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Upgrading of residues of bracts, stems and hearts of *Cynara cardunculus* L. var. *scolymus* to functional fractions enriched in soluble fiber

Eliana N. Fissore,†[a] Cinthia Santo Domingo,‡[a] Carlos A. Pujol,[b] Elsa B. Damonte,†[b] Ana M. Rojas†[a] and Lía N. Gerschenson†*[a]

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Since only the central portion of the immature flowers of artichoke (*Cynara cardunculus* L. var. *scolymus*) is consumed (<20%) it is interesting to upgrade its residues to render value added products. In this research, bracts (B), hearts (H) or stems (S) were used to isolate fractions enriched in soluble fiber. Extraction was performed in citrate buffer with or without hemicellulase. Additionally, the effect of preheating (70 °C – 5 min) prior to extraction was also tested. Polysaccharides were precipitated with ethanol and the fractions obtained were freeze-dried. The presence of the enzyme increased fiber yields and preheating produced an additional increment, especially from stems (≈21%). Isolated fibers were constituted by 70–84% of carbohydrates and 2–25% of proteins, and contained phenolics (2.1–8.2 g/100 g). Carbohydrates included uronic acids (12–25%) and neutral sugars (NS, 4–55%) of pectins, and inulin (13–55%). The lowest protein and NS contents and the highest inulin content were obtained with the enzyme and preheating. The behavior of fractions isolated with higher yields was characterized, observing a pseudoplastic behavior in water and gelation with Ca^{2+}. They also showed antioxidant activity and an inhibitory effect against herpes simplex virus type 1 without cytotoxicity. The isolated fractions retaining bioactive compounds can be useful as functional food ingredients.

1. Introduction

Globe artichoke *Cynara cardunculus* L. var. *scolymus*, an edible vegetable native to the Mediterranean area and an important component of the Mediterranean diet, has been well appreciated since ancient times as a tasty food with therapeutic properties towards human health, being a good source of natural antioxidants like cynarin (1,3-*O*-dicaffeoylquinic acid), a choleretic and liver protector, and inulin. Today, artichoke heads production is widely diffused all over the world.[1,2] About 120 000 ha are dedicated to artichoke growth in the world and 90% of it is located in the countries of the Mediterranean basin. It was brought to America by Italian immigrants and reached a remarkable development in the U.S.A. and Argentina,[3] the country that had been since 2006 and until 2009, the fifth largest producer of artichoke.[4]

[a]Industry Department, Natural and Exact Sciences School (FCEN), Buenos Aires University (UBA), Ciudad Universitaria, Intendente Güiraldes 2620, (1428) Ciudad Autónoma de Buenos Aires, Argentina. E-mail: lia@di.fcen.uba.ar; Fax: +54-11-4576-3366; Tel: +54-11-4576-3366

[b]Biological Chemistry Department, FCEN, UBA, IQUIBICEN-National Scientific and Technical Research Council (CONICET), Argentina

† Member of the National Scientific and Technical Research Council of Argentina (CONICET).

‡ Fellow of the National Scientific and Technical Research Council of Argentina (CONICET).

The edible part of the plant is the enlarged receptacle and the tender thickened bract base on the head (*capitulum*), which is the immature inflorescence, is used worldwide as both a fresh and canned delicacy product. As a consequence, the edible fraction is less than 15–20% of the total plant biomass. This percentage decreases further if the contribution to the total biomass represented by offshoots, removed from the field by common cultural procedures, is also considered.[1] Vegetables discarded at harvesting or residues developed after processing constitute a valuable and renewable source of biopolymers and bioactive compounds. Upgrading of vegetable wastes can contribute not only to reduce pollution but also to add value to the commodity production.[5]

Positive health effects along with the ability to improve rheological and nutritional properties allow inulin and pectins to be used for functional foods.[1,6] Inulin is an alternative storage carbohydrate found in the vacuole of approximately 15% of all flowering plant species. It is a non-digestible oligosaccharide constituted by D-fructose units joined by $\beta(2 \rightarrow 1)$ linkages in a linear form and terminated with a D-glucose molecule linked to fructose by an $\alpha(1 \rightarrow 2)$ bond, as in sucrose.[7] Inulin can preferentially stimulate the growth and activity of one or a limited number of desired bacteria in the colon, and thus can improve host health, functioning as a prebiotic.[8,9] Also, positive effects on blood glucose attenuation, lipid homeostasis, mineral bioavailability and immunomodulation effects, along with the ability to

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add texture and improve rheological characteristics and nutritional properties of food, allow inulin to be termed as a functional food ingredient.[10] On the other hand, pectin is an abundant, ubiquitous and multifunctional component of the cell wall of all land plants.[11] Pectic polysaccharides consist mostly of polymers rich in galacturonic acid (GalA) and often contain significant amounts of rhamnose, arabinose and galactose as well as other 13 different monosaccharides.[12] The three major pectic polysaccharides currently defined are homogalacturonan (HG), rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II).[12,13] The composition, structure, and physiological properties of pectin are in part influenced by conditions of extraction as well as sources, location and many other environmental factors. The pectin network must be in part disrupted to enable extraction from the cell wall biopolymer network. This may involve extraction with calcium-chelating agents, diluted alkali, or diluted acid. Alternatively, pectic polysaccharide fragments can be released through the use of cell wall degrading enzymes. Pectins are rheological modifiers useful for pharmaceutical and food formulation. Also, they are bioactive macromolecules.[14] In this field, natural polysaccharides were proved to be selective inhibitors of herpes simplex viruses (HSV) and also natural products from medicinal plant extracts were informed as an important source of anti-HSV agents.[15,16] HSV are disseminated worldwide and can cause a variety of inapparent, mild or severe human infections, including oral disease, keratoconjunctivitis, encephalitis and genital infections.[17] A very effective treatment for HSV has been available since the introduction of acyclovir in the 1970s and it is still the most commonly used chemotherapy.[18] However, this compound is not always well tolerated and acyclovir-resistant strains frequently emerge during long term antiviral treatment in immunocompromised patients.[19] Therefore, the discovery of new non-nucleoside antiviral compounds is of significant interest.[20]

The aim of the present work was to upgrade by-products of globe artichoke commercialization and industrialization to fractions enriched in soluble fiber. Their composition, capacity for acting as rheological modifiers and their antioxidant and antiviral activity against HSV were evaluated in order to determine the potential of the isolated fractions as functional food ingredients.

