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Adsorption of grape pomace (Vitis vinifera) and pecan shell (Carya illinoensis) phenolic compounds to insoluble dietary fiber

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Abstract

Grape pomace (GP) and pecan shell (PS) are agro-industrial by-products rich in healthy bioactives, mainly phenolic compounds (PC) and dietary fiber (DF). Raw GP and PS were chemically characterized, and the adsorption isotherms of their PC-insoluble DF (IDF) complexes were evaluated. GP and PS statistically differed ($p < 0.05$) in chemical and physicochemical properties. Total PC, total flavonoids, and IDF were higher in GP. Thirteen and eight PC were identified in GP and PS by HPLC-MS/MS chromatography, finding isoquercetin and type B dimer and trimer procyanidins as the most abundant compounds. PC-IDF (phenolic compounds-insoluble dietary fiber) adsorption isotherms were determined by both spectroscopic (Freundlich) and HPLC-MS/MS (Freundlich/Langmuir) techniques, observing that PS IDF presented higher PC adsorption at all tested concentrations. Epicatechin, isoquercetin, and quercetin were the main identified PC in both by-products, and were able to fit to both Langmuir and Freundlich isotherm by HPLC-MS/MS.

Keywords: phenolic compounds-dietary fiber interactions; adsorption isotherm; by-products; HPLC-MS/MS; Langmuir isotherm; Freundlich isotherm.

Practical Application: The evaluation of grape pomace (GP) and pecan shell (PS) by-products phenolic compounds-insoluble dietary fiber (PC-IDF) interactions will help to predict the effect of the addition of these by-products on the bioaccessibility, bioavailability, and technological effects of both types of phytochemicals on newly formulated functional foods.

1 Introduction

Plant foods are rich in bioactive compounds such as phenolic compounds (PC) and dietary fiber (DF). PC possesses antioxidant, anti-inflammatory, and anti-cancer effects, among others (Halake et al., 2016), while DF exerts health effects such as laxative, blood glucose, and lipid regulator, and acts as a prebiotic in the microbiome gut (Phan et al., 2015; Subiria-Cueto et al., 2022). PC are present in plant tissues either as free soluble forms (unbound) or bound to DF or other molecules (Zhu et al., 2018). DF, defined as non-starchy polysaccharides resistant to acid-alkaline conditions in the digestive system, that can be totally or partially fermented in the colon by intestinal microbiota, is classified in soluble (pectin, gums, oligosaccharides) and insoluble DF (cellulose, hemicellulose, and lignin) (Phan et al., 2015; Subiria-Cueto et al., 2022). Both soluble and insoluble DF can interact with PC, modifying their properties (González-Aguilar et al., 2017).

Natural PC-DF complexes may be formed during plant development (ripening), postharvest/culinary processing of plant foods, and during their gastrointestinal passage, modifying the technological and nutritional properties of plant foods (Zhu et al., 2018) by affecting the luminal bioaccessibility and further bioavailability of PC and nutrients (Phan et al., 2015). Particularly, IDF seems to protect PC from irreversible loss, transporting them intact to the colon where they may be metabolized by the resident microflora to postbiotic substrates with even better health-promoting bioactivities (Dobson et al., 2019). However, this PC-IDF complexes may also reduce the bioaccesibility and bioavailability of PC and hence, some of their beneficial activities (Martinez-Gonzalez et al., 2017). PC-IDF complexes have been characterized by studying their adsorption isotherms (Dobson et al., 2019; Koh et al., 2020; Liu et al., 2019); however these studies have used commercial cellulose and PC, and the interaction of complex PC extracts with IDF isolates from the same plant matrices has not been previously studied (Costa et al., 2015; Guo et al., 2018; Phan et al., 2017; Phan et al., 2015).

Agro-industrial wastes and by-products such as grape pomace (GP) and pecan shells (PS) are excellent sources of PC and IDF, whose health benefits have been recognized for many years (Antonić et al., 2020; Atanasov et al., 2018). Therefore, there is interest in using them as ingredients for the formulation of novel foods with enhanced health and technological properties

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(Czajkowska-González et al., 2021). However, there is limited information on the physicochemical interactions between PC and IDF in complex matrixes such as GP and PS, which could impact their biological activity, health and technological properties. Since a better knowledge of these interactions can help to optimize their use as food ingredients, this study aimed first, to chemically characterize PS and GP, their PC and DF; then, PC and IDF were extracted from both matrixes and their adsorption behavior analyzed by Langmuir and Freundlich isotherms.

