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Enzyme assisted extraction of pectin and inulin enriched fractions isolated from microwave treated *Cynara cardunculus* tissues

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ABSTRACT

Artichoke flower heads are consumed fresh or industrialized. Agroindustrial by-products of this plant can be used for the production of dietary fiber. A microwave heating procedure is proposed for polysaccharides concentration in the residues. It consumed less time (36 min vs 75 min) and solvent volume (110 mL vs 140 mL) and allowed the extraction of a higher amount of total carbohydrates (95 g/100g vs 83 g/100g) than the habitually used convective procedure. A subsequent stage involving buffer/enzymes allowed to isolate pectin and inulin enriched fractions. Their inulin content was 32-45 g/100g. The galacturonic acid content oscillated between 22 and 29 g/100g. The fractions were slightly yellow/red colored and formed gels in the presence of calcium ion. The procedure proposed is of importance in the frame of emerging biorefinery because it uses vegetable by-products to produce fractions with potential for being used in the formulation of healthy foods.

Key words: artichoke; microwave heating; enzymatic digestion; pectin; inulin.

1. Introduction

Cynara cardunculus var. *scolymus* is a perennial plant originating from the Mediterranean region. The edible portion of the plant is highly appreciated by consumers for its organoleptic properties and nutritional value. Following a general trend towards the reuse of agro-industrial and food wastes (Mirabella *et al*., 2014), attempts have been made to find ways to add value to the artichoke

waste. Artichoke is known to have a relatively high content of inulin (10.0-30.7% based on dry weight) (Supplementary file 1, SP1). It is a reserve carbohydrate with recognized prebiotic properties and used as a technological ingredient (Carlson *et al*., 2018; Lattanzio *et al*., 2009; Ronkart *et al*., 2007).

Another important polysaccharide in artichoke is pectin (Fissore *et al*., 2014). This is a high-value functional food ingredient, well recognized as soluble dietary fiber and widely used as a gelling agent and stabilizer (Willats *et al*., 2006) (SP1). Its technological performance depends critically on its average molecular weight, and its molecular weight distribution (Izydorczyk *et al*., 2005).

The positive health effects along with the ability to improve rheological and nutritional properties, allow inulin and pectin to be used for functional foods (Lattanzio *et al*., 2009; Maxwell *et al*., 2012).

The interest in the use of microwave-assisted extraction (MAE) has increased significantly in the last years. MAE has been applied to the extraction of polysaccharides such as pectins, galactomannans, arabinogalactans, xylans and sulfated polysaccharides, among others (Bélafi-Bakó *et al*., 2007; Passos and Coimbra, 2013) observing that this technique allows to reduce treatment times and solvent volumes when compared to habitually used treatments. The intermolecular friction generated by microwave heating may cause an increase in internal cell pressure in plant tissues, which, in general, gives origin to a loss of cellular integrity, as well as in tissue organization (Latorre *et al*., 2012). MAE has shown being effective for obtaining pectin of higher equivalent weight and esterification degree than conventional heating techniques (Rodsamran and Sothornvit, 2019).

Enzyme degradation of cell walls is a useful tool to facilitate the extraction of their polysaccharides. The use of enzymes has the advantage of being environmentally friendly and it has been recognized as an alternative method for natural product extraction (Li-mei Lin *et al*., 2018).

In a previous work, Santo Domingo *et al*. (2015) evaluated the use of the alcohol insoluble residue (AIR) obtained by convective heating of artichoke (*Cynara cardunculus*) stems and bracts. Enzymatic digestion of AIRs was applied in order to isolate fractions enriched in pectin and inulin.

The objective of the present work was to evaluate: a) microwave heating for the production of the alcohol insoluble residue from artichoke bracts and stems, b) buffer/enzyme (protease, hemicellulase) assisted extraction of fractions enriched in pectin and inulin from those AIRs. The

yield, chemical composition, molecular weight and rheological behaviour of the isolated fractions were studied.

