Food Chemistry 237 (2017) 312–319

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Purification of phlorotannins from *Macrocystis pyrifera* using macroporous resins

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ARTICLE INFO

Article history:
Received 9 July 2016
Received in revised form 19 May 2017
Accepted 22 May 2017
Available online 23 May 2017

Keywords:
Separation
Bioactive compounds
Brown seaweed

ABSTRACT

Phlorotannins are secondary metabolites produced by brown seaweed, which are known for their nutraceutical and pharmacological properties. The aim of this work was to determine the type of macroporous resin and the conditions of operation that improve the purification of phlorotannins extracted from brown seaweed, *Macrocystis pyrifera*. For the purification of phlorotannins, six resins (HP-20, SP-850, XAD-7, XAD-16N, XAD-4 and XAD-2) were assessed. The kinetic adsorption allowed determination of an average adsorption time for the resins of 9 h. The highest level of purification of phlorotannins was obtained with XAD-16N, 42%, with an adsorption capacity of 183 ± 18 mg PGE/g resin, and a desorption ratio of 38.2 ± 7.7%. According to the adsorption isotherm the best temperature of operation was 25 °C, and the model that best described the adsorption properties was the Freundlich model. The purification of phlorotannins might expand their use as a bioactive substance in the food, nutraceutical and pharmaceutical industries.

1. Introduction

In the last decades, the chemistry of natural products of marine origin has been the object of intense research to find new compounds with pharmacological and nutraceutical properties, where seaweed have been identified as the main source of compounds with bioactive properties (Barba, 2016; Roohinejad et al., 2016). Among the compounds with high added value present in seaweed are phlorotannins, polyphenolic compounds formed by oligomers and polymers of the phloroglucinol monomer (Shibata et al., 2004), which have been recognized as antioxidants, antiangiogenic, antiallergic, antiinflammatory and antidiabetic compounds (Kim & Himaya, 2011; Zhao, Xue, & Li, 2008; Zubia, Payri, & Deslandes, 2008; Li, Wijesekaraa, Li, & Kim, 2011; Gupta & Abu-Ghannam, 2011) and are a good candidates for use as functional ingredients in the food industry.

The extraction of phlorotannins from brown seaweed and their characterization has been reported previously (Glombitza & Pauli, 2003; Gupta, Cox, & Abu-Ghannam, 2011; Koivikko, Loponen, Pihlaja, & Jormalainen, 2007; Leyton et al., 2016; Li, Smith, & Hossain, 2006; Ortiz, Bozzo, Navarrete, Osorio, & Rios, 2006; Sanchez-Machado, Lopez-Cervantes, & Lopez-Hernandez, 2004; Tello-Ireland, Lemus-Mondaca, Vega-Gálvez, López, & Scala, 2011), although their purification from crude extracts has not been widely studied yet. Several techniques have been used to purify phlorotannins, such as chromatography using a Sephadex LH-20 column (Kantz & Singleton, 1990; Koivikko et al., 2007; Nwosu et al., 2011), ultrafiltration using membranes with cut-off between 100 and 5 KDa (Wang et al., 2012) and liquid–liquid fractionation with ethyl acetate (Cho et al., 2012; Kang et al., 2012; Shibata et al., 2004). The main drawbacks of these techniques are the use of non food-grade solvents, such as acetone, methanol and ethyl acetate, the low selectivity due to the presence of other co-extracted compounds, the precipitation of polyphenol–protein complexes (Siebert, 1999) and the high cost of these processes (Wang et al., 2012).

An alternative that overcomes most of the limitations of these techniques is macroporous resin separation (Kim et al., 2014). In this method, polyphenols in aqueous solutions are adsorbed on the resins due to hydrophobic binding and aromatic stacking. The adsorbed polyphenols are later desorbed using a mixture of

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http://dx.doi.org/10.1016/j.foodchem.2017.05.114
0308-8146/

water with an organic solvent, such as ethanol (food-grade); sugars present in the crude extract do not interact with the resins, hence, they are easily removed with water. In addition, these resins are approved by the Food and Drug Administration to produce food products (Scordino, Di Mauro, Passerini, & Maccarone, 2003).

To design the process adequately, the mechanisms of adsorption and desorption of the macroporous resin should be understood. Therefore, adsorption isotherms must be determined and fitted using isotherm models such as Langmuir or Freundlich, from where several thermodynamic parameters, such as isosteric heat, entropy and free energy, can be derived (Allen, McKay, & Porter, 2004; Sohn & Kim, 2005). In addition, kinetic data can be fitted to pseudo-first-order and pseudo-second-order kinetic to determine the adsorption efficiency (Ho & McKay, 1998).