2. Materials and methods

2.1. Sample preparation

Artichokes (*Cynara cardunculus* L. var. *scolymus*) harvested in Argentina were bought in the local market. Bracts, hearts and stems were separated, washed with distilled water, dried (85 °C, 2.5 h) in a convection oven (0.508 m s^{-1} of air rate), milled (E909, Wemir, Buenos Aires, Argentina) and sieved for obtaining powders enriched in cell wall materials (CWMs) with particle sizes in the range 420–710 μm.

Each CWM was treated as follows:

- Samples with no pre-heating:

10 g of CWM were poured into a beaker containing 1000 mL of 0.05 mol L^{-1}-sodium citrate buffer solution (pH 5.20) with 0.01 g/100 g of sodium azide (final concentration). The system was then submitted to digestion under stirring (10 rad s^{-1}), either without or with a cell wall (CW) degrading enzyme. The enzyme used was hemicellulase (H2125; SIGMA, St. Louis, USA) and 0.25 g was added when corresponded.

- Samples with pre-heating:

10 g of CWM were poured into a beaker containing 1000 mL of 0.05 mol L^{-1}-sodium citrate buffer solution (pH 5.2) with 0.01 g/100 g of sodium azide (final concentration). The system was heated at 70 °C, 5 min under stirring and then cooled to 30 °C. The digestion was performed at this temperature under constant stirring for 20 h either without (SE) or with 0.25 g hemicellulase (H1) addition. As it is known that the solubility of inulin in water remarkably increases with temperature,[21] the purpose of the heating step was to increase inulin content in fractions isolated and, as a consequence, the fraction yield.

The following fractions were obtained:

• H1S: product obtained from the treatment of artichoke stem (S) CWM with hemicellulase.

• SES: product obtained from the treatment of artichoke stem (S) CWM with no enzyme addition.

• QH1S: product obtained from the treatment of artichoke stem (S) CWM with pre-heating and with hemicellulase.

• QSES: product obtained from the treatment of artichoke stem (S) CWM with pre-heating and with no enzyme addition.

• H1H: product obtained from the treatment of artichoke heart (H) CWM with hemicellulase.

• SEH: product obtained from the treatment of artichoke heart (H) CWM with no enzyme addition.

• QH1H: product obtained from the treatment of artichoke heart (H) CWM with pre-heating and with hemicellulase.

• QSEH: product obtained from the treatment of artichoke heart (H) CWM with pre-heating and with no enzyme addition.

• H1B: product obtained from the treatment of artichoke bracts (B) CWM with hemicellulase.

• SEB: product obtained from the treatment of artichoke bracts (B) CWM with no enzyme addition.

• QH1B: product obtained from the treatment of artichoke bracts (B) CWM with pre-heating and with hemicellulase.

• QSEB: product obtained from the treatment of artichoke bracts (B) CWM with pre-heating and with no enzyme addition.

Deionized (Milli-Q™, USA) water was used for all treatments. Insoluble solid obtained after digestions was separated through filtration under vacuum, with glass fiber filter paper (Schleicher & Schuell, Dassel, Germany), and cell wall polysaccharides (CWP) were finally precipitated from each supernatant through ethanol (96%, v/v) addition (2 volumes). The precipitate was collected through filtration under vacuum using glass fiber filter paper, washed and, finally, freeze-dried.

In each case, yield was calculated as g of product obtained per 100 g of CWM used.

2.2. Chemical analyses

Deionized (Milli-Q™, USA) water and analytical grade reagents (Merck-Argentina and Sigma-Aldrich, USA) were used for preparation of all dissolutions utilized. The following analyses were performed on each sample obtained for the estimation of:

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(a) *Uronic acids* through a spectrophotometric method.[22]

(b) *Total carbohydrates* through a spectrophotometric method.[23]

(c) *Methanol* through a spectrophotometric method.[24]

(d) *Acetyl groups* through a spectrophotometric method.[25]

(e) *Degree of methylation* (DM) was calculated as the percent ratio between moles of methanol and moles of GalA in the analyzed sample, whereas the degree of acetylation (DA) was calculated as the percent ratio between moles of the acetyl group and moles of GalA in the samples.

(f) *Protein content* through a spectrophotometric method.[26]

(g) *Inulin content* was determined using the fructan assay procedure for the measurement of fructo-oligosaccharides and fructan polysaccharide (Megazyme, Ireland).

(i) *Total phenolic content* was determined using the Folin-Ciocalteau reagents after saponification of fiber products to release ester linked phenolics.[27,28]

2.3. Antioxidant activity

The antioxidant activity was measured through the DPPH assay that allows determining the ability of sample compounds to act as free radical scavengers or hydrogen donors.[29]

2.4. Rheological characterization

Systems containing a 2.00% (w/v) concentration of the different isolated products were used for comparison of their rheological behaviour.

Around 0.0400 g of each material was suspended in 1700 μl of deionized water and vortexed until complete hydration. Solutions were heated in a water bath at 65 °C until dissolution, with periodic homogenization in the vortex. Afterwards, the total volume was completed to 2000 μl either with deionized water or with a $CaCl_2$ aqueous solution at 65 °C (40 mg Ca^{2+} per g pectin) while vortexing. Then, systems were stored at 25 °C for 18 hours to attain swelling equilibrium before measurement.

Rheological characterization was performed at 25 °C using an MCR300 Paar Physica (shear) rheometer (Anton Paar, Austria) equipped with a serrated parallel plate (PP25/S) geometry (25 mm-diameter). Temperature was maintained constant with a Peltier system. A gap size of 1700 μm was set. Data points were recorded at a steady-state.