2. Materials and Methods

2.1. Sample preparation

Globe artichokes variety Madrigal, from La Plata city (Buenos Aires province), with closed heads and with the leaves forming compact layers, were purchased in the local market. The bracts and stems were removed and the heart was discarded. Bracts and stems were cut in small pieces and dried in a convection oven (85°C, 2.5 h, air rate: 0.5 m/sec). A 420 μ powder rich in cell wall material (CWM) was obtained through grinding with a domestic appliance (Wemir E909, Buenos Aires, Argentina) and sieving with an ASTM 40 sieve (Zonytest, Buenos Aires, Argentina).

2.2. Alcohol insoluble residue

The microwave treatment for obtaining the alcohol insoluble residue (AIR) for both tissues was carried out in a microwave system ETHOS Plus (Milestone Srl, Sorisole, Italy) with a magnetron of 2450 MHz. The internal temperature was controlled by means of the ATC-400 ETHOS system in the equipment.

An amount of ~ 10.0 g of CWM of bracts or stems was suspended in 50 ml of 80% (v/v) ethanol solution. Microwave conditions applied were 11 min at 250 W and 80°C. Afterwards, the suspension was filtered through a glass microfiber filter (GF/C. Whatman, UK). The solid residue obtained was treated using 30 ml of 80% (v/v) ethanol solution in the previously cited conditions and this procedure was repeated an additional time with the residue obtained after filtration. Each time, before removing the suspension from the microwave equipment, a minute was waited for safety reasons in relation to non-ionizing radiation. The total time consumption of MAE process for AIR production was 33 min plus 3 min for safety reasons. The ethanol that remained in the final residue was eliminated under a lab hood and the resultant solid was freeze dried (Stokes freeze-drier, Stokes Company, Philadelphia, MA, USA). For this procedure, the samples were frozen at -18ºC and sublimation was performed under a chamber pressure of 1000 μm Hg and at a shelf temperature of 30°C. The product was then milled and the resulting powder was vacuum packed in Cryovac ™ bags and stored at -18 ° C until later use.

2.3. Isolation of fractions

According to Santo Domingo *et al*. (2015), 5.00 g of AIR from stem (S) or bracts (B) in 500 ml of sodium citrate buffer (0.05M, pH 5.2) were heated for 5 min at 70°C under stirring, followed by cooling down to 40°C. Then, digestions with or without enzyme addition were performed at 40ºC for 5 h (**Table S1**). Enzymes used were from SIGMA (St. Louis, MO, USA): hemicellulase H2125 and protease P1236 (SP1).

2.4. Yield

Yield was calculated as g of AIR per 100g of CWM or as g of fraction isolated per 100 g of AIR used, as appropriate.

2.5. Chemical Analyses

Deionized water was used for all assays. All determinations were performed in triplicate.

The determination of cellulose, lignin, non-cellulosic polysaccharides, and protein in the AIR was performed according to Santo Domingo *et al*. (2015).

The content of total carbohydrates, uronic acids, inulin, proteins, and methanol in the isolated fractions was determined according to Fissore *et al*. (2014). The methylation degree (DM) was calculated as the percent ratio between moles of methanol and moles of galacturonic acid in the analysed sample.

2.6. Color measurement

The color was measured in triplicate by using a colorimeter (Minolta Co. Ltd., Osaka, Japan) with D65 illuminant and 2° observer angle (SP1).

2.7 Molecular mass by gel permeation chromatography

The molecular mass distribution of the isolated fiber fractions was determined using gel permeation chromatography (GPC) (SP1).

2.8 Rheological characterization

Rheological characterization was performed for calcium aqueous systems (SP1), at 25.0 ºC using an MCR300 Paar Physica (shear) rheometer (Anton Paar, Austria) equipped with a serrated parallel plate (PP25/S) geometry (25 mm diameter). A gap size of 1000 μm was set. Data points were recorded at steady-state.

2.8.1 Flow assays

Flow curves were determined, at a constant temperature of 25°C in the 0.001-100 rad s^{-1} shear rate (γ) range. The Ostwald´s power law and the Herschel-Bulkley model were used for data fitting (SP1).

2.8.2 Dynamic assays

Calcium aqueous systems of isolated fractions were submitted to oscillatory assays at 25°C and the storage (G') and loss (G'') moduli against angular frequency (ω) were recorded under linear viscoelastic conditions (SP1).

