Raspberry dietary fibre: Chemical properties, functional evaluation and prebiotic *in vitro* effect

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ABSTRACT

Raspberries have high content of antioxidants; however, there is a lack of information about the functional composition of their dietary fibre. In this work, the total dietary fibre and its soluble and insoluble fractions were analysed for extractable and hydrolysable (poly)phenols, non-starch polysaccharides and functional properties with physiological effects and health implications (fat and water retention and swelling capacity, glucose diffusion retardation index, osmotic pressure and antioxidant capacity). Additionally, their prebiotic effect was assessed by an *in vitro* fermentation model by the analysis of short chain fatty acids. Results show that raspberry fibre contained mainly hydrolysable (poly)phenols; insoluble dietary fibre was the fraction richest in these compounds and also had the highest antioxidant and fat retention capacity, whereas soluble dietary fibre underwent greater hydration. The *in vitro* fermentations showed that (poly)phenols were responsible to a great extent for the raspberry prebiotic-like effect, in comparison with the fibre fractions. The results confirm that raspberry dietary fibre fractions could be used as functional and prebiotic ingredients for the development of food products with enhanced physical and nutritional properties.

1. Introduction

Raspberry (*Rubus idaeus*), also called European raspberry or red raspberry, is a red-berry species native to Europe and northern Asia and is commonly cultivated in temperate regions (Hidalgo & Almajano, 2017). This edible fruit is rich in vitamins (C, E and K) and minerals (Ca, Mg, P, K, Mn, Cu and Fe) and has a high content of total dietary fibre (TDF) and phenolic compounds (Simmonds & Preedy, 2015). This nutrient profile makes raspberry a food with multiple functional properties, such as a high antioxidant capacity (De Souza et al., 2014) and anti-inflammatory (Jean-Gilles et al., 2012) and antimicrobial effects (Krauze-Baranowska et al., 2014). These bioactivities have been related to the prevention of many human pathologies, like degenerative, metabolic, cardiovascular or ocular illnesses and certain types of cancer (Kshatriya et al., 2019). Raspberries are consumed fresh and can be frozen or processed into puree, jam, juice or dessert toppings, and the

production of this berry has increased in recent years in Europe, particularly in Spain, due to the demands of consumers. In this sense, the agricultural industry produces an excess raspberry harvest and the processing industry generates a high amount of raspberry by-products. Taking into account the principles of the circular economy, this biomass could be used to obtain food products with high added value, by the design of productive processes that minimise the output of wastes and maximise reutilisation and recycling (Laínez & Periago, 2019). For this reason, raspberry residues could be used as ingredients rich in dietary fibre and phenolic compounds, with a high value as functional, prebiotic and/or technological components, for innovative food products.

Dietary fibre intake influences several metabolic processes, including the absorption of nutrients and carbohydrates, and fat and sterol metabolisms. It has influence on colonic fermentation and affects the production of stools (Căpriţă, Căpriţă, Simulescu, & Drehe, 2010). In

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addition, the physicochemical properties also determine the functional properties and the behavior of the fibres, when are used as ingredients in different technological process. For this reason, it is important to characterize and to know the water holding capacity, the swelling capacity, the fat absorption, the osmotic pressure, the glucose absorption retardation index and the fermentability of the new fibres used and ingredients in the development of fibre enriched foods (Cho & Samuel, 2009; López et al., 1996).

In the last few years, the definition of prebiotic has evolved into the concept of food components that affect the gut microbiota with consequences for the entire human body, and has been used to refer not only to carbohydrates, but also to other molecules, such as (poly)phenolic compounds (Espín, González-Sarrías, & Tomás-Barberán, 2017). Accordingly, the word prebiotics refers to "selectively fermented ingredients that result in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefits upon host health" (Gibson et al., 2010). The TDF of foods constitutes a prebiotic ingredient that reaches the colon and becomes fermentable by the intestinal microbiota, generating short chain fatty acids (SCFAs), such as propionic, butyric and acetic, and regulating diverse metabolic pathways, such as lipid and glucose metabolism and cholesterol production (Peris, Lesmes, Cuerda, & Álvarez, 2002). This fraction also contains bioactive compounds, such as extractable and hydrolysable phenolic compounds, that are suggested to enhance the beneficial effects of dietary fibre by their antioxidant capacities (Pérez-Jiménez et al., 2008; Quirós-Sauceda et al., 2014). In addition, the functionality of TDF can be evaluated by the extraction of two different fractions, the soluble dietary fibre (SDF) and the insoluble dietary fibre (IDF), containing different components that may be proposed as different prebiotic ingredients (Lattimer & Haub, 2010).