2.4.1. Flow assays. Fiber fractions dissolved in deionized water were submitted to these assays. The flow behavior was determined at 25 °C in the 0.001–50 s^{-1} shear rate ($\dot{\gamma}$) range. Determination of the viscosity curve over a wide range of shear rate values is essential to fit the data to different rheological models. The Cross model [eqn (1)] was considered in this work,

$$\frac{(\eta_{app} - \eta_\infty)}{(\eta_O - \eta_\infty)} = \frac{1}{1 + (\tau_{CR}\dot{\gamma})^m} \tag{1}$$

wherein η_{app} is the apparent viscosity, η_0 represents the zero shear rate or Newtonian viscosity, η_∞ represents the infinite shear rate viscosity, τ_{CR} is the time constant and m is a dimensionless parameter.

2.4.2. Dynamic assays. Fiber fractions dissolved in $CaCl_2$ aqueous solution were submitted to oscillatory assays at 25 °C. Amplitude (stress *versus* strain) sweeps were first performed at constant frequency (1 Hz) in order to determine the linear viscoelastic range for each gel system, from which the value of strain to subsequently record the mechanical spectra (frequency sweeps) was selected. These spectra were then obtained at a 0.04% constant strain. At this condition, the storage or elastic (G') and loss or viscous (G'') shear moduli as well as the tangent of the phase shift angle (δ) were recorded against angular frequency (ω). To fit the experimental data, two models were considered: (a) the power law type model,[30]

$$G'(\omega) = G'_O\omega^A \tag{2}$$

$$G''(\omega) = G''_O\omega^B \tag{3}$$

wherein A and B are the exponents related to the frequency dependence and G'_O and G''_O are the equilibrium moduli;

(b) the generalized Maxwell model, which derives from the parallel combination of "n" Maxwell elements (spring and dashpot in series) where one of them shows its characteristic relaxation time (λ_i) tending to infinity, giving rise to an equilibrium spring modulus (G_e).[31,32] The viscoelastic function exhibited by the Maxwell elements can be derived for the real and complex components of the relaxation modulus [$G(t)$], yielding the mathematical expressions for G' and G'' (shear moduli), as a discrete viscoelastic spectrum of relaxation times:

$$G'(\omega) = G_e + \sum_{i=1}^{N} G_i \frac{(\lambda_i\omega)^2}{1 + (\lambda_i\omega)^2}; G_i = \frac{\eta_i}{\lambda_i} \tag{4}$$

$$G''(\omega) = \sum_{i=1}^{N} G_i \frac{\lambda_i\omega}{1 + (\lambda_i\omega)^2}; G_i = \frac{\eta_i}{\lambda_i} \tag{5}$$

wherein λ_i is the ratio between the dashpot Newtonian viscosity (η_i) and the Hookean spring modulus (G_i). The N relaxation modes are defined by their relaxation times λ_i and strengths G_i, where G_i can be regarded as the density of the corresponding relaxation elements with respect to the relaxation spectra.[33]

The discrete viscoelastic spectra (G_i) and the relaxation times (λ_i) of the polysaccharide solutions obtained from each isolated pectin fraction were estimated from the experimental data of G' and G'' and from the values predicted by eqn (4) and (5), through an iterative process which minimizes the standard deviation (s):

$$s^2 = \frac{1}{M}\sum_{j=1}^{M}\left[\left(1 - \frac{1}{G'(\varpi_j)}\sum_{i=1}^{N}\frac{G_i(\varpi_j\lambda_i)^2}{1 + (\varpi_j\lambda_i)^2}\right)^2 + \left[1 - \frac{1}{G''(\varpi_j)}\sum_{i=1}^{N}\frac{G_i(\varpi_j\lambda_i)}{1 + (\varpi_j\lambda_i)^2}\right]^2\right] \tag{6}$$

For this purpose, the lowest but sufficient number (N) of relaxation times was maintained.[34] Hence, N parameters of G_i and λ_i were fitted, for a whole of M experimental values for $G'(\omega_j)$ and $G''(\omega_j)$, being necessarily $N < M$. The values of G_i and λ_i obtained as above indicated were applied for prediction of the G' and G'' values that allowed to obtain the curves that expressed the data trend. The percentages of mean error ($\bar{e}$%) between the experimental and predicted values for G' and G'' are also informed.

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2.5. Antiviral activity

2.5.1. Cells and viruses. Vero cells (African green monkey kidney) were grown in Eagle's minimum essential medium (MEM) supplemented with 5% calf serum. For maintenance medium (MM), the serum concentration was reduced to 1.5%. HSV-1 (herpes simplex virus type I) strain F was propagated and titrated by plaque formation in Vero cells.

2.5.2. Cytotoxicity assay. Vero cell viability was measured by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; Sigma-Aldrich) method. Confluent cultures in 96-well plates were exposed to different concentrations of the samples, with three wells for each concentration, using incubation conditions equivalent to those used in the antiviral assays. Then 10 μl of MM containing MTT (final concentration 0.5 mg ml^{-1}) was added to each well. After 2 h of incubation at 37 °C, the supernatant was removed and 200 μl of ethanol was added to each well to solubilize the formazan crystals. After vigorous shaking, absorbance was measured in a microplate reader at 595 nm. The cytotoxic concentration 50% (CC_{50}) was calculated as the compound concentration required to reduce cell viability by 50%.

2.5.3. Antiviral assay. Antitiviral activity was evaluated by a virus plaque reduction assay. Vero cell monolayers grown in 24-well plates were infected with about 50 PFU (plaque forming units) per well in the absence or presence of various concentrations of the samples. After 1 h of adsorption at 37 °C, residual inoculum was replaced by MM containing 0.7% methylcellulose and the corresponding dose of each sample. Plaques were counted after 2 days of incubation at 37 °C. The inhibitory concentration 50% (IC_{50}) was calculated as the compound concentration required to reduce virus plaques by 50%. All determinations were performed twice and each in duplicate.

2.5.4. Expression of the antiviral activity. The antiviral activity was expressed by means of the selectivity index which is calculated as the ratio between CC_{50} and IC_{50}.

2.6. Statistical analyses

Non-linear fittings were performed through Prism 5 Statistical Software for Windows (GraphPad, USA), as well as through the Solver function of the Excel program for Windows XP (Microsoft, USA). Statistical analyses for result comparisons were carried out through ANOVA (level of significance, α: 0.05) followed by pairwise multiple comparisons using Tukey's significant difference test.[35]

3. Results and discussion

3.1. Chemical composition of fractions

The yield and chemical composition of the fractions isolated are summarized in Table 1. As can be observed, in general, the yield was at least doubled when the CWM was pre-heated at 70 °C for 5 min before incubation at 30 °C with or without hemicellulase. Artichoke stems showed higher yields of biopolymers than bracts and hearts (Table 1).