2.9 Statistical Analysis

Prism 5 software (GraphPad, USA) was used for non-linear fittings. 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 (Sokal and Rohlf, 2000).

3. Results and Discussion

3.1. Chemical composition of AIRs.

The yield and composition of the stem (S) and bract (B) AIRs can be observed in **Table 2**. The yield was higher for bracts than for stems. Microwave stem AIR contained 97 g/100g carbohydrates (cellulose 8.0 g/100g; non-cellulosic carbohydrates 89g/100g of which 7.6 g/100g were galacturonic acid and 6.0 g/100g were inulin), 4.6 g/100g of proteins and 14.5 g/100g of lignin. Microwave bract AIR was mainly constituted by carbohydrates (cellulose 11 g/100g; non-cellulosic carbohydrates 82g/100g of which 8.7 g/100g were galacturonic acid and 5.4 g/100g were inulin), with 5.5 g/100g of proteins and 17.0 g/100g of lignin.

Santo Domingo (2017) and Santo Domingo *et al*. (2015) obtained AIRs from artichoke by means of convective heating at 79°C for 75 min. The relation of CWM to ethanol used was 10.0 g to 140 ml, considering that the CWM powder absorbed solvent and that, for efficient extraction, the solvent must be available for the substances of interest to solubilize in it. Results reported are informed in **Table 1** for comparison purposes. The yields were slightly lower than those obtained in the present research for both bracts and stems. MAE gave rise to an AIR with higher total carbohydrates and lower protein content. The uronic acid and inulin contents were higher when convective heating was used and the degree of methylation ranged between 32.9 and 38.45% for all the AIRs.

It is important to remark that MAE process for AIR production was less time consuming (33 min plus 3 min for safety reasons) than convective heating process (75 min) and that, for convective heating, it were used 140 ml of ethanol 80% (v/v) for each 10 g of CWM while for MAE, the necessary volume of ethanol was 110 ml.

3.2. Yield and chemical composition of isolated fractions

Yield and chemical composition of fractions isolated from microwave AIRs are reported in **Table 2**. The highest yield was obtained for fraction BM3 (≈15 %) while for the other bract fractions it was approximately 13 %. For all stem fractions, yield was ≈ 14 %. When the same systems were prepared using convection heated AIR obtained from the same cell wall material used in the present work, the yield for bract fractions was 11-13 %, while for stem fractions it was 19-23 % (Santo Domingo *et al*., 2015). It can be concluded that, from a yield point of view, the microwave treatment performed to obtain the AIR, did not generate an increase of material extracted.

As can be observed in **Table 2**, fractions were mainly composed of carbohydrates (66-89 g/100g). For both bracts and stem fractions, the main carbohydrate was inulin (31.8 – 45.0 g/100g) while the GalA content was in the order of 22 -29 g/100g.

When compared with fractions prepared with convective AIR, the pectin contents of fractions was higher for SM1, SM3, SM4, BM2, BM3 and BM4 than for the fractions isolated from convective AIR using the same hydrolysis treatments according to Santo Domingo *et al*. (2015) who reported values of GalA < 20 /100g for stem fractions with treatments 1,3,4 and values of GalA ≈ 19 g/100g for bract fractions with treatments 2,3,4. With respect to inulin, fractions SM4, BM2, BM3, and BM4 presented a higher inulin content than those isolated from convective AIR using the same hydrolysis treatments, which showed values between 22 and 30 g/100g (Santo Domingo *et al*., 2015) (**Table 2**). According to Mao *et al.* (2019), microwaves heat the material volumetrically since they can penetrate uniformly throughout the whole volume of material instantaneously and heat different components of heterogeneous systems at different rates, and this can lead to the rupture of cells within biomass, resulting in higher extraction yields. In addition, microwaves allows quicker heating avoiding pectin degradation.

In relation to the methylation degree (DM), bract fractions presented slightly higher values than stem fractions. Sabater *et al.* (2018) determined a DM of 9.1 for an industrial by-product of artichoke consisting of blanched bracts, leaves, and stems, and a DM of 19.5 for a pectin fraction isolated from the mentioned by-product by cellulase hydrolysis. In another study performed on

fresh globe artichoke (stem, receptacle, and bract) and waste of artichoke canning industry, high DM pectins were isolated from stem and bract samples while the receptacle and waste samples had low DM pectins (Ceylan *et al.* ,2017).

