Bioaccessibility of hydroxycinnamic acids and antioxidant capacity from sorghum bran thermally processed during simulated in vitro gastrointestinal digestion

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Abstract Sorghum is a source of hydroxycinnamic acids (HCA), which have shown antioxidant, anti-inflammatory and anti-proliferative capacities. However, a high proportion of them have low bioaccessibility due the complex structural disposition of the plant's cell wall. The effects of boiling and extrusion processes on sorghum bran and their effects on the antioxidant capacity and bioaccessibility of HCA during simulated in vitro gastrointestinal digestion were investigated. The bioaccessibility of phenolic compounds was significantly higher in extruded sorghum bran (38.4%) than that obtained by boiling (29.5%). This is consistent with the increase of the antioxidant capacity after in vitro digestion. In contrast, a low bioaccessibility of pure monomeric HCA was observed when they were exposed to in vitro gastrointestinal digestion. There were significant bioaccessibility reductions of 36.8, 19.5, 13.5, 62.1% for caffeic, *p*-coumaric, ferulic and sinapic acids, respectively, when unprocessed sorghum bran was added. Although the bioaccessibility of monomeric HCA was low, the total phenolic compounds and antioxidant capacity increased during the digestion simulation due to the thermal processes of extrusion and boiling. Extrusion and

boiling could be utilized to produce food based on sorghum bran with biological potential.

Keywords Bioaccessibility · Hydroxycinnamic acid · Antioxidant capacity · Sorghum

Introduction

Sorghum is an underutilized cereal with the potential to help prevent chronic diseases (Stefoska-Needham et al. 2015) due to its content of phytochemicals such as tannins, phenolic acids, anthocyanins, phytosterols and policosanols (Awika and Rooney 2004). These phytochemicals display antioxidant, anti-inflammatory and anti-proliferative effects among others (Awika et al. 2009; Dykes et al. 2013; Nguyen et al. 2015). Hydroxycinnamic acids constitute approximately 60% of the phenolic acids that are present in sorghum and are concentrated in the first layer of sorghum pericarp or bran; (Awika et al. 2005; Luthria and Liu 2013) this group includes caffeic, *p*-coumaric, ferulic and sinapic acids (Ayala-Soto et al. 2015). Although ferulic acid is the major phenolic acid in sorghum bran, a high proportion is bound to arabinoxylans and other indigestible polysaccharides that can resist digestion in the upper gastrointestinal tract, thus restricting its bioaccessibility and biological potential (Mateo-Anson et al. 2009a, b; Zhao et al. 2003). Several studies have indicated that applying processes such as fermentation, dry and wet heating and extrusion to sorghum can improve the content of its phenolic compounds and antioxidant capacity (Afify et al. 2012; Cardoso et al. 2014, 2015; Dlamini et al. 2007; Salazar Lopez et al. 2016; Zaroug et al. 2014).

In previous studies, our research group evaluated the extrusion processes on sorghum bran, it was found an

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increase in hydroxycinnamic acids and total phenolic content as well as antioxidant and anti-inflammatory capacity (Salazar Lopez et al. 2016). Similar increases in total phenolic content and antioxidant capacity were obtained when using boiled sorghum bran (data not published). However, it is necessary to perform studies on the bioaccessibility and biological potential of the phenolic compounds in sorghum after applying processes that are intended for human consumption.

We evaluated the effect of thermal processing on sorghum bran matrix and the bioaccessibility of hydroxycinnamic acids and antioxidant capacity, under simulated in vitro gastrointestinal digestion.

Materials and methods

Sample and reagents

White sorghum (*Sorghum bicolor* L. Moench cv.) free of condensed tannins was obtained from an experimental plantation in Tepic, Nayarit, Mexico during the 2015/2016 season. Pepsin from porcine gastric mucosa, pancreatin from porcine pancreas, porcine bile mixture, caffeic, *p*-coumaric, ferulic and sinapic acids were obtained from Sigma-Aldrich (Saint Louis, MO, USA). All chemicals were of analytical grade.