In general, treatment with hemicellulase produced a higher yield. After pre-heating, the yield with the enzyme showed a value of ≈10% in bracts (QH1B) and ≈21% in stems (QH1S) (Table 1). Hence, the hemicellulase activity and the heating were certainly decisive to achieve higher yields. Hemicellulase is a group of cell wall degrading enzymes such as xylanases, mannases, arabinases (both endo and exo kinds) and their corresponding glycosidases.[36] Debranching enzymes remove hemicellulose substituents and hence weaken the hemicellulose–cellulose network.[37] It is important to remark that, according to providers, the hemicellulase herein used has side cellulase activity which can result in a certain degree of cellulose framework disruption.

Inulin located in the vacuoles of artichoke cells also precipitated with the cell wall biopolymers of artichoke (Table 1) because it is soluble in hot water and insoluble after the addition of ethanol.[38] Therefore, the proportion of inulin extracted in conjunction with the cell wall polymers increased significantly (p < 0.001) from 13–25% to 38–55% when the artichoke powders were preheated at 70 °C for 5 min (Table 1).

More than 70% of the isolated biopolymers were constituted by polysaccharides including inulin, and the rest by cell wall proteins which may be ascribed to extensin type proteins rich in hydroxyproline.[39,40] The extensin extracted by the ionic aqueous medium used (citrate buffer) probably corresponds to the protein ionically related to the pectins extracted in these soluble fractions.[39] The proportion of proteins in the fractions, decreased after pre-heating (Table 1).

The uronic acid content of the biopolymers extracted varied between 14 and 25% (Table 1). It corresponds to the HG smooth regions of pectins.[12] The NS content varied between 0.8 and 54%, showing lower values in fractions obtained after pre-heating and hemicellulase treatments, especially for stems (Table 1). Pectin content, in general, decreased slightly as inulin proportion increased when the pre-heating treatment was applied and these pectins were in general characterized by a low DM (Table 1).

Phenolics can be co-extracted with the cell wall polysaccharides to which they are associated.[41] Phenolic content was detected in fractions submitted to pre-heating treatment and varied between 2.1 and 8.2 g/100 g of the fractions (Table 1). Antioxidant activity has been linked to phenolic compounds.[42] As can be observed in Fig. 1, the QHIH fraction which showed the highest phenolic content, had the greater ability to act as a free radical scavenger or a hydrogen donor as measured through the DPPH assay, whereas QSEB, QH1B and QSEH presented the lowest phenolic contents and antioxidant activity. On the other hand, QH1S was the only sample for which its phenolic content (Table 1) did not parallel its antioxidant activity when measured through the radical scavenger activity (Fig. 1). Some decrease in the percentage of DPPH free radical capture is observed beyond 300 min of assay probably due to the depletion of the compounds responsible for antioxidant activity. The advantage of this method is that DPPH is allowed to react with the whole sample and that sufficient time given in the method allows DPPH to react slowly even with weak antioxidants.[43]

3.2. Rheological characterization of the isolated fractions

Only the polymeric fractions isolated from artichoke with the highest yields (QSE and QH1 from bracts, hearts and stems;

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Table 1 Chemical composition of the soluble fibers isolated from bracts, hearts and stems of artichoke[a,b]

	Yield (%)	Total carbohydrates (%)	Uronic acids (%)	Inulin (%)	Neutral sugars (%)	Protein (%)	Total phenolics (%)	Methanol (%)	DM	Acetyl groups (%)	DA
SEB	2.7	82 ± 2	20 ± 1	20.2 ± 0.3	41.8	11 ± 3	nd	nd	nd	nd	nd
H1B	4.9	84 ± 3	17 ± 1	13.2 ± 0.3	53.8	15 ± 1	nd	nd	nd	nd	nd
QSEB	6.0	76 ± 7	14.2 ± 0.2	44.7 ± 0.3	17.1	2.7 ± 0.3	2.1 ± 0.1	0.8 ± 0.1	31	0.36 ± 0.02	10.3
QH1B	9.6	83.3 ± 0.1	14.0 ± 0.1	38 ± 1	31.3	1.8 ± 0.5	3.1 ± 0.2	0.8 ± 0.1	31	0.30 ± 0.01	8.8
SES	6.0	70.1 ± 0.5	12.1 ± 0.4	25.0 ± 0.2	33.0	25 ± 2	nd	nd	nd	nd	nd
H1S	6.7	84 ± 1	19 ± 2	20.5 ± 0.1	44.5	20.0 ± 0.1	nd	nd	nd	nd	nd
QSES	13.6	72 ± 6	18.2 ± 0.2	53.0 ± 0.1	0.8	9.2 ± 0.2	4.8 ± 0.2	1.1 ± 0.2	33	0.36 ± 0.01	8.05
QH1S	20.8	76 ± 7	15.0 ± 0.1	46.0 ± 0.4	15.0	6.8 ± 0.1	6.9 ± 0.2	0.4 ± 0.1	15	0.37 ± 0.06	10.2
H1H	3.0	74 ± 1	25 ± 1	14.9 ± 0.1	34.1	19 ± 1	nd	nd	nd	nd	nd
SEH	5.3	80 ± 3	15.0 ± 0.3	13.9 ± 0.2	51.1	14 ± 2	nd	nd	nd	nd	nd
QSEH	7.5	79 ± 6	15.1 ± 0.1	55.0 ± 0.1	8.9	5.8 ± 0.1	4.0 ± 0.1	1.6 ± 0.1	58	0.38 ± 0.05	10.3
QH1H	8.0	96.8 ± 0.3	14 ± 1	38.5 ± 0.2	44.3	7.9 ± 0.1	8.2 ± 0.2	1.0 ± 0.2	39	0.32 ± 0.01	9.3

[a] Yield was calculated as g of the product obtained per 100 g of CWM used. Chemical composition of the products is expressed as g component per 100 g of the isolated fraction. DM and DA were calculated as a ratio between moles of methanol or the acetyl group and moles of GalA (uronic acids) per 100 g of the sample, respectively. [b] nd: non determined.