The protein content in the stem fractions was higher than in bract fractions and non- significant differences were observed between different stem fractions. Microwave treatment allowed obtaining a higher amount of protein than convective heating (Santo Domingo *et al*., 2015) for stem fractions (Proteins: 1.5-2.2 g/100g AIR for stem fractions isolated from convective AIR though treatments 1,2,3,4) and for bract fraction BM2 (Proteins: 1.1 g/100g AIR for bract fraction isolated from convective AIR through treatment 2).

The results showed that all fractions isolated from microwave AIR were mainly composed of pectin and inulin, which means that they are a source of dietary fiber and of prebiotic activity. For that reason, they may be considered valuable ingredients for industrial manufacture of healthy foods. The use of MAE procedure for AIR preparation, when compared to the convective heating procedure, allowed obtaining, in general, a greater amount of pectin, inulin, and protein in the isolated fractions. Seremet *et al*. (2020) studied the effects of microwave preheating of beetroot powder in the release of bioactive compounds and concluded that microwave preheating provides increased bioavailability due to the vegetal cell disruption.

3.3. Color

The color parameters L*, a*, b* and ΔE* are reported in **Table S2**. It can be observed that, in general, fractions showed significant differences ($p<0.05$) in the parameters obtained, for each tissue and according to the treatment applied for their production.

Fractions showed L* values higher than 50% and, in general, stem fractions resulted darker that bract fractions. The parameter b* had positive values, showing that the fractions had a yellowish tone. Parameter a* took low positive values in all fractions, revealing a certain reddish tone. It could be observed that stem fractions were more yellow and red than bract fractions.

With reference to the parameter ΔE it always took positive values showing an increase of luminosity and color with enzyme usage. Fractions SM4 and BM3 showed the greatest positive variations with respect to the fractions obtained by means of the citrate buffer (BM1 and SM1). These variations could be attributed to the cell wall degrading enzymes which not only facilitated the release of carbohydrates but also pigments (Zhao *et al*., 2019).

3.4. Molecular mass distribution

Table S3 shows the number average molecular mass (Mn), weight average molecular mass (Mw), the polydispersity index (Mw/Mn) and retention time of each isolated fraction. The chromatograms can be observed in **Figure S1**.

Stem fractions showed pectin peaks of 397-806 kDa and inulin peaks of 1.5-8.0 kDa. SM1 and SM2 contained pectins of higher molecular weight than SM3 and SM4. All stem fractions presented a peak at 25.5 min, which is attributed to inulin. SM1, SM2, and SM4 presented two inulin peaks while SM3 presented only one. For bracts fractions, it could be observed pectin peaks of 100-629 kDa, lower than those of stem fractions and inulin peaks of 1.3-11.5 kDa. The polidispersity index of peaks ranged from 1.0 to 2.8, values that are in the range of natural polysaccharides (Wang and Cui, 2005), and were higher for pectin peaks. The higher molecular weights observed for pectins could be ascribed to agglomeration of the macromolecules.

Santo Domingo *et al*. (2019) reported molecular masses of 43-472 kDa (pectin) and 1.3-6.4 kDa (inulin) for fractions isolated from convection heated bracts AIR and of 45-1245 kDa (pectin) and 1.1-5.8 kDa (inulin) for fractions isolated from convection heated stems AIR. Sabater et al. (2018) informed that a pectin, isolated through cellulase hydrolysis from artichoke industrial byproducts, presented three fragments of 660, 105 and 4.8 kDa. For inulin, the molecular masses informed by Azis *et al*. (1999) ranged from 3 to 6 kDa. Ronkart *et al*. (2007) reported, for globe artichoke, the presence of inulin with a degree of polymerization of 80, which means a molecular mass of 13 kDa.

3.5. Rheological characterization of the isolated fractions

3.5.1. Flow behaviour

Flow curves were determined at 25 ºC under increasing shear rates. Since fractions contained pectin of low degree of methylation (**Table 2**), calcium was added to all systems.

