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Extraction of bioactive carbohydrates from artichoke (*Cynara scolymus* L.) external bracts using microwave assisted extraction and pressurized liquid extraction

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ABSTRACT

Microwave assisted extraction (MAE) and pressurized liquid extraction (PLE) methods using water as solvent have been optimized by means of a Box–Behnken and 3^2 composite experimental designs, respectively, for the effective extraction of bioactive carbohydrates (inositols and inulin) from artichoke (*Cynara scolymus* L.) external bracts. MAE at 60 °C for 3 min of 0.3 g of sample allowed the extraction of slightly higher concentrations of inositol than PLE at 75 °C for 26.7 min (11.6 mg/g dry sample *vs.* 7.6 mg/g dry sample). On the contrary, under these conditions, higher concentrations of inulin were extracted with the latter technique (185.4 mg/g *vs.* 96.4 mg/g dry sample), considering two successive extraction cycles for both techniques. Both methodologies can be considered appropriate for the simultaneous extraction of these bioactive carbohydrates from this particular industrial by-product. To the best of our knowledge this is the first time that these techniques are applied for this purpose.

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1. Introduction

Artichoke (*Cynara cardunculus* var. *scolymus* L.) is extensively cultivated in the Mediterranean region, constituting the 85% of world production. From this vegetable, only the inner leaves (bracts) and heart are considered edible parts, whereas external bracts, leaves and stems, are non-food industrial by-products (Christaki, Bonos, & Florou-Paneria, 2012). These by-products represent about 60% of the artichokes (Llorach, Espín, Tomás-Barberán, & Ferreres, 2002) and have been used for the extraction of bioactive components such as flavonoids and phenolic compounds (Ceccarelli et al., 2010; Lattanzio, Kroon, Linsalata, & Cardinali, 2009) or essential oils (Shimoda et al., 2003) with application in food or pharmaceutical industry. These bioactives make artichoke to be considered as a functional food, exhibiting hepatoprotective and antioxidative activities, as well as the ability to inhibit cholesterol biosynthesis and low density lipoprotein oxidation (Fallah Huseini, Kianbakht, & Heshmat, 2012; Llorach et al., 2002; Sánchez-Rabaneda et al., 2003). Artichoke is also known to have a relatively high content in inulin (10.0–30.7% based on dry weight) (Lattanzio et al., 2009; Leroy, Mabeau, Baty-Julien, & Grongnet, 2011; Ruiz-Cano et al., 2014; Van Loo, Coussement, De Leenheer, Hoebregs, & Smits, 1995), a polysaccharide composed of fructose unit chains of various lengths (linked by β-(2 → 1)-D-fructosyl-fructose bonds), terminated by a glucose unit linked by a α-(1 → 2) bond. Inulin is a reserve carbohydrate with recognized prebiotic properties and used as a technological ingredient (Gibson & Roberfroid, 1995; Lattanzio et al., 2009; Leroy, Grongnet, Mabeau, Corre, & Baty-Julien, 2010; López-Molina et al., 2005; Ronkart et al., 2007).

Up to now, the presence of inulin in artichoke has usually been evaluated on edible parts, i.e., head or capitula. Only a couple of studies have reported on the presence of this component in artichoke by-products (or agro-industrial wastes), such as external bracts or fractions resulting from industrial processes (López-Molina et al., 2005; Ruiz-Cano et al., 2014).

Apart from inulin, limited attention has been paid to the composition of other carbohydrates present in artichoke. Few studies report the total carbohydrate content on edible parts of artichoke (Dosi et al., 2013) whereas, to the best of our knowledge, the composition of the individual carbohydrate present in this vegetable has been only studied in a recent manuscript by (Hernández-Hernández, Ruiz-Aceituno, Sanz, & Martínez-Castro, 2011). In this work, low molecular weight carbohydrate (LMWC) composition of artichoke internal bracts was determined, emphasizing the content of bioactive compounds such as inositols (*chiro*-, *scyllo*- and *myo*-inositol) at levels of 2 mg/g dry weight.

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Inositols (1,2,3,4,5,6-hexahydroxycyclohexanes) are cyclic polyalcohols with molecular formula $C_6H_{12}O_6$. These compounds are mainly found in vegetables, being their presence in animal or meat sources limited (Clements & Darnell, 1980; Ruiz Aceituno, Ramos Rivero, & Sanz Murias, 2012). Several bioactive properties have been attributed to inositols and their derivatives, mainly associated with insulin resistance, and so they have been used for treating disorders such as polycystic ovary syndrome, diabetes mellitus and/or obesity (Nestler, Jakubowicz, Reamer, Gunn, & Allan, 1999; Ostlund & Sherman, 1998).