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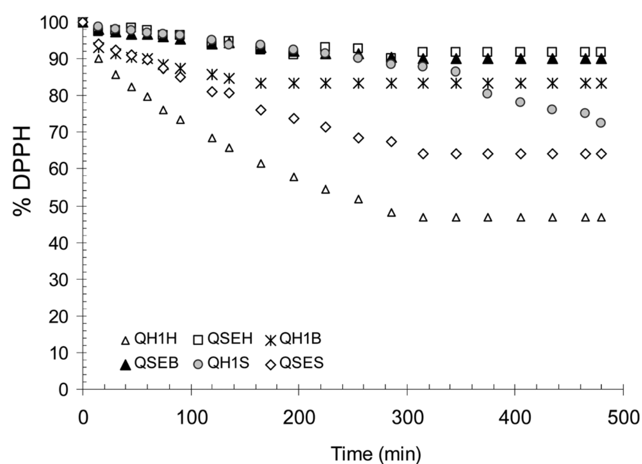

Fig. 1 Time course of scavenging of DPPH free radical by the soluble fibers extracted from bracts, hearts and stems of artichoke.

Table 1) were evaluated in relation to their rheological behavior at a 2.00% (w/v) concentration.

3.2.1. Flow assays. Viscosity was determined at 25 °C under increasing shear rates between 0.001 and 50 s^{-1} (Fig. 2A) for the artichoke fibers dissolved in deionized water. Experimental data were fitted through the Cross model [eqn (1)] and the parameters obtained are reported in Table 2. QH1H and QSEH solutions showed the highest Newtonian viscosity (η_0, 22 and 14 Pa s) at the lower shear rate values, whereas η_0 took values in the range 3–5 Pa s for the rest of the systems. Polysaccharides like pectins constitute aqueous solutions with viscoelasticity.[33,44] When individual macromolecules interpenetrate each others' domains, there is a rather sudden change in flow properties, exemplified by more pronounced increases in both the zero-shear viscosity (η_0) and the shear rate dependence of viscosity (Fig. 2A). In the shear rate range of Newtonian viscosity, there is enough experimental time to develop new interactions among the initially disrupted intermolecular entanglements.[45] At higher shear rates, a shear thinning or pseudoplastic behaviour can be clearly observed, which onset occurred when the rate of externally imposed motion became progressively greater than the rate of formation of new entanglements and, thus, the "cross-link density" of the network was depleted and viscosity was reduced.[46] The shear rate value at the onset of the shear thinning ($\dot{\gamma}_{CRIT}$) was higher for the QH1H (the lowest structural relaxation time; Table 2) trend that showed that aqueous systems constituted by this fraction were the least stable. QSEB and QSEH fractions showed the highest structural relaxation time τ derived from data fitting to the Cross equation (Table 2). This means that steady-intermolecular entanglements or macromolecular associations were more stable. A more stable network constituted by the hydrated polysaccharides needs more time for relaxation. In general, the high-shear-rate viscosity (η_∞) was experimentally inaccessible except for QSEH (Fig. 2A). This type of shear-thinning behaviour shown in Fig. 2A is usual for random coil polysaccharides as reported in the literature.[47]

3.2.2. Oscillatory assays. Artichoke fractions isolated through a pre-heating step showed, in general, a low degree of methyl-esterification (Table 1) and their aqueous solutions were then assayed in the presence of Ca^{2+}. Their mechanical spectra recorded at 25 °C showed values of G' one order of magnitude above G'' along the three decades of angular frequency swept, while tan δ (G''/G') was closer to zero (Fig. 2B). For clarity, only the mechanical spectra of some fiber fraction systems are shown in Fig. 2B. These profiles can be in general ascribed to viscoelastic solids except for the slight frequency dependence observed, which is typical of physical gels like those produced by polysaccharides swelled in water.[48] A power law type model was used (eqn (2) and (3))[30] to fit the experimental data points (Fig. 2B) in order to know the degree of frequency dependence (A and B exponents) and the parameters obtained are summarized in Table 3. Model fitting is showed by the continuous lines presented in Fig. 2B. Aqueous systems of QH1H, which showed the highest carbohydrate content, did not fit to the power law

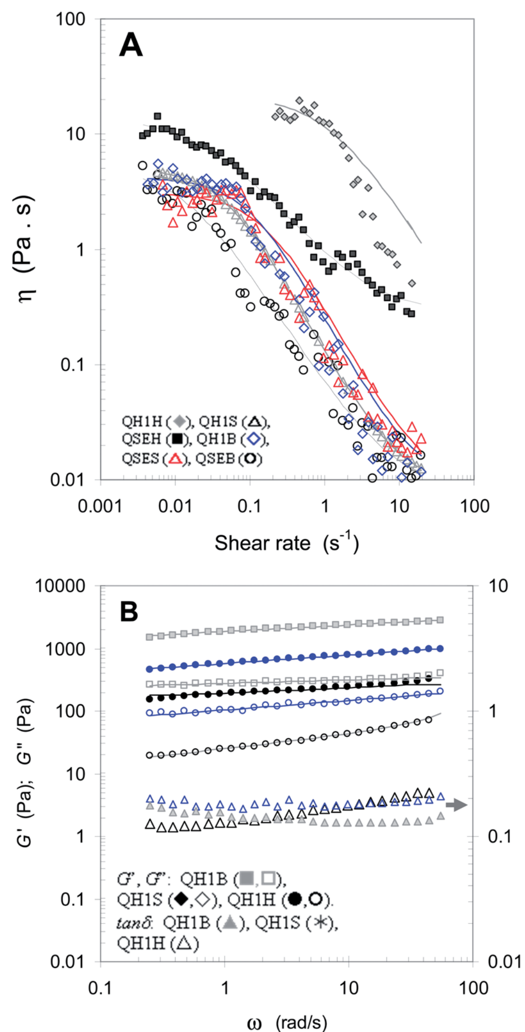

Fig. 2 Aqueous systems constituted by artichoke soluble fibers (2.0% w/v) measured through (A) flow assays, or (B) dynamic assays: mechanical spectra of QH1B, QH1S and QH1H samples, and arrow indicates the tan δ axis for the tan δ-points. Continuous lines correspond to the model fitted.