Obtention of sorghum bran

The sorghum grain was hand-cleaned and sorted to remove broken, diseased or insect infested grain, glumes and other foreign material. The grain was then decorticated (16% bran yield) using a sorghum dehuller (prototype). Floursy grain was removed from bran and discarded. Bran was milled and passed through a 0.5 mm sieve using a Pulvex 200 mill. The final product was named unprocessed sorghum bran and used in all experiments performed.

Processing of sorghum bran

Boiling

The boiling procedure was performed as reported by N'Dri et al. (2013) with slight modifications. Unprocessed sorghum bran (100 g) was weighed into a 1 L flask and 600 mL of boiling distilled water was added and sealed with aluminum foil and placed in a water bath at 100 °C. The cooking time (10 min), was recorded when the water inside of flask reached 98–100 °C. After that the boiled sorghum bran was cooled in an ice bath for 10 min and dried in a convection air oven (100°/4 h) and stored at – 20 °C until analysis.

Extrusion

The sorghum bran was extruded at 180 °C and 20% moisture according to Salazar Lopez et al. (2016). The sorghum bran was hydrated for 8 h (20% moisture) before extrusion and processed in an extruder (prototype) with a single screw with length of 45 cm and two jackets with length of 15 and 10 cm, respectively. The temperature of the jackets was controlled and set at 60 and 180 °C, respectively. The screw speed was established at 15 rpm using a die diameter of 5 mm. The extruded sorghum bran was dried in a convection oven at 60 °C to 6 h and then milled and passed through a 0.5 mm sieve and stored at – 20 °C until analysis.

In vitro digestion

The protocols used for mimic GI digestion conditions in vivo, namely mouth, gastric and intestinal phases were according to Campos-Vega et al. (2015) and Velderrain-Rodríguez et al. (2016) with slight modifications (Fig. 1). The kinetic release of hydroxycinnamic acids and antioxidant capacity from sorghum matrices (unprocessed bran, boiled bran and extruded bran), at different phases of digestion was evaluated. The bioaccessibility of total phenols and hydroxycinnamic acids and antioxidant capacity was evaluated in the different digestion phases by triplicate.

Mouth phase

Three fasting asymptomatic volunteers chewed 1 g of sample (unprocessed sorghum bran, boiled sorghum bran and extruded sorghum bran) 15 times for ~ 15 s. The subjects expelled the chewed sorghum, rinsed their mouths two times with 5 ml of water during 60 s and then expelled the liquid. The two liquid samples and the chewed sorghum were transferred into a 50 mL screw top polypropylene tube that contains mouth digesta. Blanks were prepared without the food matrix following the same simulation digestion conditions as the samples.

Gastric phase

The mouth digesta was subjected to gastric digestion by adding 0.2 M HCl–KCl buffer with the pH adjusted to 1.5. Then, 700 µL of pepsin solution (300 mg/mL) were added and incubated for 1 h in a shaking water bath (Precision Scientific Mod. 66800 Winchester, VA, USA) at 37 °C and 100 rpm to obtain gastric digesta.

Fig. 1 General diagram of in vitro digestion assay

Intestinal phase

The digestion product from the gastric phase was mixed with phosphate buffer (0.1 M, pH 7.5), and the pH was adjusted to 7.5 for intestinal simulation. Then, 1 mL pancreatin solution (17 mg/mL) and bile salts (80 mg) were added, and the mixture was incubated for 6 h in a shaking water bath at 37 °C and 100 rpm to obtain intestinal digesta.

Finally, the products of three digestion phases (mouth, gastric and intestinal) and the blanks were centrifuged for 10 min at 3000 rpm and 4 °C; then, the supernatants were

recovered and lyophilized. The lyophilized samples were dissolved in 50% methanol and filtered (Econofiltr Nylm 0.25 mm 0.45 µm, Santa Clara, CA, United States) and stored at − 20 °C until analysis.

We evaluate the effect of in vitro digestion conditions and the presence of sorghum matrix on bioaccessibility of hydroxycinnamic acids in two consecutive experiments as follows: (1) A mixture of standards (caffeic, *p*-coumaric, ferulic and sinapic acids) was prepared at concentration of 1 mg mL^{−1} for each standard and subjected to simulation in vitro digestion; (2) in parallel, 1 g of unprocessed

sorghum was added to standards mixture and subjected to the digestion conditions as previously described.