All samples showed pseudoplastic behaviour and data obtained fitted the Herschel- Bulkley model (**Table 3**), with yield stresses varying from 1.3 Pa s for fraction BM3 to 19.6 Pa s for fraction BM2. As it can be observed, stem fractions showed higher *k* values than bract fractions, probably due to the higher Mw of pectins present in stem fractions. Similar results were reported by Santo Domingo et al. (2019) for fractions isolated from convection heated AIR. Fraction SM1 showed the best thickening capacity with the highest k value (27 Pa s^n) and also high yield stress (14.5 Pa) while fraction BM4 was the least consistent (lowest *k* value). Apparent viscosities, evaluated at 20 s^{-1} were, in general, higher for stem fractions with the exception of fraction BM2. Fraction BM2 and BM4 were the less pseudoplastic ones (higher *n*) and fraction SM3 had the lowest *n* value.

It is well known that pectin is a gelling and thickening agent (Chan *et al*., 2017; Fissore, *et al*., 2013) and the increase of its content, without doubt, contributed to the rheological behaviour. With respect to inulin, Toneli *et al.* (2008) reported an increase in apparent viscosity of solutions of inulin isolated from chicory root with the increase of its concentration. According to Rodriguez-Gonzalez *et al*. (2019), the presence of long chains in chicory inulin, causes it to be less soluble, to increase the viscosity in solution and to form gels at high concentrations. Roberfroid (2004) informed that inulin can contribute to gelling by means of the synergy that it exerts with most gelling agents.

3.5.2. Oscillatory assays

For working at linear conditions, a constant strain between 1 - 7 % was applied to perform the frequency sweeps.

In general, the behaviour observed is that of gels, with G' > G'' in one order of magnitude or more and a tendency to a crossover of the moduli at higher frequencies (**Figure 1).** Santo Domingo *et al*. (2019) informed that fractions isolated from convection heated AIR, obtained from artichoke bracts or stems, formed gel-like networks of different calcium crosslinking degrees.

All stem systems showed G' > G'' with very little frequency dependence for G' and higher frequency dependence for G'' (Lefebvre and Doublier, 2005). Fraction SM1 gave the highest values of G' and G''. For bract fractions, G' showed very little frequency dependence except for fraction BM4, while G'' showed frequency dependence for all systems. Stem fractions gave stronger gels than bract fractions, probably due to the higher Mw of pectins present in stem fractions. BM3 was the one that gave the weakest gel. Fractions SM1 and SM4, with the higher GalA contents, gave the higher G' values (stronger gels).

4. Conclusions

A procedure assisted by microwaves (MAE) was developed for alcohol insoluble residue (AIR) production from stems and bracts of artichoke. When compared to the use of convective heating for AIR extraction, MAE consumed less time and solvent volume and also allowed the extraction of higher amounts of total carbohydrates and lower protein content.

A subsequent step assisted by citrate buffer or buffer/enzymatic treatment (enzyme assisted extraction, EAE) allowed obtaining artichoke fractions which showed, in general, higher GalA and inulin content for bracts and higher GalA for stems than fractions isolated from convective AIR. In the presence of calcium ions, fractions isolated from microwave AIR presented yield stress and

pseudoplastic behaviour and formed gels although fractions isolated in the presence of hemicellulase showed a weaker gel behaviour.

The isolated fiber fractions were bright and slightly colored, which has to be considered for their incorporation in food systems because of their potential effect on the final product appearance.

The isolated fractions could represent an economic source of soluble fiber and prebiotics with potential as nutritional ingredients and as thickening and gelling additives.

The benefit of the whole procedure proposed relies on:

a) the reduction of times and solvent volumes when using microwave treatment plus the advantage of the environmentally friendliness of the use of enzymes,
b) the potential of its application to other plant by-products.
c) the possibility of scaling the process which is currently under study

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The authors do not have a conflict of interest to declare.

Ethics approval

Ethics approval was not required for this research.

Data Availability Statement

Research data are not shared.

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Figure captions

Figure 1. Mechanical spectra (25°C) of calcium-aqueous systems containing a 2.00 % (w/v) concentration of the different isolated fractions. (**a**) stem fractions (**b**) bract fractions.
Filled symbols: storage modulus (G'); empty symbols: loss modulus (G''). ω: angular frequency.