The use of advanced extraction techniques such as pressurized liquid extraction (PLE) or microwave assisted extraction (MAE) to obtain extracts rich in bioactives from industrial by-products is gaining great attention in recent years (Ballard, Mallikarjunan, Zhou, & O'Keefe, 2010; Machado, Pasquel-Reátegui, Barbero, & Martínez, 2015; Paes, Dotta, Barbero, & Martínez, 2014; Pap et al., 2013; Pérez-Serradilla & Luque de Castro, 2011; Álvarez-Casas, García-Jares, Llompart, & Lores, 2014). These techniques generally provide high yields with a significant reduction of extraction times and solvent volumes as compared with conventional (i.e., non-solvent enhanced) extraction procedures. Applications reporting on the PLE of carbohydrates are still scarce in the literature. Several studies have reported on the feasibility of PLE for the extraction of inulin from Jerusalem artichoke (*Helianthus tuberosus* L.) tubers (Saengkanuk, Nuchadomrong, Jogloy, Patanothai, & Srijaranai, 2011), of inositols from pine kernels (Ruiz-Aceituno, Rodríguez-Sánchez, Sanz, Sanz, & Ramos, 2014) and of iminosugars from mulberry leaves (Rodríguez-Sánchez, Ruiz-Aceituno, Sanz, & Soria, 2013). The concurrent extraction of monosaccharides and polysaccharides from *Cordyceps* mushroom by PLE has also been evaluated (Guan, Yang, & Li, 2010). MAE has been applied to the extraction of polysaccharides (pectins, galactomannans, arabinogalactans, xylans and sulfated polysaccharides, among others) (Benkő et al., 2007; Bélafi-Bakó, Cserjési, Beszédes, Csanádi, & Hodúr, 2012; Passos & Coimbra, 2013). However, to the best of our knowledge, this technique has not been applied to the extraction of inulin in any type of vegetable and no application to the extraction of LMWC, and more specifically to inositols, has been found in the literature.

The aim of this study was to evaluate the possibility of simultaneous extraction of bioactive carbohydrates, i.e., inulin and inositols, from artichoke (*Cynara scolymus*) industrial by-product (i.e., external bracts) by PLE and MAE. The possibility of subsequent removal of other coextracted LMWC has also been evaluated.

2. Materials and methods

2.1. Samples and reagents

Fresh artichokes (var. "*Blanca de Tudela*") were acquired at local markets (Madrid, Spain), and the external bracts were taken and freeze–dried. Then, they were ground to fine particles using a domestic mill (Moulinex, Barcelona, Spain) and sieved (500 μm mesh). The sample was stored in a dry and hermetically closed recipient protected from light until analysis at ambient temperature.

Analytical standards of fructose, *chiro*-inositol, glucose, galactose, *myo*-inositol, sucrose and phenyl-β-D-glucoside were obtained from Sigma Chemical Co. (St. Louis, USA). Washed sea sand, thick grain, was acquired to Panreac (Barcelona, Spain).

Inulinase Novozym 960 from Aspergillus niger was kindly provided from Novozymes (Bagsvaerd, Denmark) with 250–400 units/g activity.

2.2. Carbohydrates extraction

2.2.1. Microwave assisted extraction (MAE)

MAE was carried out in a MARS 6 (CEM, NC, USA) system. Microwave power was set at 900 W.

In a typical experiment, dried external bracts (0.1–0.3 g) and 10 mL of ultra-pure water were placed in 100 mL Green Chem vessels (CEM) and submitted to MAE at different temperature and/or times, following experimental design conditions. The effect of three independent factors (temperature, T, °C; time, t, min; and sample amount, s, g) on carbohydrate extraction from artichoke external bracts was studied using a Box–Behnken design. A total of 15 experiments were carried out in randomized order, according to design (Table 1). The 3-level design included a subset of the runs in the full three-level factorial and 3 center points per block to estimate the experimental error. Experimental ranges for factors evaluated were: T = 50–120 °C, t = 3–30 min, and s = 0.1–0.5 g.

The quadratic model proposed was:

$$R = \beta_0 + \beta_1 T + \beta_2 t + \beta_3 s + \beta_{1,1} T^2 + \beta_{2,2} t^2 + \beta_{3,3} s^2 + \beta_{1,2} Tt + \beta_{1,3} T_s + \beta_{2,3} ts + \varepsilon \quad (1)$$

where β_0 is the intercept, β_i are the first-order coefficients, $\beta_{i,i}$ the quadratic coefficients for ith factors, $\beta_{i,j}$ the coefficients for the interaction of factors i and j and ε is the error.