Table 2 Cross model-parameters calculated from the flow assays (25 °C) for 2.0% (w/v) aqueous solutions of the artichoke soluble fractions[a,b]

Sample	η_∞ (Pa s)	η_0 (Pa s)	τ (s)	m	R^2
QSEB	0.008 ± 0.001	5.2 ± 0.9	77 ± 9	1.0 ± 0.2	0.909
QH1B	0.009 ± 0.003	4.2 ± 0.3	10 ± 2	1.1 ± 0.1	0.940
QSEH	0.27 ± 0.09	14 ± 1	34 ± 8	0.84 ± 0.09	0.943
QH1H	0.001 ± 0.002	22 ± 7	0.86 ± 0.9	1.1 ± 0.3	0.834
QSES	0.009 ± 0.002	3.1 ± 0.3	6 ± 1	1.1 ± 0.2	0.943
QH1S	0.009 ± 0.002	5.1 ± 0.9	22 ± 7	1.0 ± 0.3	0.828

[a] Mean and standard errors are shown (n = 3). R^2: goodness of fit. [b] η_0 represents the Newtonian viscosity or the viscosity at zero shear rate; η_∞ represents the infinite or residual viscosity, τ is the time constant and m is a dimensionless parameter.

but did fit to the generalized Maxwell model (eqn (4) and (5)) (continuous lines for G' and G'' in Fig. 2B) with seven relaxation times (λ_i) that resulted logarithmically spaced between 10^{-5} and

Table 3 Dynamic rheological assays. Parameters obtained by fitting experimental data to power law type model for 2.0% (w/v) aqueous (40 mg Ca^{2+} per g pectin) solutions of the artichoke fractions[a]

Artichoke fibers	G'			
	G'_O (Pa)	A	R^2	P
QSEB	1407 ± 4	0.098 ± 0.001	0.997	0.222
QH1B	1867 ± 13	0.106 ± 0.003	0.985	<0.0001
QSEH	788 ± 8	0.139 ± 0.004	0.981	0.006
QSES	1079 ± 8	0.123 ± 0.003	0.987	<0.0001
QH1S	577 ± 2	0.135 ± 0.003	0.998	0.0013
Artichoke fibers	**G''**			
	G''_O (Pa)	B	R^2	P
QSEB	191 ± 3	0.104 ± 0.007	0.909	0.021
QH1B	277 ± 2	0.056 ± 0.003	0.926	0.050
QSEH	136 ± 2	0.159 ± 0.006	0.971	0.006
QSES	202 ± 2	0.092 ± 0.004	0.965	0.306
QH1S	104 ± 2	0.153 ± 0.006	0.966	0.222

[a] Mean and standard deviation (n = 3) are shown.

10^2 s.[31] It was also necessary to consider an equilibrium spring modulus (G_e) to obtain a good adjustment between the predicted curve and the experimental G'-data. The $\bar{\varepsilon}$% between the experimental and predicted values were 2.7% for G' and 0.004% for G''.

Although pectins were present in lower proportions (14–18%) than inulin (38–55%) in these artichoke fractions (Table 1), it can be observed that pectins were able to constitute gels with different rheological characteristics in the presence of 40 mg Ca^{2+} per g pectin. At the free carboxylates of neighboring chains, calcium ions crosslinked pectins of low DM (Table 1).[49] The QH1H fraction produced a creamy and homogeneous gel in Ca^{2+} solution. This observation coincided with their adjustment to the Maxwellian model, which may be ascribed to a weak type gel. It also showed a slight minimum at 0.30 rad s^{-1} (Fig. 2B). This phenomenon may correspond to a period of oscillations which is long compared to the relaxation times of the network junction zones in water solvent but short compared to their lifetimes.[31] This system was also characterized by the lowest G' value (≈230 Pa) of the mechanical spectra (Fig. 2B) and the highest frequency dependence of its tan δ (G''/G') profile (Fig. 2B). The rest of the gel systems showed higher G' values (700–2300 Pa) and low dependence on frequency (Fig. 2B) as also shown by the "A" exponent values (Table 3), which permitted that experimental data adjusted to the power law (eqn (2) and (3)). Fractions isolated after pre-heating from bracts (QSEB, QH1B) and the fraction QSES isolated from stems were the least frequency dependent, especially for G'' (Table 3). These results coincided with rigid gels, where some degree of syneresis was observed, as well as for QH1S and QSEH. The difference observed in gel textures may be mainly ascribed to differential patterns of methyl-esterification or distribution of demethylated-blocks in the HGs of the pectin macromolecules.[11,50] Large demethylated blocks with a few points of methyl-esterification produce rigid

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and brittle gels.[11] Also, inulin might constitute a disturbing presence for gel network formation.

3.3. Antiviral activity

The antiviral activity against HSV-1 was studied on the fractions isolated with the highest yields from artichoke, which were those obtained by preheating. As seen in Table 4, all the fractions lacked cytotoxic effects for Vero cells up to the maximum concentration tested of 5000 μg ml^{-1}. More interestingly, all the fractions showed anti-HSV-1 activity. The highest inhibitory effect corresponded to the fractions obtained from stems and bracts, with selectivity indices in the range 29.5–55.4, whereas a lower but significant effectiveness was exhibited by the two heart fractions.