Table 1. Yield and chemical composition of the alcohol insoluble residues (AIRs) of globe artichoke bracts (BM-AIR) and stems (SM-AIR), obtained by microwave heating.

Composition	SM-AIR	BM-AIR	SC-AIR[4]	BC-AIR[4]
Total carbohydrates [1] (g/100g)	97 ± 5Aa	93 ± 2Aa	81 ± 8b	86 ± 3b
Cellulose (g/100g)	8.0 ± 0.6Aa	11 ± 1Ba	9 ± 1a	22 ± 3b
Galacturonic acid (g/100g)	7.6 ± 0.3Aa	8.7 ± 0.2Ba	11± 2b	10± 1b
Inulin (g/100g)	6.0 ± 0.2Aa	5.4 ± 0.1Ba	12±1b	8±1b
Lignin (g/100g)	14.5 ± 0.2Aa	17.0 ± 0.2Ba	14.8 ±1.3a	15.1 ± 0.1b
Non cellulosic carbohydrates [2] (g/100g)	89	82	72	64
Protein (g/100g)	4.6 ± 0.1Aa	5.5 ± 0.1Ba	9.0 ± 0.3b	8.5 ± 0.8b
DM (%)[3]	38.45 ± 0.03Aa	32.9 ± 0.5Ba	36.5 ±0.04Ab	38.0 ± 0.1Bb
Yield (%)	70±5Aa	84±6Bb	62±6Aa	78±4Bb

Results are expressed as mean and standard deviation (n=3). Different capital letters for the same heating method and different tissue, indicate significant differences ($p<0.05$). Different lower case letters for the same tissue and different heating method indicate significant differences ($p<0.05$).

[1] Calculated in relation to fructose calibration curve

[2] Calculated as the difference between total carbohydrates and cellulose.

[3] DM (methylation degree) was calculated as a percent ratio between moles of methanol group and moles of galacturonic acid per 100 g of sample.

[4]Data from Santo Domingo (2017) concerning the use of convective heating are also reported for comparison purposes. BC-AIR: AIR obtained from bracts by convective heating. SC-AIR: AIR obtained from stems by convective heating. Some new data concerning DM and Yield are also reported for SC-AIR and BC-AIR.

Table 2. Yield and chemical composition of fractions isolated from *Cynara cardunculus*.

Fraction	Total carbohydrates (g/100g)[1]	Galacturonic acid (g/100g)	Inulin (g/100g)	Neutral Sugars[2] (g/100g)	DM (%)[3]	Proteins (g/100g)	Yield (%)
SM1	89 ± 2 A	29.3 ± 0.1 A	42 ± 1 A	17.7	22.16±0.01 A	3.18 ± 0.03 A	14.3±1.5 A
SM2	77.7 ± 0.4 AB	24 ± 3 A	40.0 ± 0.5 B	13.7	27.2±0.1 B	3.20 ± 0.03 A	13.8±1.3 A
SM3	78 ± 6 AB	25 ± 4 A	31.8 ± 0.5 C	21.2	18.70±0.04 C	2.8 ± 0.2 A	14.2±2.1 A
SM4	66 ± 6 B	29 ± 2 A	35 ± 1 D	2.0	25.94±0.03 D	2.8 ± 0.3 A	13.8±1.2 A
BM1	71 ± 1 A	22 ± 1 A	45.0 ± 0.2 A	4.0	29.70±0.02 A	1.3 ± 0.2 A	12.9±1.3A
BM2	73 ± 2 A	26 ± 2 B	41 ± 1 B	6.0	32.33±0.01 B	2.1 ± 0.5 AB	12.8±1.6A
BM3	82 ± 2 B	25 ± 1 B	41 ± 2 B	16.0	31.81±0.04 C	1.5 ± 0.1 AB	15.2±1.6A
BM4	82 ± 5 B	26.9 ± 0.2 B	41 ± 2 B	14.1	35.1±0.1 D	2.3 ± 0.5 B	12.8±1.7A

Results are expressed as mean and standard deviation (n=3). For runs 1-4, inside each column for each tissue, different letters indicate significant differences ($p<0.05$).

[1] Calculated as fructose.