2 Material and methods

2.1 PS and GP samples

Cabernet Sauvignon (*Vitis vinifera*) GP samples were kindly donated by Grupo Alximia, in Valle de Guadalupe, Baja California, México. Samples were transported under cooling conditions to laboratory facilities. PS was kindly donated by Procesadora La Nogalera in Ciudad Juárez, Chihuahua, México. Both by-products were oven-dried (Fisher Scientific®) (55 °C) in complete darkness until constant weight, grounded (Jiawanshun®, hc-1000), sieved (420 µm) and stored in vacuum bags until use (Carmona-Jiménez et al., 2018).

2.2 Chemical composition and DF content

Proximate composition of PS and GP samples was determined by AOAC gravimetric methods for moisture, protein (920.87; Association of Official Analytical Chemists, 2000), fat (935.38; Association of Official Analytical Chemists, 2000), and ashes (923.03; Association of Official Analytical Chemists, 2000), and total carbohydrates were calculated by difference (Association of Official Analytical Chemists, 2000; Holguín-Acuña et al., 2008). Water activity (Aw) was measured with AQUA LAB® (Serie 3, Meter Food) equipment, and pH and titratable acidity by potentiometry (Fisherband™ accumet™, AB15 plus). Total, soluble (SDF), and insoluble (IDF) dietary fiber were determined following AOAC methods (985.29 and 991.42; Association of Official Analytical Chemists, 2000). Klason lignin content was determined by mixing 100 mg of IDF with 15 mL of 1.6M H_2SO_4 and heating the mixture for 90 min at 100 °C. Klason lignin samples were recovered by filtration, washed, dried at 60 °C for 5 h, and weighted (Blancas-Benitez et al., 2015; Saura-Calixto et al., 1991).

2.3 Extraction and quantification of PC by spectrophotometric methods

Soluble PC (soluble phenolic compounds) were extracted from both by-products with 80% methanol, following the method by Rosa et al. (2011). Extractable phenolic compounds (PC), flavonoids, condensed tannins, and monomeric anthocyanins were determined from the methanolic extract by Folin-Ciocalteu (Moreno-Escamilla et al., 2017), $AlCl_3$ (Rosa et al., 2011), DMAC (Dimethylacetamide) (Muñoz-Bernal et al., 2021), and pH-differential method (Muñoz-Bernal et al., 2021), respectively. Results were expressed as gallic acid equivalents (GAE) per gram of dry weight (DW) for PC, catechin equivalents (CE) per gram DW for flavonoids and condensed tannins, and cyanidin 3-glucoside equivalents (EC3G) per gram of DW for anthocyanins.

2.4 Identification of individual PC by HPLC-QTOF- MS/MS

Individual PC were identified according to the procedure of Muñoz-Bernal et al. (2021), using an Agilent Series 1200 system combined with the Agilent 6500 Series Q-TOF MS/MS system (Muñoz-Bernal et al., 2021). Separation of individual PC was carried out using a high-definition fast resolution reversed-phase C18 column (2.1 x 50 mm; 1.8 µm; ZORBAX Eclipse Plus®, Agilent, California, USA) at 25 °C with a C18 guard column cartridge. PS and GP extracts were resuspended (2 mg/mL) in acetonitrile: HPLC-grade water (50% v/v), filtered through 0.45 µm nylon filters, and injected (3.0 µL) at a flow rate of 0.4 mL/min. The mobile phase consisted of solvent A (formic acid, 0.1% v / v,) and solvent B (acetonitrile). Linear gradients were as follows: 0 to 4 min, 90% A, 4-6 min, 70% A, 6-8 min, 62% A, 8-8.5 min, 40% A, 8.5-9, 5 min, 90% A. Eluted PC were detected at 255, 275 and 320 nm. HPLC MS/MS was equipped with an electrospray ion source, operated in a negative mode. Nitrogen was used as drying gas at 340 °C, with a flow rate of 13 L/min; the nebulizer pressure was set at 60 psi, with a capillary voltage of 175 V and a mass scan range of 100 to 1000 m/z. Identification of compounds was carried out using the UV/Vis, MS and MS/MS data, and the retention times of the available standards.