Therefore, the aim of this work was to determine the type of macroporous resin and the operating conditions that improve the purification of phlorotannins extracted from brown seaweed *Macrocystis pyrifera*. Adsorption kinetics, static adsorption and desorption, the adsorption isotherm and thermodynamic adsorption parameters of phlorotannins will be evaluated.

2. Materials and methods

2.1. Preparation of phlorotannin extract

The brown seaweed employed was *Macrocystis pyrifera* collected from Chiloe, 30 km southeast of Puerto Montt, Chile, in June 2013, donated by Prof. A. Buschmann from the Universidad de Los Lagos. The alga was dried at 40 °C and ground size <0.5 mm prior to extraction. The extraction condition was optimized in our previous work: solution 0.5 M of NaOH, solid/liquid ratio of 1/20 at 100 °C for 3 h. The mixer was filtered using Whatmann N°1 paper and the liquid phase stored at 4 °C until use. The phlorotannin concentration present in the extract was of 1800 mg phloroglucinol equivalent (PGE)/L (Leyton, Pezoa-Conte, Mäki-Arvela, Mikkola, & Lienqueo, 2017).

2.2. Adsorbents

Six macroporous resins were tested to choose the one with the best adsorption/desorption behavior: Diaion HP-20, Sepabeads SP-850, Amberlite XAD-7, XAD-16N, XAD-4 and XAD-2 (from Sigma-Aldrich). Their physicochemical characteristics are shown in Table 1. All resins were washed with ethanol 70% at 25 °C for 12 h, priori to use.

2.3. Adsorption kinetics of phlorotannins

The adsorption kinetics determination was carried out as follows: 0.2 g of each resin was mixed with 30 mL of phlorotannin extract under continuous agitation (300 rpm) at 25 °C, 1 mL of solution was removed at different time intervals to determine the concentration of phlorotannins in the aqueous solution. The adsorption kinetics allow us to determine the time of adsorption equilibrium. The adsorption capacity of phlorotannins on the resins at a given contact time was calculated with the following equation:

$$q_t = (C_0 - C_t) \times \frac{V_i}{W} \quad (1)$$

where q_t is the adsorption capacity at the given contact time t (mg PGE/g dry resin), C_t is the concentration of phlorotannins in the solution at the given contact time (mg PGE/mL), C_0 is the initial concentrations (mg PGE/mL) of phlorotannins in the solution, V_i is the volume of the initial sample solution (mL), and W is the resin weight (g).

To determine the adsorption efficiency of different resins, the kinetic models of pseudo-first-order (Eq. (2)) and pseudo-second-order (Eq. (3)) were adopted (Ho, 2016; Ho & McKay, 1998; Simonin, 2016):

Pseudo first order:

$$Lg(q_e - q_t) = \frac{K_1}{2.303} t + Lg\ q_e \quad (2)$$

Pseudo second order:

$$\frac{t}{q_t} = \frac{1}{q_e} t + \frac{1}{K_2 q_e^2}) \quad (3)$$

where K_1 and K_2 are the constant rate of the pseudo-first-order-model and pseudo-second-order-model, respectively; q_e is the adsorption capacity at the adsorption equilibrium (mg PGE/g dry resin) and t is time.

2.4. Adsorption of phlorotannins on the resins

In order to determine the best macroporous resin for the adsorption of phlorotannins, a static adsorption was performed, where 2 g of each resin was put into a tube with 30 mL of phlorotannin extract. The tube was shaken using a shaking incubator, at 300 rpm, at 25 °C to reach adsorption equilibrium. After adsorption, the resins were filtered for the subsequent desorption of phlorotannins, and the concentration of phlorotannins in the

Table 1
Physicochemical properties and kinetic parameters of phlorotannin adsorption on XAD-7, XAD-16N, HP-20, XAD-4, XAD-2 and SP-850 at 25 °C.

Resins		HP-20	SP-850	XAD-7	XAD-16N	XAD-4	XAD-2
Physicochemical properties							
Structure		SDVB[a]	SDVB[a]	Acrylic ester	SDVB[a]	SDVB[a]	SDVB[a]
Porosity (mL/g)		1.3	1.2	1.14	0.55	1.0	0.65
Surface area (m^2/g)		600	930	450	800	725	330
Pore radius (A)		260	38	90	200	20	90
Particle size (mm)		0.25–0.60	0.30–0.80	0.25–0.84	0.56–0.71	0.4–0.6	0.25–0.84
Kinetic parameters of phlorotannin adsorption							
Pseudo first order model	q_e[b]	70.3	75.1	79.0	72.2	53.1	55.1
	K_1	0.03	0.03	0.02	0.02	0.01	0.01
	q_1[b]	70.3	75.1	79.0	72.2	51.2	52.4
	R^2	0.88	0.83	0.92	0.89	0.95	0.90
Pseudo second order model	K_2	3.9E−04	3.3E−04	2.5E−04	2.4E−04	6.8E−05	5.5E−05
	q_2[b]	68.6	73.0	76.4	69.4	44.5	44.9
	R^2	0.87	0.85	0.91	0.84	0.90	0.85