Given the above, the aim of this work was to describe the chemical composition and the functional properties, from a technological and nutritional point of view, of raspberry and raspberry fibre fractions (TDF, SDF and IDF), through the determination of sugar composition, extractable and hydrolysable phenolic compounds, antioxidant capacities and technological properties (water retention capacity (WRC), swelling capacity (SWC), fat absorption capacity (FAC), glucose diffusion retardation index (GDRI) and osmotic pressure). In addition, the prebiotic *in vitro* effect of the raspberry fractions was evaluated using human faecal bacteria fermentation model as we hypothesized that the different composition of the raspberry fibre fractions could modify the metabolism of the colonic microbiota.

2. Material and methods

2.1. Samples

Frozen raspberries (*Rubus idaeus*) were purchased from a local supermarket in Murcia (Spain) and were transported directly to the laboratory. Some samples were conserved frozen for the evaluation of the proximate composition and others were freeze-dried for 72 h before being ground using a Thermomix TM-3. The raspberry powder was stored at −20 °C prior to extractions and analyses.

2.2. Proximate composition of raspberry

Frozen raspberry samples were analysed to determine the proximate composition (moisture, protein, fat and ash) using the AOAC Official methods (AOAC, 2016). The crude protein content was determined by the Kjeldahl method; the crude fat was analysed with petroleum ether using a Soxhlet extractor; the ash content was determined by incineration at 525 °C. Total carbohydrates were calculated by difference. Total dietary fibre (TDF) was determined by following the enzymatic and gravimetric method described by Prosky, Asp, Schweizer, DeVries, and Furda (1988), using a Fibertec E 1023 system (Hogänas, Sweden). The quantification of soluble sugars was estimated by the difference between total carbohydrates and total dietary fibre.

2.3. Extraction of raspberry dietary fibre

Three different fibre fractions (TDF, SDF and IDF) were extracted from the freeze-dried raspberries, following the procedure described in Fig. 1. Firstly, raspberry powder samples were dissolved in phosphate buffer solution, and immediately, samples were digested following an *in vitro* intestinal digestion with α-amylase heat-stable (A3306 Sigma, St. Louis, USA) at 95 °C for 30 min, with protease from *Bacillus licheniformis* (P3910 Sigma, St. Louis, USA) at 95 °C for 35 min, and with α-amyloglucosidase from *Aspergillus niger* (A9913 Sigma, St. Louis, USA) at 60 °C for 35 min. After complete the digestion, samples containing the dietary fibre residues were treated in different ways to obtain the total dietary fibre and their fractions (soluble and insoluble fibre). For total dietary fibre (TDF) isolation, 70% ethanol was added and maintained overnight to allow precipitation of soluble components. After that, samples were centrifuged at 8820g, 20 °C, for 30 min in a Centrifuge Beckman J2-21 (Indianapolis, USA), supernatants were discarded and the precipitates, containing total dietary fibre, were stored at −20 C° and freeze-dried. For soluble and insoluble fibre isolation (SDF and IDF), digested samples were centrifuged at 25920g for 30 min. The precipitates were taken as IDF. The supernatants were collected and were mixed with 95% ethanol to allow soluble fibre precipitation, during all night. Samples were centrifuged and the precipitates were taken as SDF. Both fractions insoluble and soluble were stored at −20° and freeze-dried.