2.5 Antioxidant capacity

The antioxidant capacity of GP and PS was evaluated by the ferric ion reducing antioxidant power (FRAP) (Moreno-Escamilla et al., 2017), the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) (Rosa et al., 2011), and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate (ABTS) (Brand-Williams et al., 1995) methods. Results were reported as micromoles of Trolox equivalents (TE)/g dry weight.

2.6 Obtention of Insoluble Dietary Fiber (IDF)

IDF of both by-products was obtained according to the methodology proposed by Punnadiyil et al. (2016) and Vieyra et al. (2015). Ten grams of each dry by-product were mixed with 300 mL of 2% NaOH at 95 °C for 2 h. The dark paste obtained was filtered, washed with distilled water, and dried at 60 °C for 3 h. Then, 100 mL of 50% (v/v) acetic acid: H_2O_2 was added and mixed for 4 h, at room temperature (25 °C), washed and dried again under the previous conditions. The resulting material was transferred to a ball flask and refluxed at 105 °C with 100 mL of 1.5N HCl - 5% NH_4OH for 1 h. Finally, the insoluble dietary fiber isolate was filtered, washed with distilled water, and dried (60 °C) until constant weight.

2.7 IDF water and oil holding capacity

The water and oil retention capacity of IDF in both by-products was determined according to the methodology of Ul-Islam et al. (2012). The results were expressed as grams of water or oil retained per gram of IDF.

2.8 Purification of PC extracts

PS and GP lyophilized methanol extracts were purified with C18 cartridges (Supelclean™ ENVI™-18) to remove low molecular weight polar compounds before the adsorption experiments. One hundred mg of each methanolic extract was dissolved in 10 mL of distilled water and passed through C-18 SPE cartridges. Two volumes of water were used to remove low molecular weight compounds (organic acids, amino acids, sugars), and then 2 mL of methanol was used to recover the purified PC. Methanol was partially removed with rotary evaporation, water was added and lyophilized (Freezone 6 Labconco) to remove the remaining methanol and dry purified extracts were stored at -20 °C (Correa-Betanzo et al., 2014).

2.9 Determination of PC-IDF adsorption isotherms

PC-IDF adsorption isotherms were determined by quantifying the remaining (soluble) PC concentration of solutions containing different PC: IDF ratios. Following the method of Phan et al. (2015) with slightly modification, purified GP and PS extracts were dissolved at different concentrations (0.3 to 7.0 mg/mL) in 0.1M citrate-phosphate buffer pH 5.0. Then, 5 mL of each solution were mixed with 100 mg of their respective IDF isolates (PS or GP) in plastic tubes with lid and kept in a rotary shaking at 20 rpm and 25.0 °C in complete darkness. Equilibrium was achieved after 24 h, and tubes were removed from the shaker, the dispersed particles were allowed to precipitate for 15 min, and aliquots were taken from each tube to quantify PC using the Folin-Ciocalteu reagent. Samples at the same PC concentrations without IDF were used as control. The amount of adsorbed PC was calculated as the difference in the final PC concentration ([PC]*f*) between sample and control at each initial PC concentration (Equation 1) (Phan et al., 2017, 2015).

$$Adsorbed\ PC(Q) = [([PC]\ f\ in\ control - [PC]\ f\ in\ sample) \quad (1)$$

Langmuir and Freundlich isotherms were used to predict the binding capacity of PC to IDF at equilibrium. Langmuir isotherm was linearized according to equation (Equation 2):

$$\frac{1}{Q} = \frac{1}{Qmax} + \frac{1}{Qmax\ x\ KL} x \frac{1}{C} \quad (2)$$

where Q is the amount adsorbed at a certain initial PC concentration (expressed as mg GAE/100 mg of adsorbent); Qmax (mg GAE/100 mg of IDF) is the maximum adsorption capacity of the monolayer; KL is the apparent binding affinity constant, and C is the PC concentration in the solution at equilibrium (mg GAE/mL).