[a] SDVB, styrene divinyl-benzene (Information obtained from provider).
[b] q_e, q_1 and q_2 are the experimental and theoretical adsorption capacity of each model, respectively.

extract was measured. The adsorption capacity was calculated according to the following equation:

$$q_e = \frac{(C_0 - C_e)V_i}{W} \quad (4)$$

where q_e is the adsorption capacity at the adsorption equilibrium (mg PGE/g dry resin), and C_e is the equilibrium concentration (mg PGE/mL) of phlorotannins in the solution. C_0, V_i and W are the same as defined above.

2.5. Desorption of phlorotannins from the resins

In order to determine the best macroporous resin for desorption of phlorotannins, a static desorption was performed. A first step was to determine the best ethanol concentration for desorption of phlorotannins, and different concentrations of ethanol (10, 20, 40, 60, 80, 90 and 100% v/v) were screened in XAD-7HP. Once the best ethanol concentration was determined, 30 mL of solvent was added to the different resins, and shaken at 300 rpm and 25 °C to reach desorption equilibrium. After desorption, the resins were filtered and the concentration of phlorotannins in the extract was measured. Desorption ratio, capacity and purification level were calculated according to the following equations:

Desorption ratio

$$D = \frac{C_d V_d}{(C_0 - C_e)V_i} 100 \quad (5)$$

Desorption capacity

$$q_d = C_d \cdot \frac{V_d}{W} \quad (6)$$

Purification level

$$P = \frac{q_e}{q_d} 100 \quad (7)$$

where D is the desorption ratio (%), C_d is the concentration of phlorotannins in the desorption solution (mg PGE/mL), and V_d is the volume of the desorption solution (mL). C_0, C_e and W are the same as defined above, q_d is the desorption capacity of phlorotannins (mg PGE/g) and P is the level of purification.

2.6. Adsorption isotherms of phlorotannins

In order to determine the optimum temperature for the adsorption of phlorotannins, three adsorption isotherms were determined, for which 30 mL of extract of phlorotannins at different initial concentrations were mixed with 2 g of resin and shaken at 300 rpm at different temperatures, specifically 25, 35 and 45 °C, to reach adsorption equilibrium. After the adsorption, the concentration of phlorotannins in the extract was measured.

In order to select a suitable model for describing the adsorption properties of the different resins, the Langmuir and Freundlich equations were tested:

Langmuir:

$$q_e = \frac{K_L C_e q_m}{1 + K_L C_e} \quad (8)$$

Freundlich:

$$q_e = K_F C_e^{\frac{1}{n}} \quad (9)$$

where q_m is the maximum adsorption capacity of the adsorbent (mg/g dry resin), K_L is the parameter related to the adsorption energy (L/mg), K_F reflects the adsorption capacity of an adsorbent (mg/g (L/mg)1/n), and the parameter n represents the affinity of the adsorbent for a given adsorbate.

2.7. Thermodynamic parameters

In order to determine the thermodynamic behavior of the adsorption on the resins, changes in thermodynamic parameters, such as enthalpy (ΔH), entropy (ΔS), and free energy (ΔG) were determined. The change of ΔH and ΔS can be obtained from the slope and intercept of the plot of the natural logarithm of the constant of adsorption equilibrium (K_{eq}) and 1/absolute temperature (1/T). ΔG was determined using the following equations:

$$G = -RTlnK_{eq} \quad (10)$$

$$lnK_{eq} = -\frac{G}{RT} = -\frac{H}{RT} + \frac{S}{R} \quad (11)$$

where R is the gas constant (J/mol K) and T is absolute temperature (°K).

2.8. Determining the phlorotannin content

The concentration of phlorotannins in the extracts was determined according to the Folin–Ciocalteu assay (Singleton & Rossi, 1965) adapted to 96-well plates, as well as standards containing phloroglucinol with concentrations varying from 20 to 100 mg/L. Samples and standards (20 μl) were introduced separately into 96-well plates, each containing 100 μl of Folin–Ciocalteu's reagent diluted with water (10 times) and 80 μl of sodium carbonate (7.5% w/v). The plates were mixed and incubated at 45 °C for 15 min. The absorbance was measured at 765 nm using a UV–Visible spectrophotometer. The phlorotannin concentration was determined by the regression equation of the calibration curve and expressed as mg of phloroglucinol equivalent (mg PGE/L).