2.4. Analysis of phenolic compounds by HPLC-DAD

Three different extracts were prepared from each sample (freeze-dried raspberry, TDF, IDF and SDF) to determine the extractable and hydrolysable phenolic compounds. For the extraction process, the method described by Arranz, Saura-Calixto, Shaha, and Kroon (2009) was followed with some modifications. Briefly, for the extractable phenolic compounds (EPC), 5 mL of MeOH/H$_2$O/H$^+$ (79/20/1, v/v/v) were added to 100 mg of sample. Then, samples were shacked and centrifuged at 4500g for 10 min. The supernatant obtained from the extraction was dried under vacuum at 35 °C in a Laborota-4002 rotatory evaporator (Heidolph, Schwabach, Germany). Afterthought, the residue was re-dissolved in 10 mL of milli-Q water and 10 μl of 98% formic acid were added, then, samples were loaded into pre-aconditioned Sep-Pak C$_{18}$ cartridge (Waters, Milford, Massachusetts, USA), which was washed with 10 mL of mili-Q water before elution with 1 mL of methanol. For the hydrolysable phenolic compounds (HPC) the precipitate was treated with 10% sulphuric acid (MeOH/H$_2$O, 9/1, v/v) and incubated in a shaking incubator (VorTemp 1550, LabNet Biotécnica, Spain) during 20 h at 85 °C. Then, the supernatant was treated following the same method described above.

Phenolic compounds in the samples were quantified following the method described previously by González-Barrio, Periago, Luna-Recio, García-Alonso, and Navarro-González (2018). Briefly, an HPLC 1200 series with diode array detector (DAD) (Agilent Technologies, Waldbronn, Germany) was used, scanning from 200 to 600 nm. Separation of the different phenolic compounds was performed using a LiChroCART RP-18 column (250 × 4.6 mm, i.d. 5 μm), with a pre-column (4 × 4 mm) of the same material (Merck, Darmstadt, Germany). The mobile phases used were 1% aqueous formic acid (solvent A) and acetonitrile (solvent B), at a flow rate of 1 ml min^{-1}. Elution began with a linear gradient from 2 to 40% B in 50 min, followed by washing and then a return to the initial conditions. Chromatograms were recorded at 280, 320, 360, and 520 nm. Anthocyanins were quantified by comparison with the standard cyanidin-3-*O*-glucoside at 520 nm, flavonols as quercetin-3-*O*-rutinoside at 360 nm, caffeic acid derivatives at 320 nm as caffeic acid, ellagic acid derivatives at 360 nm as ellagic acid and ellagitannins at 280 nm, using punicalagin as standard.
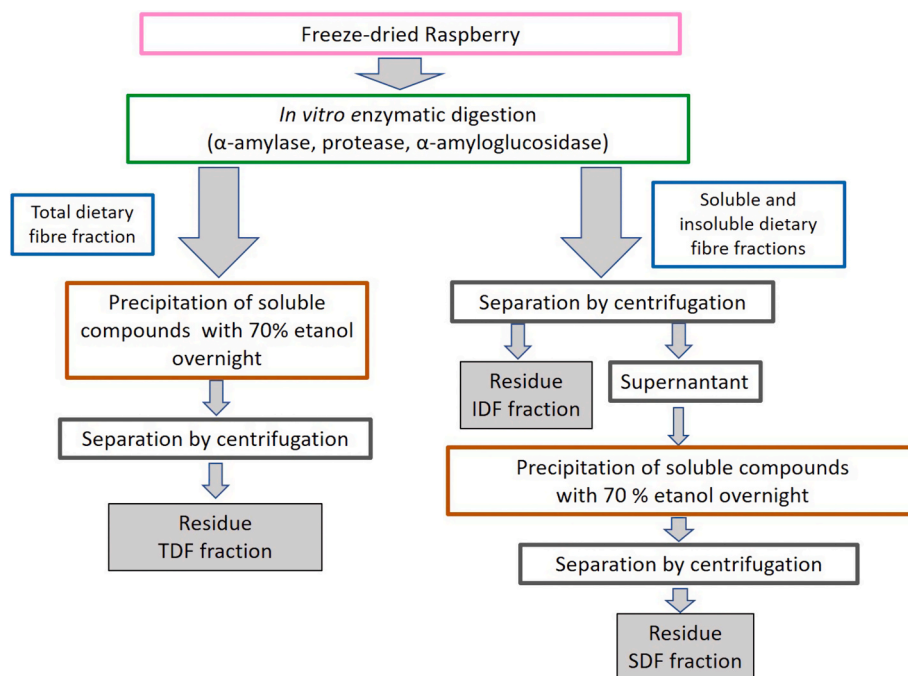
Fig. 1. Flow diagram of the procedure used to obtain the three different fractions (TDF, IDF and SDF) of dietary fibre from raspberry.