[2] Calculated by difference between total carbohydrates, galacturonic acids and inulin

[3]DM (methylation degree) was calculated as a percent ratio between moles of methanol group and moles of galacturonic acid per 100 g of sample.

Table 3. Herschel Bulkley model[1] parameters calculated after fitting flow experimental data for stem and bract isolated fractions.

Fraction	η_a (Pa s) 20 s^{-1}	τ_0 (Pa)	k (Pa s^n)	n	R_{adj}^2	Sy.x (%)
SM1	5.3	14.5 ± 0.8A	27 ± 1A	0.41 ± 0.02A	0.989	4.25
SM2	2.7	1.7 ± 0.4B	17. 1 ± 0.4B	0.37 ± 0.02A	0.989	6.23
SM3	1.9	12 ± 1C	11 ± 1C	0.28 ± 0.05B	0.936	4.34
SM4	3.0	7.0 ± 0.5D	16 ± 1B	0.40 ± 0.03A	0.979	4.83
BM1	0.6	5.2 ± 0.1 D	1.8 ± 0.1D	0.45 ± 0.02A	0.979	4.72
BM2	4.2	19.6 ± 0.5	9.0 ± 0.6C	0.66 ± 0.08C	0.926	4.40
BM3	0.5	1.3 ± 0.1B	2.9 ± 0.1E	0.39 ± 0.01A	0.989	4.67
BM4	0.8	5.7 ± 0.7D	1.2 ± 0.3D	0.71 ± 0.06C	0.948	13.15

[1]Mean and standard error of fittings are shown.

η_a: apparent viscosity, τ_0: yield stress, k: consistency index; n: pseudoplasticity index; R_{adj}^2: adjusted coefficient of determination (α:0.05); Sy.x: residual standard deviation in percentage, calculated as $(Syx/\tau_{average}).100$. $\tau_{average}$: average shear stress.

Inside each column, different letters indicate significant differences ($p<0.05$).

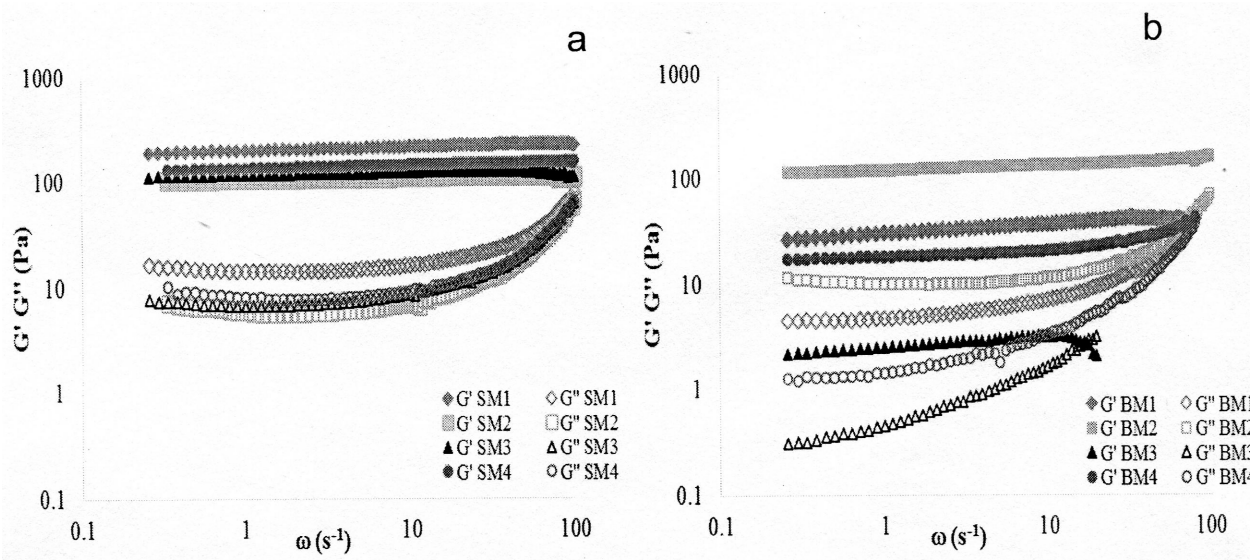

Figure 1

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