Freundlich isotherm was linearized according to Equation 3:

$$log\ Q = log\ KL + \frac{1}{n} logC \quad (3)$$

where Q is the amount of substrate (PC) adsorbed (expressed in mg GAE/100 mg of adsorbent), after reaching equilibrium; C is the initial PC concentration; KL is the adsorption capacity constant (mg/100 mg IDF) and n is a constant that expresses adsorption intensity (Costa et al., 2015; Phan et al., 2015).

2.10 Determination of PC-IDF adsorption isotherms for individual PC, by HPLC-MS/MS

Adsorption isotherms of individual PC identified in the extracts were determined by HPLC-MS/MS. Experiments were carried out exactly as previously described. At the end of the experiment (24 h), samples were centrifugated at 1000 G for 3 min. One mL aliquots were filtered through a .45 µm nylon filters and placed in HPLC vials. Samples were injected into the HPLC-MS/MS as described in section 2.4. Specific PC were selected if they were quantified in both experimental samples (PC + IDF) and controls. PC final concentrations (at equilibrium) were determined by HPLC-MS/MS, adsorbed individual PC were calculated according to and adjusted to Langmuir and Freundlich isotherms (Equations 2-3) (Costa et al., 2015; Phan et al., 2015).

2.11 Statistical analysis

Experiments were carried out in triplicate and average ± standard deviation was reported. Adsorption isotherms were fitted by linearized Langmuir and Freundlich equations, using total phenol content (Folin-Ciocalteu) or HPLC-MS/MS quantification for individual polyphenols. t- Student was performed using XLSTAT program, version 2021.2 (Addinsoft®). A $p < 0.05$ was used to determine significant differences.

3 Results and discussion

3.1 Physicochemical and DF characterization

The proximal composition of GP and PS samples is shown in Table 1. Moisture, lipids, proteins, total and soluble DF values were between the ranges reported for these by-products by other authors (Prado et al., 2009; Sousa et al., 2014; Tseng & Zhao, 2013; Valiente et al., 1995; Zhang et al., 2017). As expected, and considering that PS is the hard external protective layer of pecan

Table 1. Chemical composition and physicochemical characteristics of raw samples.

	GP	PS
Moisture (g/100 g)	3.3 ± 0.1[a]	1.8 ± 0.0[b]
Ashes (g/100 g)	7.0 ± 0.0[a]	2.2 ± 0.0[b]
Fat (g/100 g)	10.8 ± 0.0[a]	0.9 ± 0.1[b]
Protein (g/100 g)	10.9 ± 0.4[a]	1.5 ± 0.2[b]
Total carbohydrates (g/100 g)	68.0 ± 0.3[b]	93.6 ± 0.2[a]
Total dietary fiber (g/100 g)	41.7 ± 1.2[b]	85.3 ± 0.5[a]
Soluble fiber (g/100 g)	9.9 ± 0.1[b]	22.3 ± 0.0[a]
Insoluble fiber (g/100 g)	31.9 ± 0.7[b]	63.1 ± 0.8[a]
Titratable acidity (% CAE)	0.001 ± 0.0[a]	0.004 ± 0.0[a]
Water activity (Aw)	0.15 ± 0.0[a]	0.06 ± 0.0[a]
pH	3.7 ± 0.2[b]	5.3 ± 0.1[a]
Water holding capacity of IDF (g/g)	2.7 ± 0.0[b]	3.8 ± 0.0[a]
Oil holding capacity of IDF (g/g)	2.5 ± 0.1[b]	3.5 ± 0.4[a]

Values are expressed as mean ± standard deviation. Citric acid equivalents (CAE, 0.064), raw grape pomace (GP), pecan shell (PS). Different superscript per line indicates statistical differences ($p < 0.05$).

nut, it presented higher total dietary, soluble, and insoluble fiber content than GP, while GP contained higher ashes, fat and protein. The content of total DF was 85.3% in PS and 41.7% in GP. The ratio SDF:IDF in both by-products was close to 0.3, which is recommended soluble to insoluble fiber ratio to exert health benefits (Álvarez & González, 2006).