Three response (R) variables were individually considered in the optimization of the MAE method: $R1$, total inositols amount/g of dry sample; $R2$, inulin amount/g of dry sample; and $R3$, total sugar amount/g of dry sample. The experimental conditions that independently maximized $R1$ and $R2$, and minimized $R3$ were obtained from the fitted models. Under optimized conditions, the number of cycles (first, second and third cycle, named as C1, C2 and C3, respectively) were also evaluated. Obtained extracts were immediately cooled down on ice, centrifuged at 4400g for 10 min at 4 °C and keep in a freezer at −18 °C until analysis.

2.2.2. Pressurized liquid extraction (PLE)

Extracts were obtained using a PSE ONE system from Applied separations (PA, USA). Dried artichoke bracts (0.3 g) were introduced in a 11-mL stainless steel extraction cell in between sand layers. Milli-Q water was used as extraction solvent and a single static extraction cycle was done in all instances. Extractions were carried out under a pressure of 100 bar, and a purge of nitrogen gas of 2 min was done at the end of the extraction cycle to ensure the complete removal of the extraction solvent from the cell and

Table 1
Box–Behnken experimental design and obtained concentrations (mg/g dry weight of bracts) of the MAE of carbohydrates.

T (°C)	t (min)	Sample amount (g)	Inositols	Sugars	Inulin
50	3	0.3	9.3 (0.2)*	66.3 (0.2)	84.5 (8.7)
50	16.5	0.1	8.7 (0.5)	66.3 (0.9)	77.7 (6.0)
50	16.5	0.5	8.2 (0.4)	57.0 (4.4)	79.4 (13.4)
50	30	0.3	8.2 (0.4)	57.3 (3.0)	69.8 (0.6)
85	3	0.1	8.8 (1.5)	64.5 (9.3)	85.9 (11.0)
85	3	0.5	7.5 (0.4)	56.7 (3.1)	80.8 (8.2)
85	16.5	0.3	6.9 (1.0)	53.6 (2.4)	89.8 (6.0)
85	16.5	0.3	7.2 (0.4)	50.4 (9.7)	98.3 (7.3)
85	16.5	0.3	8.0 (1.0)	66.7 (4.9)	96.3 (13.9)
85	30	0.1	6.8 (0.6)	50.6 (2.3)	78.3 (7.5)
85	30	0.5	7.6 (0.3)	59.1 (0.2)	78.4 (10.2)
120	3	0.3	7.5 (1.6)	58.6 (8.4)	114.6 (2.8)
120	16.5	0.1	7.6 (0.7)	56.9 (6.9)	86.0 (4.9)
120	16.5	0.5	7.6 (1.1)	56.0 (6.6)	83.0 (19.7)
120	30	0.3	6.7 (0.7)	56.9 (5.7)	87.6 (10.8)

* Standard deviations are in parentheses.

the lines. The effect of two independent factors (T and t) on carbohydrate extraction from artichoke external bracts was studied using a 3^2 full factorial design with 4 replicates at the center point. Experimental ranges for factors evaluated were: T = 40–120 °C and t = 3–30 min. The quadratic model proposed was:

$$R = \beta_0 + \beta_1 T + \beta_2 t + \beta_{1,1} T^2 + \beta_{2,2} t^2 + \beta_{1,2} T_t + \varepsilon \quad (2)$$

Acronyms are similar to Eq. (1).

For comparative purposes, the same response variables as in MAE, i.e., $R1$, $R2$ and $R3$, corresponding to the total inositols amount/g of dry sample, inulin amount/g of dry sample and total sugars amount/g of dry sample, respectively, were considered. In total, 12 experiments were performed according to design (Table 2).

2.3. Enzymatic hydrolysis of inulin

The inulin concentration was calculated taking into account the fructose, glucose and sucrose contents in the artichoke bracts extracts before and after hydrolysis with inulinase, as previously carried out by other authors (Jaime, Martín-Cabrejas, Mollá, López-Andréu, & Esteban, 2001; Prosky & Hoebregs, 1999; Schütz, Muks, Carle, & Schieber, 2006).

MAE and PLE artichoke bract extracts (0.4 mL) were evaporated and re-dissolved in 1 mL acetate buffer (pH 4.6). A volume of 50 μL of inulinase was added and the mixtures were incubated at 55 °C while shaking at 730 *g* in a Thermomixer (Eppendorf, Hamburg, Germany). Temperature and pH conditions were chosen as the optimal for the enzyme according to the manufacturer's recommendations. Subsamples were taken at 0, 8, 24, 48, 72 and 96 h and the enzymatic reaction was stopped by immersing the sample for 90 s on boiling water. Then, samples were cooling on ice and stored in a freezer until analysis by GC as indicated in Section 2.5.