Antioxidant capacity

ABTS assay

The Trolox equivalent antioxidant capacity (TEAC) assay is based on the ability of antioxidant molecules to scavenge the 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid radical (ABTS^{•+}), which produces a change in its color that can be spectrophotometrically quantified (Blancas-Benitez et al. 2015). A stable stock solution of ABTS was prepared by mixing 5 mL of an aqueous solution of ABTS (7 mM) with 0.088 mL of sodium persulfate (148 mM) and incubating it in the dark at room temperature for 16–18 h. The ABTS^{•+} working solution was prepared immediately before use by diluting the stock solution in ethanol (~ 1:88, v/v), and its absorbance was adjusted to 0.7 ± 0.02 at 734 nm on a microplate reader FLUOstar® Omega (Ortenberg, Germany). Then, 280 µL of the ABTS working solution was combined with 10 µL of the each digestion product in a microplate well. The changes in absorbance were recorded as ABTS radical-scavenging capacity. A standard curve was prepared using Trolox as a standard, which was used to convert the changes in absorbance of the samples to µmol of Trolox equivalents (TE) g⁻¹ of sample.

DPPH assay

The DPPH radical-scavenging assay was performed as described by Robles-Sánchez et al. (2009). The assay is based on the scavenging of the DPPH radical, which changes its coloration from dark purple to yellow. For this assay, 20 µL of each sample was pipetted into a microplate well, and 280 µL of a methanolic DPPH solution (0.025 mg mL⁻¹) was added. The mixtures were incubated with constant shaking for 30 min at room temperature in the dark, and the changes in absorbance were subsequently monitored at 515 nm on a microplate reader. A standard curve of Trolox was prepared, and the changes in absorbance of the samples were converted to µmol TE g⁻¹ of sample.

Determination of total phenolic content

The total phenolic content of digested products was measured spectrophotometrically by the Folin–Ciocalteu assay with some modifications (Quirós-Sauceda et al. 2014). Briefly, in a 96-well microplate, 30 µL of digested product was mixed with 150 µL of Folin–Ciocalteu reagent (diluted tenfold before use) and 120 µL of 7.5% Na₂CO₃ solution.

The mixture was incubated for 30 min at room temperature, and the absorbance was measured at 765 nm. The results were expressed as micrograms of gallic acid equivalents per gram of sample of dry weight (µg GAE g⁻¹).

Quantification of hydroxycinnamic acids by UHPLC-DAD

The hydroxycinnamic acid content of digestion products was quantified using a UHPLC-DAD system (Agilent Technologies, Germany) with a diode array detector. The hydroxycinnamic acids were identified by their retention time and the absorbance of external standards of caffeic, *p*-coumaric, ferulic, and sinapic acids at 280 nm. The results were expressed as micrograms of hydroxycinnamic acid per gram of dry weight (Salazar Lopez et al. 2016).

Bioaccessibility determination

The estimation of bioaccessibility (%) of total phenols and caffeic acid, *p*-coumaric acid, ferulic acid and sinapic acid for each digestion phases (mouth, gastric and intestinal) of unprocessed, boiled and extruded sorghum bran was calculated as follow:

$$\text{Bioaccessibility (\%)} = \left[\frac{A - C}{B - C} \right] \times 100$$

where *A* represent the total phenolics content or hydroxycinnamic acid content found in the digestion product from unprocessed, boiled or extruded sorghum bran. *B* represent the total phenolic content or hydroxycinnamic acid content found on non-digested sorghum bran (unprocessed sorghum bran). *C* represent the total phenolic content or hydroxycinnamic acid content found in the blank digestion product of each digestion phases.

Statistical analysis

Values are given as mean ± SD of three measurements. The data were tested by ANOVA using statistical software JMP 5.0.1 (USA, SAS institute, Inc.), followed by Tukey test. Differences of *p* < 0.05 were considered significant.