3.2 PC content and antioxidant capacity

Total soluble phenols, flavonoids, condensed tannins, and anthocyanins content of methanolic GP and PS extracts are presented in Table 2. Under the experimental conditions GP presented higher content of total soluble PC, flavonoids and monomeric anthocyanins than PS. This was expected because PS contains a high amount of high molecular weight proanthocyanidins, which are better extracted with acetone (Rosa et al., 2011). However, in the present work, methanol was selected as the extracting solvent to study the interactions between low molecular weight PC with IDF. GP PC, anthocyanin, and tannin contents were within those reported by other studies, while flavonoids showed higher values (Iora et al., 2015; Reis et al., 2016; Xu et al., 2016). GP antioxidant capacity (FRAP, DPPH, and ABTS) was similar to previous reports (Rockenbach et al., 2011). For PS soluble PC, tannins and antioxidant capacity were lower than in other studies due to the use of methanol as extracting solvent (Rosa et al., 2011; Prado et al., 2013).

Table 2. Phenolic Compounds (PC) and antioxidant capacity of raw materials[1,2].

	GP	PS
Soluble PC (mg GAE/g)	99.1 ± 5.9[a]	55.9 ± 0.2[b]
Soluble flavonoids (mg CE/g)	54.3 ± 5.2[a]	24.5 ± 9.6[b]
Monomeric anthocyanin (EC_3G mg/g)	0.3 ± 0.01[a]	0.09 ± 0.01[b]
Condensed tannins (mg CE/g)	13.2 ± 1.9[a]	12.9 ± 2.0[a]
FRAP (μmol TEAC/g)	474.9 ± 47.5[a]	519.1 ± 26.7[a]
ABTS (μmol TEAC/g)	805.7 ± 65.2[a]	890.8 ± 47.2[a]
DPPH (μmol TEAC/g)	596.5 ± 30.1[b]	642.1 ± 11.9[a]

[1]Data is expressed as mean ± SD in dry basis; [2]Grape pomace (GP), pecan shell (PS) methanolic extracts. Soluble phenolic compounds (Soluble PC), 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate assay (ABTS), ferric ion reducing antioxidant power assay (FRAP), gallic acid (GAE), catechin (CE), Cyanidin-3-O- glycoside (C_3G) equivalents (E), Trolox equivalent antioxidant capacity (TEAC). Different superscript letters indicate statistical differences between samples ($p < 0.05$).

3.3 Identification and quantification of individual PC by HPLC MS/MS

Purified (C-18 SPE) PS and GP methanolic PC extracts were injected into an HPLC-MS/MS. Seventeen compounds were identified, and/or quantified, and results are presented in Table 3. Isoquercetin was the main compound quantified in GP, followed by and oligomeric (procyanidin dimers and trimers) and monomeric (catechin and epicatechin) flavan-3-ols, and the flavanones eriodictyol and naringenin. These results are in agreement with those previously published, in which monomeric and oligomeric flavan-3-ols, were reported in GP (Iora et al., 2015; Muñoz-Bernal et al., 2021; Yu & Ahmedna, 2013). Fewer PC were identified and/or quantified in the PS extract. Vanillic acid and procyanidin B1(dimer) were the main compounds quantified in PS. Hydroxybenzoic acid derivatives, flavonols, and flavan-3-ols were found at lower concentrations. In agreement with our results, the presence of hydroxybenzoic acid derivatives and flavan-3-ols monomers has been previously reported in aqueous and ethanol PS extracts (Prado et al., 2014). Interestingly, under

Table 3. Phenolic profile of GP and PS methanolic extracts.