The antiviral activity of these polysaccharide-enriched fractions may be ascribed either to the carbohydrate component or to the associated polyphenolic compounds. A large number of small molecules, like phenolics, polyphenols, terpenes, flavonoids, sugar-containing compounds, were found to be promising anti-herpetic agents.[6] Caffeic acid was the most active compound against HSV-1 (IC_{50} = 15.3 μg mL^{-1}) with a selectivity index (SI) of 67.1 when the antiviral activity of aqueous extracts and pure compounds of *Plantago major* was examined.[51] Furthermore, the anti-herpectic activity attributable to phenolic compounds present in diverse medicinal plant extracts has been recently characterized.[52,53] With respect to polysaccharides, it is well known that the highest antiviral effectiveness of these polymers is directly related to their sulfate content, with carrageenans, dextran sulfate, mannans and fucoidans as very selective and potent inhibitors of HSV.[54] However, nonsulfated carbohydrate derivatives have been reported as active HSV inhibitors. In particular, a pectic polysaccharide obtained from *Portulaca oleracea* exhibited antiviral activity against HSV-2 by affecting virus penetration into the cell and a pectic arabinogalactan from leaves of *Stevia rebaudiana* showed antiviral activity *in vitro* against HSV-1.[55,56] A fructan with antiviral activity *in vitro* and *in vivo*, against the HSV-2 agent, was isolated from Chikuyo-Sekko-To (aerial parts of *Lophatherum gracile*, roots of *Panax ginseng*, roots of *Glycyrrhiza glabra* or *Glycyrrhiza uralensis*, tuberous roots of *Ophiopogon japonicus*, tuberous roots of *Pinellia ternate*, seeds of *Oryza sativa*and gypsum).[57] Also a fructan with antiviral activity against the HSV-2 agent, was isolated from a traditional Chinese medicinal herb, *Polygonatum cyrtonema* Hua.[58]

Further research is required to assess the mechanism of anti-HSV-1 activity of fractions studied here as well as to identify the active principle.

Table 4 Antiviral activity of the soluble fractions extracted with the highest yields from artichoke stems (QH1S, QSES), bracts (QH1B, QSEB) and hearts (QH1H, QSEH)[a,b]

Sample	CC_{50} (μg ml^{-1})	IC_{50} (μg ml^{-1})	SI (CC_{50}/IC_{50})
QH1S	>5000	169.4 ± 18.6	>29.5
QSES	>5000	90.2 ± 5.5	>55.4
QH1B	>5000	108.3 ± 0.4	>46.1
QSEB	>5000	150.0 ± 15.5	>33.3
QH1H	>5000	475.5 ± 43.2	>10.5
QSEH	>5000	728.9 ± 147.0	>6.8

[a] CC_{50}: cytotoxic concentration 50%. [b] IC_{50}: inhibitory concentration 50%. SI (CC_{50}/IC_{50}): selectivity index. Mean and standard deviation ($n = 3$) are reported.

4. Conclusions

Hemicellulase enhanced the extraction of soluble fibers mainly from stems of globe artichoke and pre-heating treatment (70 °C – 5 min) doubled the enzymatic yield because of the important increase in inulin, a polysaccharide with important immunomodulation effects. Cell wall network disorganization produced by hemicellulase decisively helped the extraction with or without pre-heating. Aqueous systems of the isolated fractions showed pseudoplastic behavior and constituted gels with different rheological characteristics in the presence of 40 mg Ca^{2+} per g pectin. In this way, the QH1H fraction produced a creamy and homogeneous weak gel in Ca^{2+} solution whereas the other soluble fiber fractions produced rigid gels. Different gel textures of the soluble fibers extracted by pre-heating may be ascribed to differential patterns of demethylated-blocks in the HGs of the pectin macromolecules, and also probably to the disturbing presence of inulin. The antioxidant capacity showed by the fractions can be attributed to the co-extracted phenolic compounds. The antiviral activity against HSV-1 might be ascribed to phenolic compounds and/or to polysaccharides. Residues of globe artichoke bracts, stems and hearts can be upgraded as fractions rich in soluble fibers and valuable as functional food ingredients that can also deliver phenolics into the gut exerting positive health effects.

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References

1 V. Lattanzio, P. A. Kroon, V. Linsalata and A. Cardinali, *J. Funct. Foods*, 2009, **1**, 131–144.
2 S. Lombardo, G. Pandino, R. Mauro and G. Mauromicale, *Ital. J. Agron.*, 2009, **4**, 181–189.
3 A. Castagnino, *Manual de Cultivos Hortícolas Innovadores*, ed. Hemisferio Sur, Buenos Aires, 2009, in Spanish.
4 FAO, *Food and Agriculture Organization of the United Nations*, FAOSTAT, 2011, http://faostat.fao.org/site/567/Desktop Default.aspx?PageID=567#ancor.
5 G. Laufenberg, B. Kunz and M. Nystroem, *Bioresour. Technol.*, 2003, **87**, 167–198.
6 S. Parkar, E. Redgate, R. Wibisono, X. Luo, E. Koh and R. Schröder, *J. Funct. Foods*, 2010, **2**, 210–218.
7 F. Miremadi and N. Shah, *Int. Food Res. J.*, 2012, **19**, 1337–1350.