Results and discussion

Several studies have demonstrated a positive correlation between the total phenol content and antioxidant capacity in sorghum and other cereals (Chávez et al. 2017; Guo and Beta 2013). However many of these studies have been carried out on organic solvent extracts, whereby there is a clear need to know the behavior of the bioactive

compounds that are present on the food matrix processed or unprocessed after their intake, as well as its biological potential. Bioaccessibility and stability assays of bioactive compounds have been used to enhance the knowledge of the behavior of these compounds. In this study, we investigated the effect of thermal processing of sorghum bran on antioxidant capacity and bioaccessibility of total phenols and hydroxycinnamic acids during simulated *in vitro* gastrointestinal digestion.

Antioxidant capacity

The effects on the antioxidant capacity of extruded, boiled and unprocessed sorghum bran during the simulated *in vitro* gastrointestinal digestion were measured by the DPPH and ABTS radical scavenging capacity assays (Fig. 2). Both the different conditions for each *in vitro* digestion phase and the applied thermal processes affected the antioxidant capacity ($p < 0.05$) measured with both the ABTS and DPPH assays.

The ABTS values during the intestinal digestion phase in unprocessed, boiled and extruded sorghum bran were up to 2.9, 4.8, and 5.2 times higher than those measured for the mouth phase, respectively (Fig. 2a). Similar behavior was observed when DPPH was evaluated. The digestion product of the sorghum bran processed by extrusion displayed the highest scavenger radical capacity ($12.44 \pm 0.55 \mu\text{mol TE g}^{-1}$) followed by boiled ($8.27 \pm 0.74 \mu\text{mol TE g}^{-1}$) and unprocessed sorghum bran ($7.7 \pm 0.48 \mu\text{mol TE g}^{-1}$) (Fig. 2b). The highest release of phenolic compounds the highest scavenger radical capacity. These results could be explained by considering that during the extrusion process, the sorghum bran matrix was

exposed to high pressure, shear and high temperature (180°C); these conditions can cause mechanical and linkage breakdowns such as glycosidic and ether linkages. Such breakdowns can result in an increase in the porosity of bran, the exposure of bioactive compounds or the production of new molecules with biological activity (Chiramba et al. 2012; Salazar Lopez et al. 2016).

Bioaccessibility of total phenolic compounds

Changes in the total phenolic contents of unprocessed, boiled and extruded sorghum bran were evaluated by the Folin–Ciocalteu method during the simulation of digestion, and the results are summarized in Table 1. Significant differences were observed in the total phenolic contents of unprocessed, boiled and extruded sorghum bran during digestion simulation ($p < 0.05$). The mouth digesta of extruded and boiled sorghum bran had a higher total phenolic content than did that from unprocessed sorghum bran. However, when the digestion products of the mouth phase were exposed to simulated gastric digestion, the total phenolic content was the highest for the extruded sorghum bran ($3604.86 \pm 69.93 \mu\text{g GAE g}^{-1}$) compared to the boiled and unprocessed samples (3141.45 ± 82.46 and $2878.57 \pm 130.22 \mu\text{g GAE g}^{-1}$, respectively). At the end of the gastrointestinal simulation, there were no significant changes due to the intestinal digestion between unprocessed and boiled sorghum bran ($p > 0.05$), but a significant effect was identified when extrusion processes was applied. The processing sorghum bran by extrusion increased the bioaccessibility of the phenolic compounds for up to 38.43%. This finding is consistent with the increase of the antioxidant capacity after the simulation of

Fig. 2 Antioxidant capacity of unprocessed, boiled and extruded sorghum bran during *in vitro* digestion; **a** ABTS, **b** DPPH. Each value represents the mean of three replicates $\pm$ SD

Table 1 Hydroxycinnamic acid content ($\mu\text{g g}^{-1}$) and bioaccessibility (%) in unprocessed, boiled and extruded sorghum bran