Compound	Formula	RT	Mass	RM	Content*	
					GP	PS
Gallic acid	$C_7H_6O_5$	0.451	170.0217	170.0215	--	--
Vanillic acid	$C_8H_8O_4$	0.519	168.0416	168.0423	--	14.8 ± 2.0
m-Hydroxybenzoic acid	$C_7H_6O_3$	0.99	138.0318	138.0317	--	5.8 ± 0.2
Epigallocatechin	$C_{15}H_{14}O_7$	0.485	306.074	306.074	--	2.3 ± 0.1
Procyanidin B1(dimer)	$C_{30}H_{26}O_{12}$	0.754	578.1414	578.1424	42.6 ± 7.0	14.5 ± 1.0
B-type Procyanidin trimer 1	$C_{45}H_{38}O_{18}$	0.854	866.2048	866.2058	26.2 ± 4.4	4.4 ± 1.0
Catechin	$C_{15}H_{14}O_6$	0.889	290.0792	290.079	18.3 ± 0.0	--
Epicatechin	$C_{15}H_{14}O_6$	1.127	290.079	290.079	11.7 ± 0.2	--
B-type Procyanidin trimer 2	$C_{45}H_{38}O_{18}$	1.225	866.2042	866.2058	2.8 ± 0.5	4.3 ± 0.4
Eriodictyol	$C_{15}H_{12}O_6$	1.663	288.0639	288.0634	11.7 ± 0.5	--
Procyanidin B2 (dimer)	$C_{30}H_{26}O_{12}$	1.798	578.142	578.1424	24.8 ± 0.1	--
B-type Procyanidin trimer 3	$C_{45}H_{38}O_{18}$	2.269	866.2039	866.2058	3.4 ± 0.7	--
B-type Procyanidin trimer 4	$C_{45}H_{38}O_{18}$	2.639	866.2048	866.2058	26.4 ± 2.0	--.
Naringenin glucoside	$C_{21}H_{22}O_{10}$	3.616	434.1213	434.1213	8.3 ± 1.0	--
Isoquercetin	$C_{21}H_{20}O_{12}$	3.784	464.0953	464.0955	310.0 ± 13.0	7.0 ± 0.4
Methyl ellagic acid	$C_{15}H_8O_8$	4.088	316.0217	318.0376	--	1.3 ± 0.2
Isorhamnetin 3-O-glucoside	$C_{22}H_{22}O_{12}$	4.255	478.1113	478.1111	0.3 ± 0.0	--
Naringenin	$C_{15}H_{12}O_5$	5.838	272.069	272.0685	16.1 ± 1.2	--

*Data reported as mg/g of extract. Below detection limit or absent (--); grape pomace (GP); pecan shell (PS); reference mass (RM); retention time (RT).

the extraction conditions, proanthocyanidin dimers and trimers were quantified in higher content in GP than in PS.

3.4 Adsorption of GP and PS PC to their respective IDF

Considering the interest to evaluate the IDF - PC interactions, an IDF fraction, constituted mainly by cellulose, was extracted from each by-product. The IDF isolate content was higher in PS (49.82%) than in GP (18.75%). These results agree with those of IDF content reported in Table 1 for both by-products, although the recovered IDF isolates were lower than the total IDF contents. It has been reported that fiber isolates are mainly conformed of cellulosic compounds (Punnadiyil et al., 2016; Vieyra et al., 2015), which can retain water and oil, however, the presence of Klason lignin has also been detected (Dhingra et al., 2012). PS IDF presented higher Klason lignin content (15.6%) compared to GP IDF (11.3%). The water and oil holding capacity of the PS IDF isolate was also higher than that of the GP IDF isolate (Table 1). This could be related to the higher lignin content and could indicate a higher tendency of PS IDF to interact and adsorb other compounds, such as PC (Khazraji & Robert, 2013; Ul-Islam et al., 2012).

To determine the adsorption isotherms of GP and PS PC with their respective IDF isolates, a kinetic study was first carried out to determine the time needed to reach equilibrium (data not shown). Equilibrium was achieved after 24 h, so different concentrations of raw or SPE-purified PC extracts (GP or PS) were incubated with 100 mg of its corresponding IDF isolate for 24 h. The amount of adsorbed PC was calculated for each initial PC concentration, and experimental data were adjusted to both Langmuir and Freundlich isotherms. Contrary to previous studies that used pure PC standards and purified commercial cellulose, Langmuir demonstrated a better adjustment for the adsorption (Padayachee et al., 2012; Phan et al., 2015), in the present study, Freundlich isotherm model best fitted experimental data for both raw and purified extracts (Table 4). Langmuir isotherm could only be adjusted to PS extracts, especially for the PS raw extract, while Freundlich isotherm could be adjusted to both systems. These results indicate that PC-IDF adsorption followed a multilayer behavior (Soetaredjo et al., 2013). This may be explained by considering that, in the present study, complex PC extracts were used, and consequently, the observed isotherm may be the combination of several individual PC isotherms, which overall may result in a multilayer isotherm (Koh et al., 2020; Phan et al., 2017, 2015). These multilayer isotherms can also be explained by considering that the presence of lignin in the isolates may generate a ternary cellulose-lignin-PC adsorption complex (Ganguly et al., 2020; Punnadiyil et al., 2016; Vieyra et al., 2015).