2.4. Carbohydrate fractionation

MAE extracts corresponding to C1 and C2 were mixed and incubated with 1% (w/v) yeasts (*Saccharomyces cerevisiae*, type II, Sigma Chemical Co.) at 37 °C under stirring (90 g). Aliquots were taken at 0 h (corresponding to the beginning of the experiment) and after 3, 8 and 24 h of treatment. All assays were carried out in triplicate. Samples were centrifuged at 4400*g* (MiniSpin; Eppendorf, Madrid, Spain) for 10 min to remove yeasts. Samples were then treated as indicated in Section 2.5.

Table 2
Central 3^2 composite design for optimization of carbohydrate concentration (mg/g dry weight of bracts) extraction using PLE.

T (°C)	t (min)	Inositols	Sugars	Inulin
40	3	5.4 (0.1)*	49.6 (0.7)	67.7 (2.6)
40	17	6.1 (0.3)	55.5 (1.2)	104.8 (20.6)
40	30	6.3 (0.4)	58.4 (2.7)	123.0 (30.8)
80	3	5.3 (0.2)	54.3 (0.3)	77.6 (6.9)
80	17	6.1 (0.7)	59.5 (6.4)	137.2 (18.9)
80	17	5.8 (0.0)	54.4 (4.1)	140.3 (15.2)
80	17	6.1 (0.2)	53.9 (1.3)	142.4 (4.6)
80	17	6.0 (0.1)	54.8 (0.4)	140.1 (19.0)
80	30	6.7 (0.0)	58.5 (7.3)	130.3 (6.8)
120	3	5.7 (0.2)	55.0 (1.3)	97.1 (5.2)
120	17	6.1 (0.2)	64.5 (0.3)	85.3 (5.2)
120	30	5.4 (0.1)	57.7 (0.2)	117.9 (11.9)

* Standard deviations are in parentheses.

2.5. Instrumental section

Concentration of sugars in artichoke bract extracts (before and after enzymatic treatment) was determined by GC-FID.

2.5.1. Derivatization procedure

1 mL of artichoke bracts extract was mixed with 0.5 mL of a 70% ethanolic solution of the internal standard (phenyl-β-D-glucoside, 1 mg/mL) and evaporated (38-40 °C) prior to carbohydrate derivatization. Trimethylsilyl (TMS) oxime derivatives were prepared according to Sanz, Gonzalez, de Lorenzo, Sanz, and Martınez-Castro (2005). In brief, oximes were obtained by addition of 350 μL of a solution 2.5% hydroxylamine chloride in pyridine after 30 min at 75 °C. They were then silylated with hexamethyldisilazane (350 μL) and trifluoroacetic acid (35 μL) at 45 °C for 30 min. After reaction, samples were centrifuged at 4400*g* for 10 min, and the supernatant was subjected to GC analysis. This derivatization procedure gives rise to a single chromatographic peak for non-reducing sugars (corresponding to their TMS ethers) and two peaks for reducing sugars (corresponding to their *syn* (*E*) and *anti* (*Z*) oxime isomers).

2.5.2. GC analysis

GC analysis of derivatized samples was carried out using a 7890A gas chromatograph equipped with FID from Agilent Technologies (Palo Alto, CA, USA). Chromatographic analyses were carried out on a Zebron ZB-1MS methylsilicone capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness; Phenomenex, CA, USA), using nitrogen at 1 mL/min as carrier gas.

The oven temperature was programmed as follows: 200 °C (14.5 min), then at 20 °C/min to 220 °C (3 min), and finally at 25 °C/min to 270 °C (20 min). Injections (1 μL) were carried out in split mode (1:20) at 300 °C. FID temperature was 300 °C. Data acquisition was done using HP ChemStation software (Agilent Technologies).

Carbohydrates were identified by using available standards and previous data from literature (Hernández-Hernández et al., 2011). Quantitative data were directly obtained from peak areas. Standard solutions of LMWC were prepared using calibration curves in the 0.1–1 mg/mL range (5 calibration points) to calculate the response factor relative to phenyl-β-D-glucoside (n = 3).

2.6. Statistical analysis

The experimental designs and the parameters of the models (Eqs. (1) and (2)) were estimated by multiple linear regression (MLR) using StatGraphics Centurion XV software (Statistical Graphics Corporation, Rockville, MD, USA).