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8 G. Gibson, *J. Nutr.*, 1999, **129**, 1438S–1441S.
9 D. López-Molina, M. Navarro-Martínez, F. Rojas-Melgarejo, A. Hiner, S. Chazarra and J. Rodríguez-López, *Phytochemistry*, 2005, **66**, 1476–1484.
10 W. Lingyun, W. Jianhua, Z. Xiaodong, T. Da, Y. Yalin, C. Chenggang, F. Tianhua and Z. Fan, *J. Food Eng.*, 2007, **79**, 1087–1093.
11 W. Willats, J. Knox and J. Mikkelsen, *Trends Food Sci. Technol.*, 2006, **17**, 97–104.
12 J. Vincken, H. Schols, R. Oomen, G. Beldman, R. Visser and A. Voragen, in *Advances in Pectin and Pectinase Research*, ed. F. Voragen, H. Schols and R. Visser, Kluwer Academic Publishers, Boston, 2003, pp. 47–59.
13 K. Waldron, M. Parker and A. Smith, *Compr. Rev. Food Sci. Food Saf.*, 2003, **2**, 128–146.
14 E. Maxwell, N. Belshaw, K. Waldron and V. Morris, *Trends Food Sci. Technol.*, 2012, **24**, 64–73.
15 M. Abad Martinez, L. Bedoya del Olmo and P. Bermejo Benito, in *Studies in Natural Products Chemistry*, ed. Atta-ur-Rahman, Elsevier, USA, 2005, vol. 30, pp. 393–418.
16 M. Khan, A. Ather, K. Thompson and R. Gambari, *Antiviral Res.*, 2005, **67**, 107–119.
17 R. J. Whitley and B. Roizman, in *Clinical Virology*, ed. D. D. Richmann, R. J. Whitley and F. G. Hayden, ASM Press, Washington, 2009, pp. 409–436.
18 R. C. Brady and D. J. Bernstein, *Antiviral Res.*, 2004, **61**, 73–81.
19 J. Piret and G. Boivin, *Antimicrob. Agents Chemother.*, 2011, **55**, 459–472.
20 G. Kleymann, *Expert Opin. Invest. Drugs*, 2005, **14**, 135–161.
21 Y. Kim, M. Faqih and S. Wang, *Carbohydr. Polym.*, 2001, **46**, 135–145.
22 T. Filisetti-Cozzi and N. Carpita, *Anal. Biochem.*, 1991, **197**, 157–162.
23 M. Dubois, K. Gilles, J. Hamilton, P. Robers and F. Smith, *Anal. Chem.*, 1956, **28**, 350–356.
24 P. Wood and I. Siddiqui, *Anal. Biochem.*, 1971, **39**, 418–428.
25 I. Naumenko and M. Phillipov, *Akademi Nauk Republi Moldova Biologi i Khimie Nauki*, 1992, vol. 1, pp. 56–59, in Russian.
26 O. Lowry, N. Rosebrough, A. Farr and R. Randall, *J. Biol. Chem.*, 1951, **193**, 265–275.
27 V. Singleton and J. Rossi Jr, *Am. J. Enol. Vitic.*, 1965, **16**, 144–158.
28 M. Bunzel, J. Ralph, J. Marita and H. Steinhart, *J. Agric. Food Chem.*, 2000, **48**, 3166–3169.
29 W. Brand-Williams, M. Cuvelier and C. Berset, *Food Sci. Technol.*, 1995, **28**, 25–30.
30 C. Kim and B. Yoo, *J. Food Eng.*, 2006, **75**, 120–128.
31 M. Grassi, R. Lapasin and S. Pricl, *Carbohydr. Polym.*, 1996, **29**, 169–181.
32 J. Ferry, in *Viscoelastic Properties of Polymers*, John Wiley & Sons, New York, 2nd edn, 1980.
33 R. Lapasin and S. Pricl, in *Rheology of industrial polysaccharides. Theory and applications*, Chapman & Hall, Blackie Academic and Professional, London, 1995.
34 J. M. Quintana, PhD thesis, Universidad Nacional de La Plata, 2003, Buenos Aires,in Spanish.
35 R. Sokal and J. Rohlf, in *Biometry. The principles and practice of statistics in biological research*, WH Freeman and Company, San Francisco, 2000, pp. 253–380.
36 T. Ghose and V. Bisaria, *Pure Appl. Chem.*, 1987, **59**, 1739–1752.
37 J. Brigham, W. Adney and M. Himmel, in *Handbook of Bioethanol: Production and Utilization*, ed. C. E. Wyman, Taylor and Francis, Washington DC, 1996, pp. 119–142.
38 I. Vijn and S. Smeekens, *Plant Physiol.*, 1999, **120**, 351–359.
39 S. Fry, *Annu. Rev. Plant Physiol.*, 1986, **37**, 165–186.
40 A. Nuñez, M. Fishman, L. Fortis, P. Cooke and A. Hotchkiss, *J. Agric. Food Chem.*, 2009, **57**, 10951–10958.
41 M. Marry, K. Roberts, S. Jopson, I. Huxham, M. Jarvis, J. Corsar, E. Robertson and M. McCann, *Physiol. Plant.*, 2006, **126**, 243–256.
42 L. Bravo, L. Goya and E. Lecumberri, *Food Res. Int.*, 2007, **40**, 393–405.
43 S. Kedare and R. Singh, *J. Food Sci. Technol.*, 2011, **48**, 412–422.
44 S. Jampen, I. Britt and M. Tung, *Food Res. Int.*, 2000, **33**, 579–586.
45 S. Ross-Murphy, in *Physical Techniques for the Study of Food Biopolymers*, ed. S. Ross-Murphy, Blackie Academic & Professional, Chapman & Hall, London, 1994, pp. 343–393.
46 E. Morris, A. Cutler, S. Ross-Murphy, D. Rees and J. Price, *Carbohydr. Polym.*, 1981, **1**, 5–21.
47 S. Lee, K. Warner and G. Inglett, *J. Agric. Food Chem.*, 2005, **53**, 9805–9809.
48 J. Lefebvre and J. Doublier, in *Polysaccharides: Structural diversity and functional versatility*, ed. S. Dimitriu, Marcel Dekker, USA, 2005.
49 I. Braccini and S. Pérez, *Biomacromolecules*, 2001, **2**, 1089–1096.
50 R. Cameron, G. Luzio, P. Vasu, B. Savary and M. Williams, *J. Agric. Food Chem.*, 2011, **59**, 2717–2724.
51 L. Chiang, W. Chiang, M. Chang, L. Ng and C. Lin, *Antiviral Res.*, 2002, **55**, 53–62.
52 A. Alvarez, K. Dalton, I. Nicieza, I. Diñeiro, A. Picinelli, S. Melón, A. Roque, B. Suárez and F. Parra, *Phytother. Res.*, 2012, **26**, 1513–1520.
53 P. Schnitzler, S. Schneider, F. Stintzing, R. Carle and J. Reichling, *Phytomedicine*, 2008, **15**, 1108–1116.
54 E. Damonte, M. Matulewicz and A. Cerezo, *Curr. Med. Chem.*, 2004, **11**, 2399–2419.
55 C. Dong, K. Hayashi, J. Lee and T. Hayashi, *Chem. Pharm. Bull.*, 2010, **58**, 507–510.
56 A. Oliveira, L. Cordeiro, R. Gonalvesa, L. Ceole, T. Ueda-Nakamura and M. Iacomini, *Carbohydr. Polym.*, 2013, **94**, 179–184.
57 J. Lee, T. Fukai, K. Hayashi and T. Hayashi, *Carbohydr. Polym.*, 2011, **85**, 408–412.
58 F. Liu, Y. Liu, Y. Meng, M. Yang and K. He, *Antiviral Res.*, 2004, **63**, 183–189.

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