To evaluate the effect of non-phenolic low molecular weight compounds that can be present in the methanolic extract (such as organic acids, sugars, and amino acids), on the binding behavior of PC to IDF, adsorption isotherms were determined with raw and SPE-purified PC extracts. Figure 1 shows the Freundlich isotherm for raw and purified GP (Figure 1A) and PS (Figure 1B) PC extracts, with their IDF. The raw GP extract showed a higher adsorption isotherm than the purified extract, while PS extracts showed an opposite behavior. This can also be observed in the Freundlich KL parameters (Table 4): KL value was higher for purified *vs* raw PS extracts and higher for raw *vs* purified GP extracts. This could be explained by differences in both the PC extract composition and IDF isolate characteristics of both by-products. In GP the presence of non-phenolic molecules, such as organic acids or amino acids, in the raw extract seem to enhance adsorption of PC to IDF, while in PS non-phenolic molecules interfere with PC adsorption to IDF. It is also worth

Table 4. Langmuir and Freundlich binding parameters for the PC-IDF complexes.

Sample	Langmuir			Freundlich		
	Qmax	KL	R^2	n	KL	R^2
Raw GPE	--	--	--	1.02	0.54	0.98
Purified GPE	--	--	--	1.15	0.39	0.97
Isoquercetin in GPE	0.01	18.35	0.92	1.31	0.03	0.93
Raw PSE	0.87	1.10	0.98	1.53	0.40	0.96
Purified PSE	0.50	0.47	0.99	1.10	0.60	0.99
Catechin derivative in PSE	--	--	--	0.71	5.08	0.98
Ellagic acid derivative in PSE	--	--	--	0.75	2.77	0.99
Quercetin derivative in PSE	0.08	11.83	0.73	0.89	0.7	0.89
Epicatechin in PSE	0.01	169.8	0.93	1.01	0.76	0.96

Phenolic compounds (PC); insoluble dietary fiber (IDF); Affinity constant (KL); Adsorption intensity constant (n); maximum PC adsorbed (Qmax; mg GAE/100 mg of IDF); grape pomace extract (GPE); pecan shell extract (PSE); not fitted (--).

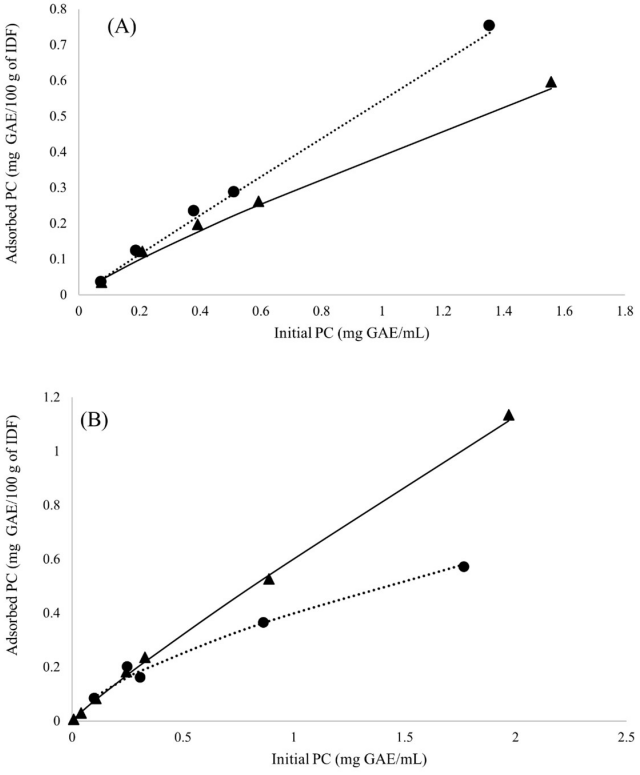

Figure 1. Freundlich adsorption isotherms for GP (A) and PS (B) IDF-PC complexes at 24 h. Freundlich adjustment for raw extract (●) and purified extract (▲). Lines represent the adjusted isotherm with experimental data of raw (·····) and purified (—) extracts.

noting that adsorption of the raw PS extract was the only one better fitted by the Langmuir isotherm, which may indicate that this system also behaved as a monolayer. Another possible explanation is the presence of reducing carbohydrates in the raw extracts, which interact with the Folin-Ciocalteu reagent (Bourvellec et al., 2005; Muñoz-Bernal et al., 2017; Phan et al., 2017, 2015), causing interference in the determination of adsorbed PC and hence, on the calculated parameters.