2.9. Determination of radical scavenging activity, total antioxidant activity

The free radical scavenging activity was measured using the modified method of Von Gadow, Joubert, and Hansmann (1997). 40 μl of 0.4 M 1,1-diphenyl-2-picryl-hydrazyl (DPPH) solution in ethanol was added to 50 μl of the sample solution, supplemented with 110 μl of ethanol. The plates were mixed and allowed to stand for 30 min in the absence of UV light to avoid decomposition. The absorbance was measured at 520 nm against an ethanol blank. Calibration curves of Trolox (0–24 mg/L) were prepared and the results were expressed as the number of equivalents of Trolox (mg TE/L).

2.10. High precision liquid chromatography (HPLC) detection of carbohydrate and phloroglucinol

The quantification of phloroglucinol was carried out using a reverse phase C18 column (150 × 4.6 mm, 5 μm) and an HPLC system (Shimadzu, Model LC-10A) with ultraviolet (UV) detector (operating at 280 nm). 20 μL of extract was analyzed using as mobile phase 1% v/v formic acid in deionized water (solvent A) and acetonitrile (solvent B), fed at a flow rate and temperature of 1 mL/min and 25 °C according to the following elution gradient: 0–15 min, 5% B; 15–75 min, 5–100% B; 75–85 min, 100% B and 85–90 min, 100–5% B (Sundberg, Pranovich, & Holmbom, 2003). Phloroglucinol (Sigma) was used as standard.

The quantification of carbohydrate was carried out using a HPX-87H column and a Refractive Index Detector (RID) in HPLC. 10 μL of extract was analyzed using as mobile phase of sulfuric acid 50 mM, fed at a flow rate and temperature of 0.5 mL/min and 55 °C with

lineal elution. Glucose, mannitol, galactose, fucoidan, fucose and laminarin were used as standard.

2.11. Statistical analysis

Each experiment was carried out in triplicate. The measurements were presented as average ± standard deviation. The significance and relative influence of each resin in the static adsorption and desorption of phlorotannins were determined using the analysis of variance (ANOVA). The significance of the factors was determined at a 5% confidence level.

3. Results

3.1. The adsorption kinetics of phlorotannins

The curves of the adsorption kinetics for each resin are presented in Fig. 1. In this figure a gradual increase in the adsorption of phlorotannins with time was observed, where all resins reached adsorption equilibrium after approximately 8 to 9 h of contact time. Kinetic data was fitted to pseudo-first-order and pseudo-second-order models as shown in Table 1. According to the correlation coefficient, the pseudo-first-order model best describes the adsorption kinetics of phlorotannins (Table 1).

3.2. The phlorotannin adsorption isotherm

The phlorotannin adsorption isotherms for different resins at 25, 35 and 45 °C are shown in Fig. 2. The phlorotannin data for adsorption was fitted to the Langmuir and Freundlich isotherm equations; the equation constants and correlation coefficients obtained for each models are listed in Table 2. According to the correlation coefficient obtained from each model, the model that better described the adsorption properties was the Freundlich model; the correlation coefficients obtained were 0.80 for XAD-16N, 0.90 for HP-20 and 0.91 for SP-850. The best temperature for adsorption of phlorotannins by different resins was 25 °C.

3.3. Static adsorption and desorption of phlorotannins

The best time and temperature for phlorotannin adsorption were 8 h and 25 °C, respectively. Static adsorption and desorption under these conditions were carried out. The adsorption and desorption capacity of phlorotannins obtained for the resins tested are shown in Fig. 3. The highest adsorption capacity was obtained with HP-20 followed closely by XAD-16N and SP-850 with 190 ± 10, 183 ± 18 and 183 ± 31 mg PGE/g, respectively. The lowest adsorption capacity was obtained with XAD-2, (156 ± 27 mg PGE/g). The adsorption efficiency obtained for each polymer was similar, showing values of 70.6 ± 1.1, 69.5 ± 2.0, 70.3 ± 0.2, 69.7 ± 0.1,

