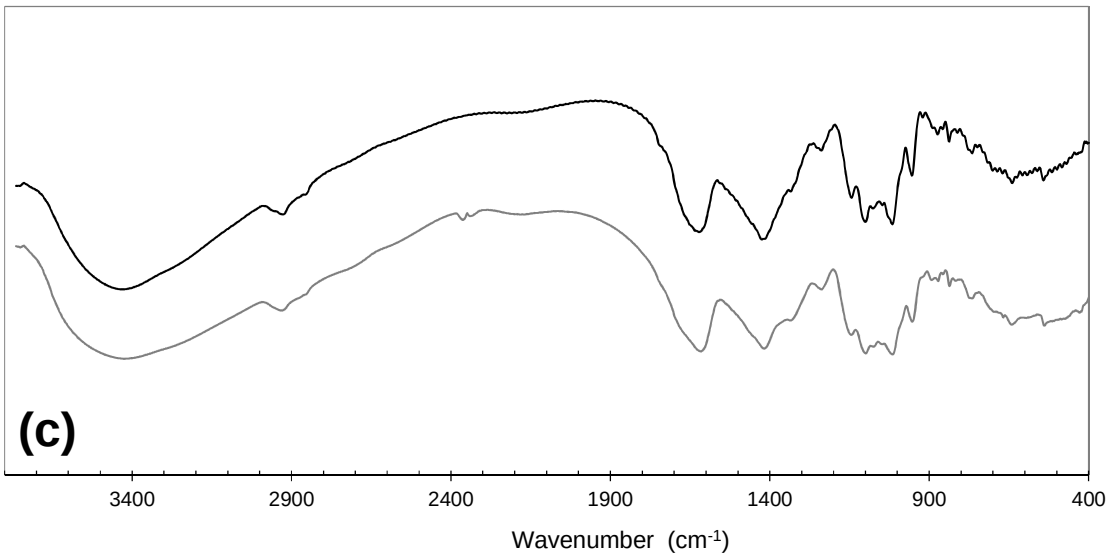
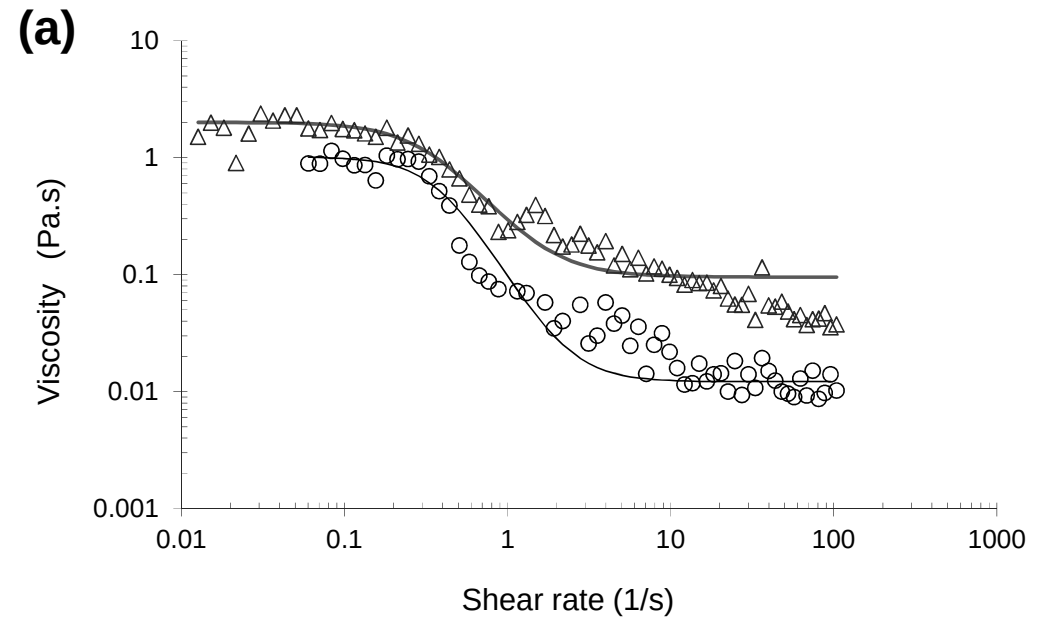
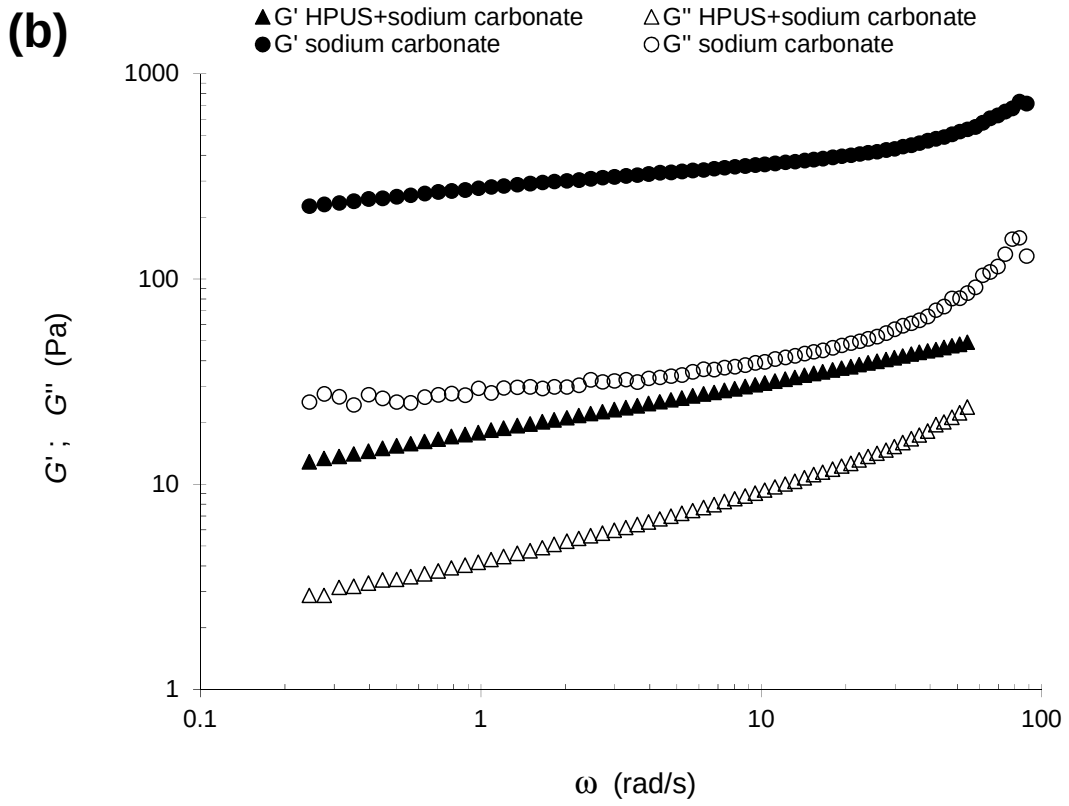


Fig. 3.


(a)
Viscosity (Pa.s)
10
1
0.1
0.01
0.001
0.01
0.1
1
10
100
1000
Shear rate (1/s)


(b)
▲G' HPUS+sodium carbonate
△G'' HPUS+sodium carbonate
●G' sodium carbonate
○G'' sodium carbonate
G' ; G'' (Pa)
1000
100
10
1
0.1
1
10
100
ω (rad/s)

HIGHLIGHTS

Discarded carrots produced gelling pectin-enriched fractions (PEF) with antioxidants

High-power ultrasound (HPUS) power intensity > 10W/cm^2 altered carrot powder matrix

HPUS pre-treatment and 1h-sodium carbonate extracted the whole powder pectin content

HPUS increased PEF yield and decreased methylation degree and molecular weight of PEF

HPUS-PEF produced higher Newtonian viscosity and calcium-gels of lower elastic *G*'