Phase digestion	Sorghum	Caffeic $\mu\text{g g}^{-1}$	ρ -Coumaric		Ferulic		Sinapic $\mu\text{g g}^{-1}$	Total phenol content	
			$\mu\text{g g}^{-1}$	%B	$\mu\text{g g}^{-1}$	%B		$\mu\text{g GAE g}^{-1}$	%B
Mouth	Unprocessed	ND	$25.18 \pm 1.29^{\text{cd}}$	$14.17 \pm 0.73^{\text{cd}}$	$24.45 \pm 2.30^{\text{bcd}}$	$0.80 \pm 0.08^{\text{bcd}}$	ND	$1720.24 \pm 25.37^{\text{d}}$	$17.35 \pm 0.26^{\text{d}}$
	Boiled	ND	$32.45 \pm 0.55^{\text{bc}}$	$18.26 \pm 0.31^{\text{bc}}$	$23.60 \pm 0.09^{\text{cd}}$	$0.77 \pm 0.00^{\text{cd}}$	ND	$1991.01 \pm 92.80^{\text{c}}$	$20.08 \pm 0.94^{\text{c}}$
	Extruded	ND	$23.78 \pm 2.63^{\text{d}}$	$13.38 \pm 1.48^{\text{d}}$	$29.04 \pm 3.14^{\text{abc}}$	$0.95 \pm 0.10^{\text{abc}}$	ND	$2163.86 \pm 68.62^{\text{c}}$	$21.83 \pm 0.69^{\text{c}}$
Gastric	Unprocessed	ND	$33.09 \pm 2.23^{\text{ab}}$	$18.62 \pm 1.26^{\text{ab}}$	$26.33 \pm 2.04^{\text{abc}}$	$0.86 \pm 0.07^{\text{abc}}$	ND	$2878.57 \pm 130.22^{\text{b}}$	$29.04 \pm 1.31^{\text{b}}$
	Boiled	ND	$39.28 \pm 2.70^{\text{a}}$	$22.10 \pm 1.52^{\text{a}}$	$20.14 \pm 1.49^{\text{d}}$	$0.66 \pm 0.05^{\text{d}}$	ND	$3141.45 \pm 82.46^{\text{b}}$	$31.69 \pm 0.83^{\text{b}}$
	Extruded	ND	$36.60 \pm 1.42^{\text{ab}}$	$20.59 \pm 0.80^{\text{ab}}$	$31.42 \pm 0.48^{\text{a}}$	$1.03 \pm 0.02^{\text{a}}$	ND	$3604.86 \pm 69.93^{\text{a}}$	$36.36 \pm 0.71^{\text{a}}$
Intestinal	Unprocessed	ND	ND	ND	$18.55 \pm 1.69^{\text{d}}$	$0.61 \pm 0.06^{\text{d}}$	ND	$2873.60 \pm 149.29^{\text{b}}$	$28.99 \pm 1.51^{\text{b}}$
	Boiled	ND	ND	ND	$30.67 \pm 0.72^{\text{a}}$	$1.00 \pm 0.02^{\text{a}}$	ND	$2930.84 \pm 183.44^{\text{b}}$	$29.56 \pm 1.86^{\text{b}}$
	Extruded	ND	ND	ND	$29.36 \pm 0.37^{\text{ab}}$	$0.96 \pm 0.01^{\text{ab}}$	ND	$3809.58 \pm 261.75^{\text{a}}$	$38.43 \pm 2.64^{\text{a}}$

%Bioaccessibility (%B); total phenol content (TFC); ND not detectable or calculable. Each value represents the mean of three replicates $\pm$ SD. Different letters in column represent significant differences between treatments and digestion phase ($p < 0.05$). Bioaccessibility was calculated as amount of hydroxycinnamic acid or total phenols content found in each digestion phases in relation to amount of hydroxycinnamic acid (caffeic acid: $16.0 \pm 0.54 \text{ ug g}^{-1}$; ρ -coumaric acid: $177.7 \pm 3.53 \text{ ug g}^{-1}$; ferulic acid: $3093.0 \pm 87.46 \text{ ug g}^{-1}$; sinapic acid: $3.4 \pm 0.12 \text{ ug g}^{-1}$) or total phenols content found in unprocessed sorghum bran (no digested sorghum bran) ($9914.4 \pm 509.43 \text{ ug GAE g}^{-1}$). The aforementioned values correspond to the sum of free and bound fractions for hydroxycinnamic acids or total phenols

gastrointestinal digestion. The Folin Ciocalteu assay used in this study is designed to quantify particularly substances with phenolic structures among others. Then bioaccessibility obtained in this study for total phenolics could be attributed to several phenolic compounds present in the food matrix including hydroxycinnamic acids.