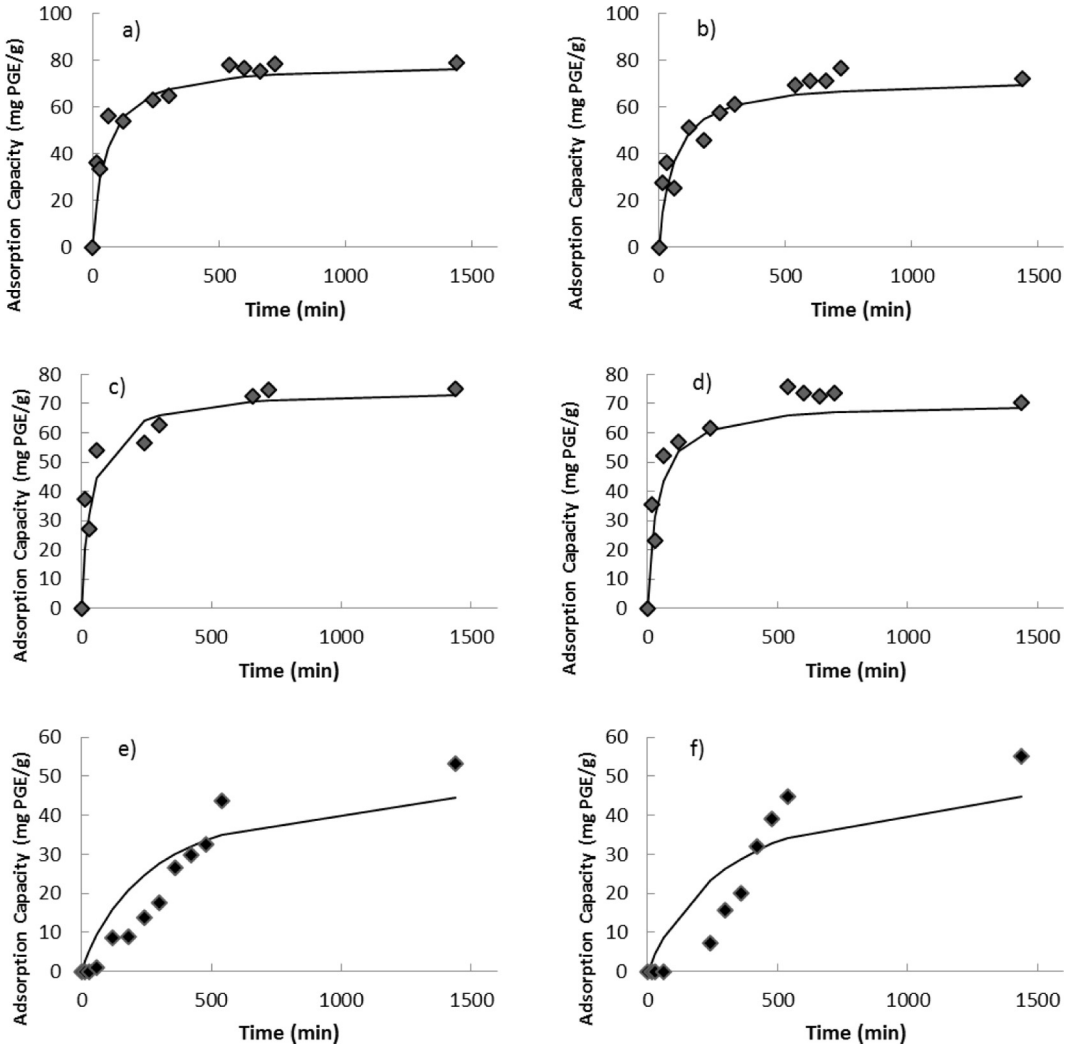

Fig. 1. Adsorption kinetics of phlorotannins on: a) XAD-7, b) XAD-16N, c) SP-850, d) HP-20, e) XAD-4 and f) XAD-2 at 25 °C. The continuous line is for pseudo-first-order.