3. Results and discussion

3.1. Qualitative carbohydrates composition of artichoke external bracts

Prior to the optimization of PLE and MAE experimental parameters, characterization of the composition of artichoke external bract extracts was required. For this purpose, both a PLE and a MAE extracts (obtained at 80 °C and 17 min, and 85 °C and 16.5 min, respectively) were analyzed by GC, after a previous derivatization step as indicated in Section 2.5.1. Similar chromatographic profiles of carbohydrates were obtained for both extraction procedures. Fig. 1A shows the GC profiles of the extracted carbohydrates from artichoke bracts using MAE. Glucose, fructose and sucrose were detected in these extracts. Regarding inositols, *chiro*-inositol was the most abundant, followed by *myo*- and

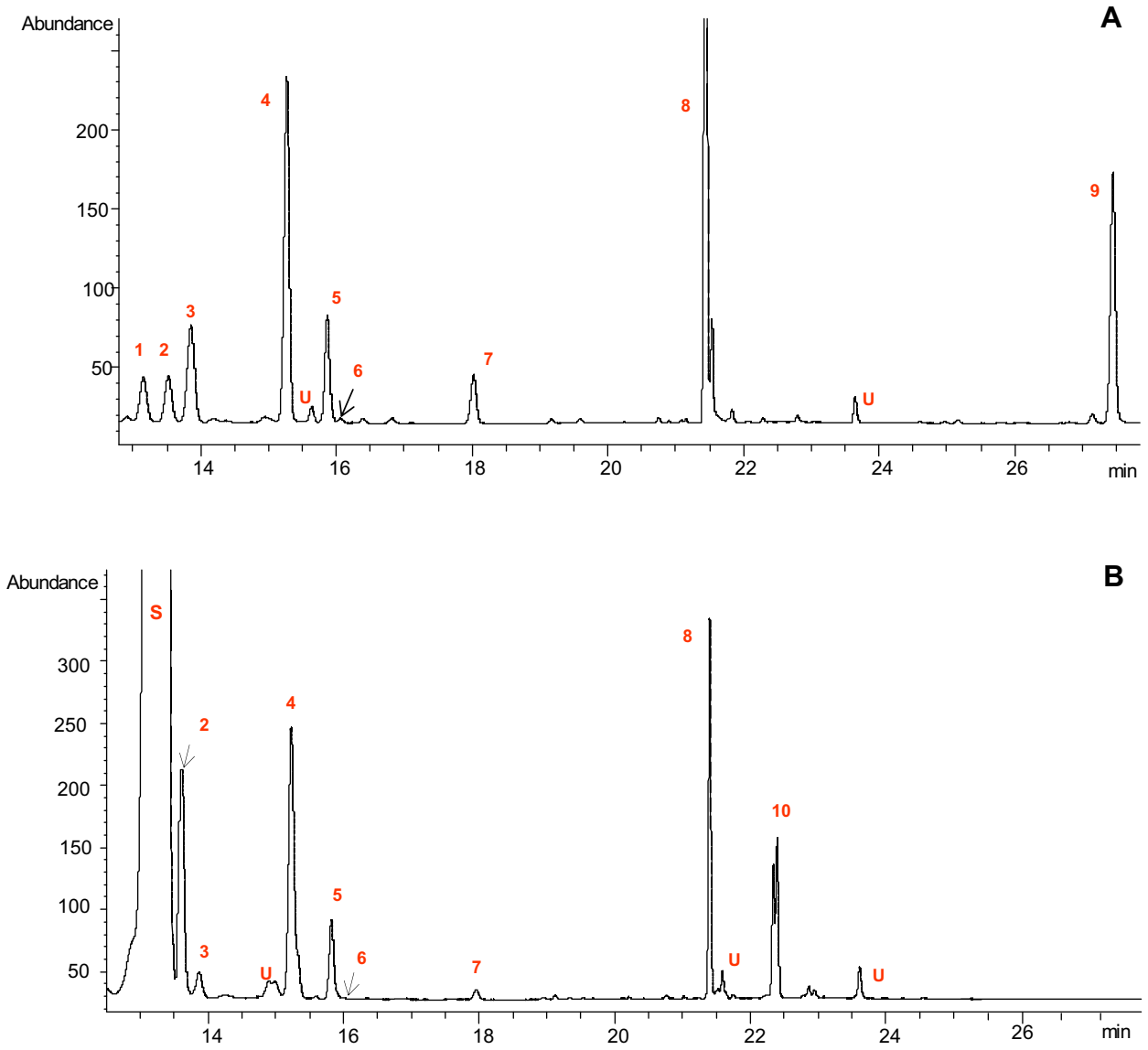


Fig. 1. GC-FID profile of low molecular weight carbohydrates (as TMSO derivatives) extracted from artichoke external bracts using MAE before (A) and after (B) inulinase treatment. Peak identification: 1, 2: fructose; 3: *chiro*-inositol; 4, 5: glucose; 6: *scyllo*-inositol; 7: *myo*-inositol; 8: phenyl-β-D-glucoside (internal standard); 9: sucrose; 10: glycosyl-glycerol: U: unknown compounds; S: sorbitol, present in the commercial inulinase.

scyllo-inositol. These results are consistent with those previously reported by Hernández-Hernández et al. (2011) in artichoke internal bracts.