2.5. Functional properties of raspberry fibre fractions

The water retention capacity (WRC), swelling capacity (SWC), fat absorption capacity (FAC), glucose diffusion retardation index (GDRI) and osmotic pressure were analysed as functional properties of the fibre fractions (TDF, IDF and SDF), following the methodology previously described by our research group (Navarro-González, García-Valverde, García-Alonso, & Periago, 2011).

2.6. Antioxidant capacity of raspberry and its fibre fractions

For the antioxidant capacity analysis, 1 g of raspberries and 0.2 g of raspberry TDF, SDF and IDF were mixed with 80% MeOH, sonicated during 10 min at RT using an ultrasonic bath (Branson Digital model 250 (Danbury, USA) and centrifuged (4500g, 10 min, room temperature). The supernatants were evaporated with a vacuum concentrator (Eppendorf model 5301, Hamburg, Germany) until they reached a volume of 8 mL for raspberry samples, 2.5 mL for TDF and IDF and 2 mL for SDF. The antioxidant capacity of the samples was evaluated by two different antioxidant assay methods: the ferric reducing antioxidant power (FRAP) assay, as described by Benzie and Strain (1996), using a UV–visible spectrophotometer (Evolution 300, Thermo-Scientific, England); and the oxygen radical absorbance capacity (ORAC) method, performed according to Ou, Hampsch-Woodill, and Prior (2001), in a microplate spectrophotometer (BioTek Instruments, Winooski, USA). Trolox was used as the standard, and the results were expressed as mg Trolox equivalents (TE) g^{-1} sample dry weight (DW).

2.7. Neutral sugars, cellulose and uronic acids in raspberry fibre fractions

For the characterisation of neutral sugars (rhamnose, fucose, arabinose, xylose, mannose, galactose and glucose), the gas–liquid chromatography method described by Englyst, Quigley, Hudson, and Cummings (1992) was used. The polysaccharides were first hydrolysed, then alditol acetate derivatisation neutral sugars were identified and quantified by gas chromatography (Agilent, 7890 B). A neutral sugars mix solution was used as standards, using allose as internal standard. The content of individual neutral sugars were expressed as percentage. The cellulose

content was calculated according to the method of Englyst et al. (1992), as the difference between the total glucose before and after complete hydrolysis of cellulose. The determination of uronic acids was carried out by the colorimetric method described by Scott (1979), using galacturonic acid as the standard and expressing the results in g 100 g^{-1} of sample.

2.8. Prebiotic in vitro effect of raspberry fractions

The prebiotic effect of raspberry and its extracted fractions (TDF, SDF, IDF and the fraction rich in phenolic compounds) was evaluated by performing *in vitro* fermentations, using human faecal bacteria. The total phenolic compounds (EPC and HPC) were extracted as described in section 2.4.; then, the supernatants were combined and evaporated to be dissolved in water. Faecal samples were collected from a healthy normal-weight woman (a non-smoker, with stable food habits, who did not present any symptoms of gastrointestinal disease and had not taken antibiotics for at least 3 months before the study). Written informed consent was obtained from the subject. The present study was conducted according to guidelines and procedures approved by the Committee of Ethics of Research of the University of Murcia (ref. nº 1434/2017). Fresh faeces were collected in a tub containing an AnaeroGen™ Sacket (AN35, Oxoid®, UK), to produce anaerobic conditions and avoid microbial modifications, and were processed in the 2 h following deposition according to the method of Gonzalez-Barrio, Edwards, and Crozier (2011). Briefly, samples of fresh faeces were homogenized with phosphate buffer to obtain 32% faecal suspensions. Five mL of suspension was added to 44 mL of fermentation medium and placed in a 100 mL McCartney bottle. Freeze-dried digested raspberries (200 mg) and the corresponding fractions of TDF (94 mg), SDF (28 mg), IDF (67 mg) and phenolic compounds (~8.5 mg) were dissolved in 1 mL of water and added to each fermentation bottle. After this, the fermentation bottles were purged and then incubated for 48 h at 37 °C, simulating colonic lumen conditions. The prebiotic activity of the substrates was measured by evaluation of the formation of the main SCFAs (acetic, propionic and butyric acid) and the minor ones (isobutyric, isovaleric, valeric, isocaproic, caproic and heptanoic acid), as metabolites produced by the gut microbiota. The SCFAs were analysed in aliquots of the faecal