Bioaccessibility and stability of hydroxycinnamic acids

The hydroxycinnamic acid content from unprocessed, boiled and extruded sorghum bran during simulated gastrointestinal digestion was evaluated (Table 1). Intestinal digestion revealed a significantly greater content of ferulic acid in boiled and extruded sorghum bran (30.67 ± 0.72 and $29.36 \pm 0.37 \mu\text{g g}^{-1}$, respectively) compared to unprocessed sorghum bran ($18.55 \pm 1.69 \mu\text{g g}^{-1}$). In contrast, *p*-coumaric acid did not differ significantly between the sorghum bran that was processed (boiled or extruded) and unprocessed in the gastric phase, and it was not detected during the intestinal phase. Caffeic and sinapic acids were not detected during any of the gastrointestinal simulation phases. The bioaccessibility (%B) of ferulic acid changed minimally through in vitro digestion. However, in the intestinal phase, the bioaccessibility of ferulic acid from boiled ($1.0 \pm 0.02\%$) and extruded ($0.96 \pm 0.01\%$) sorghum bran was higher than that obtained from unprocessed bran ($0.64 \pm 0.02\%$) ($p < 0.05$). According to these results it is possible to establish that both boiling and extrusion process applied to sorghum bran increase the bioaccessibility of ferulic acid by 34–36% in relation to unprocessed sorghum bran. These results describe that it was not possible to associate the increase in total phenolic content and antioxidant capacity in the digested products of bran sorghum with the monomeric hydroxycinnamic acids, because these acids showed low bioaccessibility during the digestion process. Therefore, studies focused on determining other compounds with biological potential derived from sorghum bran processing are necessary.

Previous studies have reported that thermal processes such as microwaving at 180 or 200 °C produced feruloylated arabinoxylo-oligosaccharides from maize accompanied by the production of monosaccharides, free ferulic acid, and furfural (Rose and Inglett 2010). Similarly, Dall'Asta et al. (2016) showed that 59.5% of the total phenolic acids in whole wheat bread are constituted of dimers and trimers of ferulic acid.

In our study, we evaluated only monomers of hydroxycinnamic acids. Therefore, we postulate that ferulated residues could be responsible for the increase in the total phenolic content and DPPH and ABTS radical scavenging capacity found in the digestion products of sorghum bran,

however other forms present, including benzoic and cinnamic acid derivatives, flavonoids and others, could also contribute to the antioxidant capacity (Arrieta-Baez et al. 2012; Awika et al. 2009).

Several studies have shown that the bioaccessibility of hydroxycinnamic acids could be attributed to several factors, such as their interactions with food matrix constituents (proteins, lipids, carbohydrates), the chemical nature of these compounds (dimers, trimers, oligomers), and their stability throughout gastrointestinal digestion (Domínguez-Avila et al. 2017; Jakobek 2015; Mateo-Anson et al. 2009a, b; Sun et al. 2016).