Previous to the analysis of inulin extracted by MAE and PLE, the optimization of the enzymatic treatment was mandatory to achieve its complete hydrolysis into glucose and fructose. Extracts were treated with inulinase as previously reported (Leroy et al., 2010; Ronkart et al., 2007), at 55 °C and subsamples were taken at different times up to 96 h. Fructose, glucose and sucrose were analyzed by GC before and after inulin hydrolysis. After 48 h treatment, inulin was totally hydrolyzed into fructose and glucose and stable concentrations of these monosaccharides were obtained at following hydrolysis times; therefore this time was chosen for further analyses. Fig. 1B shows the chromatographic profile of LMWC of artichoke bracts extract using MAE after inulinase treatment. As consequence of the hydrolysis, a noticeable increase of fructose (peak 2) was detected whereas glucose only increased slightly. It is worth to mention that an abundant peak (peak S) eluting at 13.5 min and corresponding to sorbitol was detected in these samples. As described by Novozymes in their product data sheet, inulinase is supplied as a solution in 20% glycerol and 20% sorbitol. This polyalcohol coeluted with the first isomer of fructose (peak 1), hampering its direct quantitation. Therefore, quantitation of fructose in extracts treated with inulinase was done considering peak 2 and the abundance ratio of the isomeric forms (peak 1 and peak 2) of the commercial standard.

3.2. Optimization of the extraction conditions

The efficiency of two techniques (MAE and PLE) for the extraction of bioactive carbohydrates (inositols and inulin) from artichoke external bracts was compared.

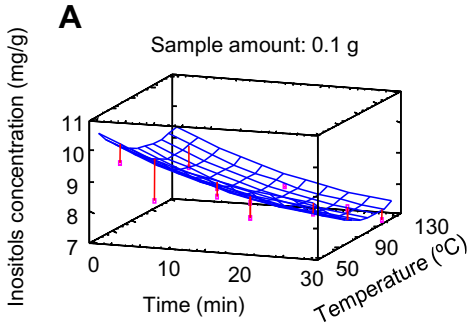
3.2.1. MAE

The solvent nature, the extraction temperature and time, and the solid/liquid ratio have commonly been identified as the most relevant parameters affecting the MAE efficiency (Soria, Ruiz-Aceituno, Ramos, & Sanz, 2014). Considering previous studies

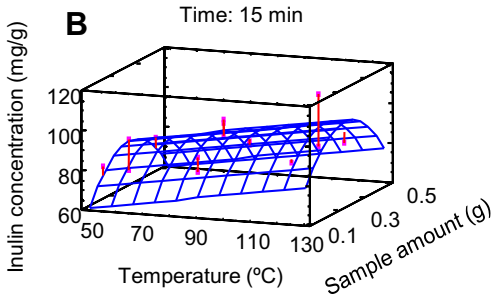
regarding conventional extraction of carbohydrates (López-Molina et al., 2005; Ruiz-Aceituno, Ramos, Martinez-Castro, & Sanz, 2012; Saengkanuk et al., 2011), water was selected as solvent to achieve the highest yields. The influence of three independent variables (extraction time, temperature and sample amount) on MAE of carbohydrates from artichoke bracts was studied using a Box–Behnken design. *R*1 (total inositols amount) and *R*2 (inulin amount) were considered as response variables to be maximized, and *R*3 (total sugar amount) as variable to minimize, taking into account that these co-extracted carbohydrates (glucose, fructose and sucrose) may interfere in the bioactivity related to carbohydrate metabolism of artichoke extracts.

Concentrations (mg/g dry weight) of total inositols (*chiro*-, *scyllo*- and *myo*-inositol), total sugars (glucose, fructose and sucrose) and inulin in artichoke bract extracts obtained by MAE at the different conditions evaluated by the experimental design are shown in Table 1. Inositol concentrations varied between 6.7 and 9.3 mg/g dry sample, total sugars between 50.4 and 66.7 mg/g dry sample and inulin concentrations ranged from 69.8 to 114.6 mg/g dry sample.