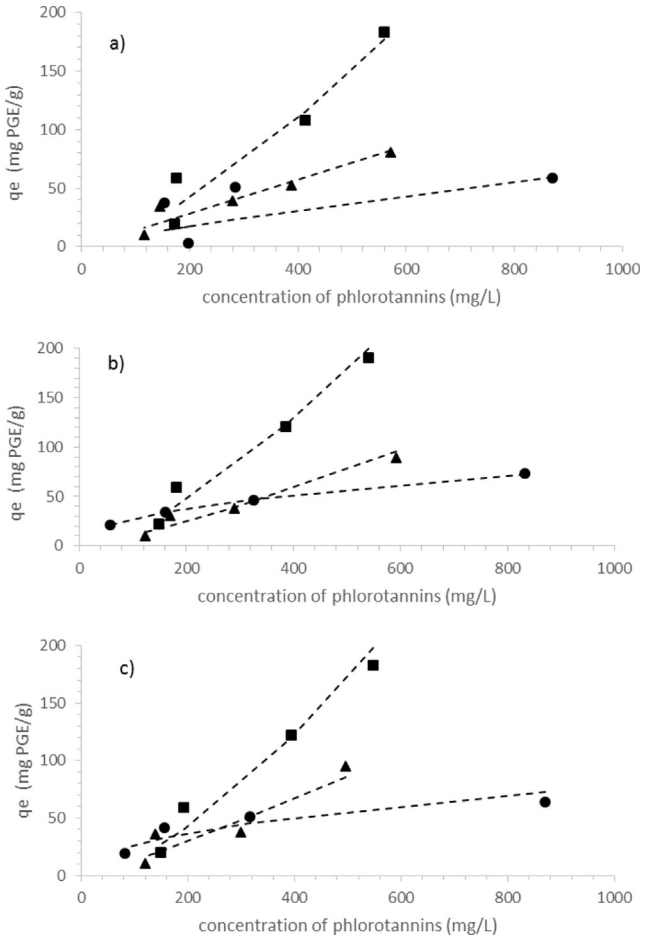

Fig. 2. Equilibrium adsorption isotherm of phlorotannin adsorption on: a) XAD-16N, b) HP-20 and c) SP-850 at 25 °C (■), 35 °C (▲) and 45 °C (●). The segmented line is for the Freundlich model. q_e is the adsorption capacity of phlorotannins.

70.6 ± 0.9 and 69.1 ± 3.3% for HP-20, XAD-16N, SP-850, XAD-2, XAD-4 and XAD-7, respectively.

The best desorption of phlorotannins was achieved (data not shown) with ethanol at 90% v/v; hence, this concentration was employed for the desorption experiments. The desorption ratios obtained for HP-20, XAD-16N and SP-850 were 32.8 ± 2.1, 38.2 ± 7.7 and 27.7 ± 4.6%, respectively, with a desorption capacity of 47.3 ± 0.2, 77 ± 13 and 53 ± 14 mg PGE/g, respectively. The purification levels (recovery of phlorotannins) reached with the resins were 24.9, 42.0, 29.0, 36.0, 32.4 and 32.4% for HP-20, XAD-16N, SP-850, XAD-2, XAD-4 and XAD-7, respectively. The statistical analysis (ANOVA) showed that the resins do not present a significant effect ($p < 0.05$) on the adsorption and desorption capacity (Table 3), therefore the criteria for selecting the best resin was the level of purification which was XAD-16N at 42%.

3.4. Thermodynamic parameters

The thermodynamic parameters were calculated using the constant of adsorption equilibrium of the Freundlich model (K_F) for each resin, the value of enthalpy (ΔH), entropy (ΔS), and free energy (ΔG) are presented in Table 2. Adsorption with XAD-16N, HP-20 and SP-850 show positive enthalpy values of 87, 200 and 215 kJ/mol, respectively, which suggests an endothermic adsorption process (Montgomery, 1985; Özcan, Özcan, Tunali, Akar, & Kiran, 2005). The free energy of all resins was negative, which suggests spontaneous and physical adsorption (Han et al., 2009; Karakaya, 2011). The entropy values for XAD-16N, HP-20 and SP-850 were positive at 262, 632 and 683 kJ/mol K respectively, which suggests increased randomness at the solid/solution interface during the adsorption of phlorotannins (Özcan et al., 2005).

3.5. Characterization of the fraction of phlorotannins desorbed using XAD-16N macroporous resin

The fraction of phlorotannins desorbed from XAD-16N presented an antioxidant activity of 632.88 mg TE/L with a phloroglucinol concentration of 8.6 g/L (0.78 g/g dry sample, DS) and carbohydrate concentration of 0.38 g/L (0.034 g/g DS). For the initial solution (before static adsorption and desorption) the antioxidant activity was 3860 mg TE/L with a concentration of phloroglucinol and carbohydrate of 12.8 and 43.6 g/L (0.26 and 0.87 g/g DS), respectively. Therefore, the use of XAD-16 N allowed enrichment (30 times) of phloroglucinol and a decrease in carbohydrate concentration of 92% of the fraction desorbed respect to solution initial.

4. Discussion

The most commonly used methods for the purification of crude extracts of phlorotannins have been liquid-liquid or solid-liquid separation based on the polarity of the molecules (Cérantola, Breton, Ar Gall, et al., 2006; Kubanek, Lester, Fenical, et al., 2004; Zubia, Fabre, Kerjean, et al., 2009), and discrimination of molecular size through dialysis, and/or ultrafiltration steps (Arnold & Targett, 1998; Breton, Cérantola, & Ar Gall, 2011; Le Lann, Ferret, VanMee, et al., 2012; Tierney, Smyth, Rai, et al., 2013), which use toxic solvents incompatible with the food industry, as well as complex processes that hinder their scaling (Siebert, 1999; Wang et al., 2012). On the other hand, the use of resins such as Sephadex LH-20 and silica columns have been used for the separation of phlorotannins

Table 2
Isotherm parameters and thermodynamic parameters for phlorotannin adsorption on XAD-7, XAD-16 N, HP-20, XAD-4, XAD-2 and SP-850 at 25, 35 and 45 °C.