On the other hands, the individual behavior of caffeic, *p*-coumaric, ferulic and sinapic acids during the simulated gastrointestinal digestion, as well as their bioaccessibility after their addition to the food matrix (unprocessed sorghum bran) was evaluated (Fig. 3). It was observed that in vitro digestion conditions reduced the hydroxycinnamic acids content, which it was reflected on a reduced bioaccessibility. This was particularly observed for gastric digestion, which had bioaccessibility values for caffeic, *p*-coumaric, ferulic and sinapic acids of 73.27 ± 2.35 , 68.89 ± 2.53 , 79.45 ± 3.45 , and $70.29 \pm 4.38\%$, respectively. Additionally, when the unprocessed sorghum bran was added to the mixture of hydroxycinnamic acids the bioaccessibility during gastric simulation, was lower than individual hydroxycinnamic acids (57.88 ± 3.19 , 52.10 ± 2.03 , 57.6 ± 2.89 , $50.44 \pm 4.04\%$ for caffeic, *p*-coumaric, ferulic and sinapic acids, respectively). The values obtained after the intestinal phase corresponding to *p*-coumaric and ferulic acids (Fig. 3b, c) showed greater bioaccessibility (%) than did those found in gastric digestion, in contrast to the results obtained for caffeic and sinapic acids, which apparently were affected not only during the gastric phase but also following intestinal digestion (Fig. 3a, d). The above results showed that the food matrix is a factor that contribute to the low bioaccessibility of hydroxycinnamic acids from sorghum, which combined with the changes of pH during the simulation of gastrointestinal digestion, could promote the adsorption of hydroxycinnamic acids limiting their bioaccessibility. Previous studies have shown that adsorption grade of caffeic and ferulic acids by cellulose and xylan varies with the pH, reaching their maximum adsorption at pH 2.0 after 10 min of incubation, and it is reversible at pH 7.0. Caffeic acid displayed a greater affinity for adsorption to cellulose ($8.8 \pm 1.02 \text{ mg g}^{-1}$) and xylan ($9.78 \pm 0.60 \text{ mg g}^{-1}$) compared to ferulic acid (5.29 ± 0.15 and $5.68 \pm 0.28 \text{ mg g}^{-1}$, respectively) (Costa et al. 2015). Similar behavior was observed for caffeic and ferulic acids at pH 4 on cellulose systems (Padayachee et al. 2012).

Based on the adsorption process, different physico-chemical interactions between complex polysaccharides

Fig. 3 Effect of *in vitro* digestion conditions and unprocessed sorghum bran matrix on the bioaccessibility and stability of hydroxycinnamic acids: **a** caffeic acid; **b** p-coumaric acid; **c** ferulic acid; **d** sinapic acid. Each value represents the mean of three replicates $\pm$ SD

and hydroxycinnamic acids can be formed through hydrogen bonds, ester bonds, hydrophobic interactions, and covalent bonds or simply by a physical entrapment (Quirós-Sauceda et al. 2014). Furthermore, the number and position of hydroxyl groups or the native charge of phenolic compounds are determinant factors for that interactions can be presented (hydrogen bonds, non-covalent and hydrophobic) (Domínguez-Avila et al. 2017; Jakobek 2015; Phan et al. 2015) and could be explain the different degrees of adsorption obtained for the hydroxycinnamic acids evaluated.

The bioaccessibility of hydroxycinnamic acids could also be affected by their interaction with digestive enzymes that affect the protein digestibility and bioaccessibility of the phenolic acids. For instance, chlorogenic acid could interact with pepsin forming a chlorogenic-pepsin complex through Van der Waals' forces that occur between

chlorogenic acid and pepsin in the area of the hydrophobic cavity and hydrogen bonds that change the conformation of pepsin and result in the inhibition of pepsin activity (Zeng et al. 2014). The interaction of α -amylase and caffeic acid to form new compounds has been demonstrated (Sun et al. 2016). This could reduce the bioaccessibility of caffeic acid. In addition, ferulic and caffeic acids could interact with bovine serum albumin (BSA). The hydrogen bond force may occur between the hydroxyl groups of phenols and the polar groups at BSA surface (Sun et al. 2016).

Conclusion

The present investigation shows that extrusion and boiling process applied in sorghum bran improves the release of total phenols and hydroxycinnamic acids and increase the

antioxidant capacity. Ferulic acid from boiled or extruded sorghum bran showed the highest bioaccessibility in intestinal digesta. We found that food matrix and in vitro digestion conditions, plays an important role in the release of phenolic acids, additionally to the technological processes applied. These conditions apparently are affecting the release and bioaccessibility of free phenolics as well as those linked to the food matrix. Further studies could be focusing on the possible interactions of the hydroxycinnamic acids and their derivatives with the different constituents of the sorghum bran matrix, under in vitro and in vivo digestion.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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