Response surface methodology was used to calculate the coefficients of the quadratic models proposed and to estimate the statistical significance of the regression coefficients. Regarding *R*1 model, the most significant coefficients ($P < 0.05$) were *t* and *T*, whereas *T* and s^2 were the most significant ($P < 0.05$) for *R*2. Fig. 2 shows the surface plots for *R*1 and *R*2 of the combined effect of parameters and the model equations after excluding non-significant ($P > 0.05$) terms in the model. These quadratic models appropriately described the variability of both *R*1 and *R*2 (R^2 = 83% and 80%, respectively). The highest inositol yields were obtained at low temperatures and short extraction times (optimal conditions: 50 °C, 3 min and 0.1 g of dry sample) (Fig. 2A), whereas the highest inulin yields were achieved when using 120 °C, 3 min and 0.3 g dry sample (Fig. 2B). Regarding *R*3, experimental values could not be adjusted to an appropriate model (R^2 = 32%), which probably could be explained by the low variability of total sugar concentrations at the different conditions assayed. Therefore, this variable could not be minimized under the experimental conditions in this study and was not further considered.



$y = 13.152 - 0.078*T - 0.093*t - 3.208*s + 0.0003*T^2 + 0.194*t*s$



$y = 57.9705 + 0.213571*T - 0.478704*t + 155.545*s - 259.241*s^2$

Fig. 2. MAE response surface plots for the extraction of inositols (A) and inulin (B).

A multiple response analysis was then performed to maximize the extraction of the bioactive carbohydrates (*R*1 and *R*2). The optimal extraction parameters were 60 °C, 3 min and 0.26 g dry sample. Temperature was the factor showing the main differences regarding optimal conditions for *R*1 and *R*2. The increase of extraction temperatures usually improves recoveries of carbohydrates (Passos & Coimbra, 2013); however, high temperatures frequently result in an increase of undesirable coextracted materials. Under the optimal conditions cited above, 9 mg of inositols and 92 mg of inulin per gram of dried artichoke bracts were extracted. These inulin concentrations are in the range of those found in dry artichoke bracts by other authors (Leroy et al., 2011; Ruiz-Cano et al., 2014).

After selection of the most appropriate extraction conditions, 3 successive extraction cycles were carried out for the exhaustive extraction of inositols and inulin from the matrix. Percentage of inositols (8.6%) and inulin (3.0%) found in the third cycle were very small. Since this value may be considered negligible compared with those obtained in the first (70.8% and 92.6%, respectively) and second cycle (20.6% and 4.4%, respectively), only these two cycles were considered for further experiments. Therefore, 11.3 mg of inositols/g and 96.7 mg inulin/g dry samples were obtained under optimized MAE process.

3.2.2. PLE

Due to the intended comparison between MAE and PLE, a sample amount similar to that used in MAE experiments was selected for PLE, i.e., 0.3 g. Under the proposed working conditions and at a selected pressure of 100 bar, after PLE, a total solvent volume of 9–10 mL was collected. This volume was also in the order of the 'sample/solvent' ratio used in the MAE experiment so allowing direct comparison among both sets of results. Therefore, in this case a 3^2 full factorial design was used. The experimental results obtained after PLE of inositols, sugars and inulin from artichoke bracts are shown in Table 2.

Fig. 3 presents the results of fitting the experimental data to the model of Eq. (2) for inositols (*R*1, Fig. 3A) and for inulin (*R*2, Fig. 3B). Regarding *R*1, the most significant parameter ($P < 0.05$) was *t*, whereas both *t* and T^2 were the most significant ($P < 0.05$) for *R*2. These adjusted models explained 68% and 79% of the variability of *R*1 and *R*2, respectively. Under these conditions, optimal inositol yields were achieved at 40 °C and 30 min, whereas the highest inulin yields were extracted at 75 °C and 26.7 min. The slightly decrease in the concentration of inulin at higher temperatures could be associated with changes in the dielectric constant of water under these conditions. Regarding *R*3 (total sugar extraction), the most significant parameter was *T* followed by *t*; the optimal conditions to minimize this response were 40 °C and 3 min. However, the proposed model ($R3 = 48.5769 + 0.0570833T + 0.193827t$) explained only 48% of the observed variability. Consequently, this parameter was not considered for further optimization studies.