		Isotherm parameters of phlorotannin adsorption						Thermodynamic parameters for phlorotannin adsorption		
		Langmuir equation			Freundlich equation					
Resin	Temperature (°C/K)	K_L	q_m	R^2	K_F	n	R^2	ΔH (kJ/mol)	ΔG (kJ/mol)	ΔS (kJ/mol K)
XAD-16N	25/298.15	1.2E−03	117	0.59	0.02	0.70	0.80		−78174	
	35/308.15	9.4E−04	110	0.71	0.13	0.98	0.79	87	−80799	262
	45/318.15	5.9E−04	79	0.10	0.20	1.18	0.21		−83424	
HP-20	25/298.15	1.5E−03	98	0.79	0.02	0.68	0.90		−188294	
	35/308.15	1.5E−03	51	0.80	0.02	0.74	0.89	200	−194616	632
	45/318.15	7.7E−03	69	0.96	3.59	2.25	1.00		−200939	
SP-850	25/298.15	1.6E−03	77	0.81	0.01	0.65	0.91		−203472	
	35/308.15	1.1E−03	96	0.60	0.06	0.86	0.72	215	−210303	683
	45/318.15	2.8E−03	111	0.94	3.05	2.13	0.84		−217135	

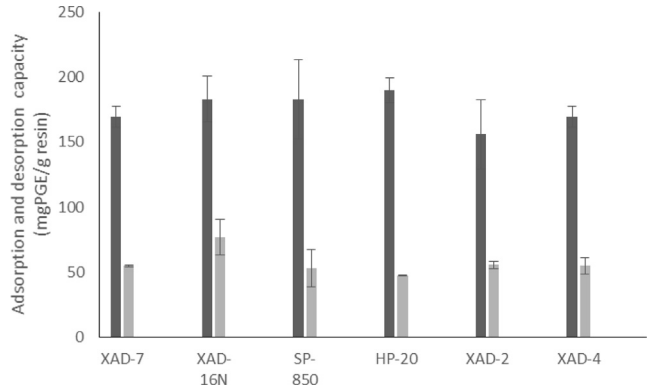

Fig. 3. The static adsorption (black) and desorption (gray) capacities of different resins at 25 °C.

obtaining low yields due to loss of sample by irreversible adsorption (Lee et al., 2014)

The use of macroporous resins for the purification of phlorotannins was proposed as an environmentally friendly alternative due to the use of food-grade organic solvent, low cost due to the reuse of the resins, and safety due to their compatibility with food products according to the FDA. Macroporous adsorption resins have been used to purify bioactive compounds from food and plant extracts (Alexandratos, 2009; Kim et al., 2014; Ma et al., 2009). The macroporous resins screened for adsorption and desorption of phlorotannins were XAD-7, HP-20, XAD-16N, XAD-2, XAD-4 and SP-850, which have been used in the purification of plant polyphenols in previous studies (Bretag, Kammerer, Jensen, & Carle, 2009; Hui et al., 2010; Lin, Zhao, Dong, Yang, & Zhao, 2012; Soto et al., 2012). Only (Kim et al., 2014) have done prior studies of the purification of phlorotannins from brown seaweed with macroporous resins.

The concentration and molecular size of phlorotannins vary according to both intrinsic factors (reproductive condition, age and size of the algae), and extrinsic factors (environmental and ecological stimuli). They are derived from the polymerization of phloroglucinol units and depend on the types of interlinkage present. Phlorotannins can be classified into various subclasses, i.e. phlorotannins with phenyl linkages, ether linkages or ether and phenyl linkages that are characteristics for different types of phlorotannins. Phlorotannins are hydrophilic compounds whose molecular size varies between 126 Da (phloroglucinol) and 650 kDa (phlorofucoroeckol) (Glombitza & Pauli, 2003; Ortiz et al., 2006; Sanchez-Machado et al., 2004).

According to the hydrophilic nature and molecular size of phlorotannins, macroporous resins of styrene divinyl-benzene and acrylic ester structure were used, since they have hydrophilic surface of high and intermediate polarity (Information obtained from provider), respectively, favoring the interaction between phlorotannins and resins. Furthermore, the different pore sizes of the resins, see Table 1, allow the separation of phlorotannins with different polymerization grades, which is reflected in the adsorption efficiency obtained for each resin, 70% on average, indicating the adsorption of phlorotannins of small and intermediate molecular size and polymerization grade.