Comparing the purified extracts, PS showed a higher binding constant (KL) than GP, indicating a higher affinity between PS PC and their insoluble fiber. This higher PS binding constant could be due to the molecular weight and branching degree of polysaccharides found in IDF, as well as the higher Klason lignin content (Guo et al., 2018; Phan et al., 2017), which is also related to the higher water and oil retention capacity of PS IDF isolate (Table 1). The solubility of PC has also been reported to play an important role in their binding to insoluble fractions of other compounds (Doğan & Gökmen, 2015). The obtained results may help to predict the bioaccesibility and bioavailability of PC during consumption of foods fortified with GP and PS, suggesting that both properties would be lower for PS compounds.

3.5 Adsorption of individual PC identified in the extracts

Finally, the binding behavior of individual PC identified by HPLC-MS/MS in PS and GP purified extracts was evaluated using both Langmuir and Freundlich isotherms (Table 4). In the GP extract, isoquercetin, the main PC quantified, was the only individual PC that could be found in all the samples and controls and was adjusted to both Langmuir and Freundlich isotherms. Other individual PC (gallic acid, caffeic acid, isoquercetin) were quantified at low concentrations in the absence of IDF (controls), but they were not detected in the samples containing IDF. This may indicate that they were highly adsorbed by IDF, but its isotherm could not be determined. In the PS extract, one ellagic acid derivative, one quercetin derivative, one catechin derivative, and epicatechin were analyzed with both Langmuir and Freundlich models; however, only epicatechin and the quercetin derivative could be adjusted to both isotherms. The ellagic acid and catechin derivatives were only adjusted to Freundlich (Table 4). In all cases where the PC adsorption could be adjusted to both models, Langmuir showed the higher binding constants (KL), which may indicate that the monolayer mechanism was favored. This is in agreement with spectroscopic studies carried out with individual PC (Costa et al., 2015; Guo et al., 2018; Phan et al., 2017, 2015), and corroborates the suggested hypothesis that when the PC-IDF interaction was evaluated using the total phenolic content (Folin-Ciocalteu method), the combination of several individual PC-IDF complexes had to be explained as a complex multilayer system through the Freundlich isotherm. In the case of isoquercetin, its high concentration in the GP extract (Table 3), may also explain the favorable formation of a monolayer (Dobson et al., 2019; Halake et al., 2016).

The catechin derivative in the PS extract could not be adjusted to the Langmuir isotherm, in contrast to epicatechin, which showed a good fit to both models but a much higher KL value in Langmuir. This suggests the relevance of isomeric structures on the stability of binding isotherms, and could indicate that, while epicatechin forms monolayers, catechin will form multilayer complexes. These results agree with those reported by Soetaredjo et al., that analyzed pure catechin and epicatechin binding with fiber, using the Freundlich isotherm; they found that catechin showed higher binding than epicatechin and described that the aromatic rings of catechin are adsorbed parallel to the surface of the adsorbent, avoiding the union of other compounds, resulting in multiple overlapping layers (multilayers) (Soetaredjo et al., 2013).

4 Conclusion

GP and PS are important sources of PC and DF. GP showed a higher content of methanol-soluble PC while PS showed higher total, soluble and insoluble DF. Isoquercetin was the main PC found in GP, while procyanidin B1 was the main PC in PS. Adsorption of total PC in both extracts to their respective IDF was best fitted to the Freundlich isotherm model, indicating multilayer binding, and the PC-IDF complexes of PS showed a higher binding constant. In agreement with PC extracts, all individual PC identified were best fitted to the Freundlich isotherm model. Interesting, all identified flavonoids (except catechin) could also be fitted to the Langmuir isotherm, which could explain why PS extract (rich in these compounds) could also be fitted to this isotherm model. These results show that the non-covalent binding of PC to IDF depends on the structural characteristics of both PC and IDF and that the presence of multiple compounds in extracts alters the adsorption behavior. The nature and type of the diverse bioactive compounds are fundamental factors to understand how they can bind with each other, and thus predict possible health effects or food applications.

Conflict of interest

The authors declare no conflict of interest.

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