A multiple response analysis was carried out to maximize *R*1 and *R*2, alike to MAE. Optimal conditions for inositols and inulin extraction were 75 °C and 26.7 min, whereas the extraction yield of these bioactive carbohydrates was 6.2 mg inositols/g dry sample and 141.0 mg inulin/g dry sample. At these optimal conditions, three PLE cycles were carried out. Regarding the carbohydrates extracted in each cycle (79%, 19% and 3% for inositols, and 73%, 21% and 6% for inulin, corresponding to first, second and third cycle, respectively), the third one was discarded due the negligible

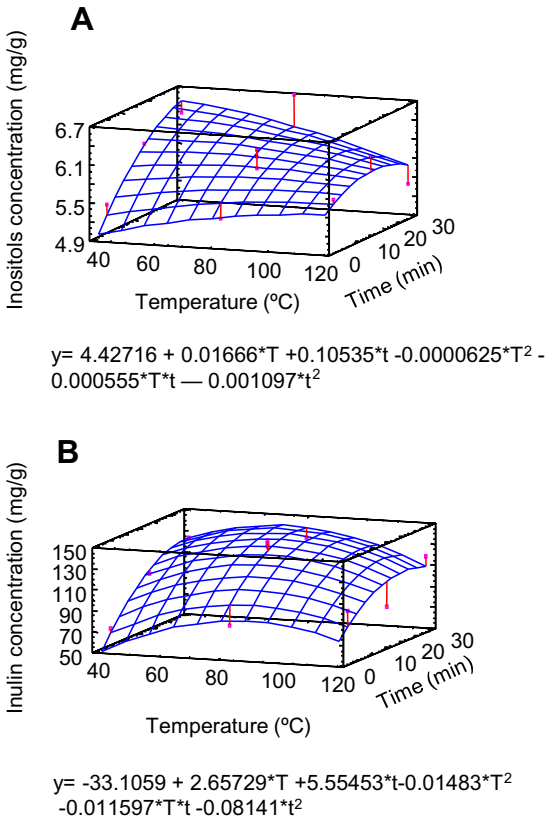


Fig. 3. PLE response surface plots for the extraction of inositols (A) and inulin (B).

amount that represented. Consequently, 7.6 mg inositols/g and 185.4 mg inulin/g dry sample were obtained using optimized conditions with this technique.

3.2.3. Comparison of extraction techniques

Under optimized conditions, MAE allowed the extraction of higher yields of inositols than PLE (11.6 mg/g *vs*. 7.6 mg/g dry sample). On the contrary, the highest yields of inulin were obtained using PLE (185.4 mg/g *vs.* 96.4 mg/g dry sample). In both cases, relatively soft extraction temperatures (60–75 °C) were used. Extraction times were shorter with MAE (3 min), although those of PLE can still be considered acceptable (26.7 min). In consequence, the choice of the extraction method would depend on the specific goal of the treatment.

The comparison of our results with those reported in literature regarding artichoke content of inulin (Leroy et al., 2011; Ruiz-Cano et al., 2014) leads to an extraction of this carbohydrate within the range of the previously reported data. Regarding inositols, higher concentrations were extracted by the evaluated extraction techniques as compared to those concentrations previously described by Hernández-Hernández et al. (2011) (ca. 2 mg/g dry sample of total inositols with solid-liquid extraction *vs*. 11.3 mg/g or 7.6 mg/g dry sample for MAE and PLE, respectively). However, more studies should be done regarding variability of these carbohydrates depending on the maturity, origin, etc. of the artichoke.

3.3. Fractionation assays using yeast

Considering the unavoidable coextraction of LMWC which could interfere in the bioactive properties of inositols and inulin, and the impossibility to minimize their extraction either by MAE or PLE, a fractionation procedure using *S. cerevisiae* was applied to cycles 1 + 2 of MAE artichoke bracts extracts for their selective removal (Rodríguez-Sánchez et al., 2013; Ruiz-Aceituno et al., 2013).

After 3 h of treatment, 40% of sugars remained in the extracts, but after 8 h of treatment all of them were totally removed, except trehalose (produced as consequence of yeast metabolism), which remained at trace level. Concentration of inositols and inulin remained unaltered during this fractionation process. Consequently, these experimental conditions were selected as optimal.

4. Conclusions

Overall, this is the first time that MAE and PLE are proposed for the effective extraction of bioactive carbohydrates (inositols and inulin) from artichoke external bracts, as an agro-industrial by-product. To the best of our knowledge, this is also the first time that the simultaneous extraction of these carbohydrates is optimized by these techniques. In particular, MAE was less time-consuming than PLE. Although co-extraction of other interfering sugars could not be avoided with the proposed extraction procedures, yeast treatment demonstrated to be effective for their subsequent removal, allowing the enrichment of extracts on these bioactive compounds. These extracts could represent an economic source of these bioactives with a potential positive combined activity to be used in food or pharmaceutical products.

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