From the macroporous resins tested, we found that XAD-16N was the best to purify phlorotannins extracted from *M. pyrifera*. In addition, the optimum operating conditions found were 25 °C and 9 h of adsorption and 9 h of desorption. Under these conditions, the adsorption capacity for XAD-16N was 183 ± 18 mg PGE/g resin, the desorption ratio was 38 ± 8% and the purification level was 42%. These values are higher than those reported by Kim et al. (2014), using HP-20 for the purification of phlorotannins from *E. cava*, with an adsorption and desorption capacity of 38 and 35 mg PGE/g resin, respectively. This difference could be attributable to a higher surface area and pore size of XAD-16N in comparison to HP-20 (see Table 1), which could allow the migration of polymeric units of phloroglucinol that form the different types of phlorotannin compounds present in *M. pyrifera* (Leyton et al., 2016). On the other hand, the purification level can be further improved by employing a continuous or dynamic desorption processes (Kim et al., 2014).

The model that best described the adsorption isotherms for XAD-16N, HP-20 and SP-850 resins was the Freundlich model. This model assumes that the surface of the resin is heterogeneous and characterized by sorption sites at different energy levels (Duran, Ozdes, Gundogdu, & Senturk, 2011). Fig. 2 shows that the capacity of the resins to adsorb phlorotannins decreases with temperature, suggesting a physisorption mechanism defined by Van der Waals forces (Duran et al., 2011; Montgomery, 1985). The thermodynamic parameters provide information on the inherent energy change of adsorbents after adsorption, and also the mechanism involved in the adsorption process. The positive value of ΔS obtained for XAD-16N, HP-20 and SP-850, suggests that some structural changes occur on the solid/liquid interface where randomness increases as the adsorption process progresses (Gupta, 1998). The value of ΔG for XAD-16N, HP-20 and SP-850 increases with temperature, indicating that the adsorption becomes more favorable at lower temperatures, which is consistent with the behavior shown in Fig. 2 (Gao, Yu, Yue, & Quek, 2013).

On the other hand, in the market there is widespread use of antioxidants of synthetic origin, the most widely used being: hidroxybutylanisol (BHA), butylated hydroxy toluene (BHT), propyl gallate (PG), Octylgalate (OG) and tertiary butyl hydroquinone (TBHQ), used to preserve oils, fats or other compounds from deterioration by oxidation. However, the use of these compounds has been linked to increased tumor activity (particularly BHA and BHT) (Kahl & Kappus, 1993) resulting in strict regulation for use in foods (Hettiarachchy, Glenn, Gnanasambandan, & Johnson, 1996). Consequently, the interest in natural sources of antioxidants has increased. The antioxidant activity present in the phlorotannin

Table 3
Analysis of variance (ANOVA) for static adsorption and desorption capacity of phlorotannins.

Factors	DOF[a]	Sum of Squares	Variance	F-ratio	P-value
Adsorption capacity					
Resins	5	1552	310	0.85	0.56
Others	6	2181	363		
Total	11	3733			
Desorption capacity					
Resins	5	1031	206	2.85	0.12
Others	6	435	724		
Total	11	1465			

[a] DOF, degree of freedom.

extract desorbed from the resin XAD-16N could be used as a natural antioxidant to prevent lipid oxidation in substitution of synthetic compounds.

The high concentration of phenolic compounds in marine algae species contribute to their antioxidant properties, which can be of benefit in reducing oxidative reactions deleterious in food (Gupta & Abu-Ghannam, 2011). Although the relationship between the structures of phlorotannins and their radical scavenging activities are unclear, it may be that the phenolic hydroxyl groups attached to the eckol skeleton play an important role (Shibata, Ishimaru, Kawaguchi, Yoshikawa, & Hama, 2008). Therefore is expected that a high concentration of polyphenols is related to a high antioxidant activity as was the case in this study

5. Conclusions

The best macroporous resin for the purification of phlorotannins extracted from *M. pyrifera* was XAD-16N. The optimum operating conditions determined from kinetic and isotherm of adsorption were 25 °C for 9 h of adsorption followed by desorption with ethanol 90% v/v at 25 °C for 9 h. Under these conditions, the adsorption capacity was 183 ± 18 mg PGE/g resin, with a desorption ratio of 38 ± 8% and a purification level of 42%. To improve the purification level, further studies applying dynamic adsorption and desorption are needed. The purification of phlorotannin compounds from crude extracts could expand their use as nutraceuticals and increase the commercial value of the product.

Acknowledgments

The authors would like to acknowledge the financial support from AKA-ERNC 009 PROJECT, Centre for Biotechnology and Bioengineering (CeBiB) FB-0001; FONDECYT through Postdoc Grant 3150622; and Anillo en Ciencia y Tecnología, Grant ACT1105, CONICYT, Chile.

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