suspensions after 0, 4, 6, 24 and 48 h of fermentation, by GC-FID. The protocol used to determine the SCFAs in the faecal suspensions was that of Anson et al., 2011; Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, & Hawkins Byrne, 2006, with some modifications. Briefly, the faecal suspensions were mixed for 5 min with a solution of formic acid (20%), MeOH and 2-ethyl butyric acid (internal standard, 2 mg mL^{-1} in methanol) (1/4.5/1, v/v). After that, samples were centrifuged at 16110g for 15 min at RT and each supernatant obtained was filtered (Ø 13 mm, pore size 0.22 μm, PTFE, VWR International, USA) and analysed by GC-FID. Chromatographic analysis was carried out using an Agilent 7890A GC system equipped with a flame ionization detector (FID) and a 7683B automatic injector (Agilent Technologies, USA). A fused-silica capillary column (Nukol TM, Supelco, USA) of 30 m × 0.25 mm I.D., 0.25 μm coated, was used to separate the SCFAs. Helium was supplied as the carrier gas at a flow rate of 25 mL min^{-1}. The initial oven temperature was 80 °C and it was kept constant for 5 min and then raised to 185 °C at a rate of 5 °C min^{-1}. Samples (2 μL) were injected in splitless mode, with an injection port temperature of 220 °C. The flow rates of hydrogen, and air as makeup gas were 30 and 400 mL min^{-1}, respectively. The temperature of the FID was 220 °C and the running time for each analysis was 26 min. The SCFAs were identified by comparison with the retention times of authentic standards (Supelco, USA). Quantification was based on calibration curves constructed for a set of SCFAs standards (acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, isocaproic acid, caproic acid and heptanoic acid). The concentration, expressed as mmol L^{-1}, of each SCFA was calculated using linear regression equations (R^2 0.99) derived from the corresponding standard curves. All chemicals were at least GC grade quality.

2.9. Statistical analysis

The data were processed using the SPSS 24.0 software package (LEAD Technologies, Inc., Chicago, IL, USA). All assays were conducted in triplicate. Normality was determined by the Shapiro-Wilk test. The homogeneity of variances was analysed using the Bartlett test. Multifactorial analysis of variance (ANOVA) and Tukey's multiple-range post-hoc test were carried out to determine significant differences at P values < 0.05.

3. Results and discussion

3.1. Nutritional composition of raspberry

The data of the proximate composition of the raspberries are shown in Table 1. These fruits showed a high moisture content (87%) and lower contents of total proteins and fats (0.05 and 0.01%, respectively) compared to the values presented by the USDA database (USDA, 2018) and other authors (De Souza et al., 2014; Probst, 2015). With regard to

Table 1
Red raspberries chemical composition (g 100 g^{-1} FW).

Parameters	Content
Moisture	87 ± 11*
Proteins	0.05 ± 0.01
Fats	0.02 ± 0.01
Ash	0.8 ± 0.1
Total carbohydrates	12 ± 0.3
Soluble sugars	5.8 ± 0.1
TDF	6.1 ± 0.6
SDF	1.9 ± 0.1
IDF	4.3 ± 0.2

*Mean values of three determinations ± standard deviation (SD). The results are expressed on a fresh weight (FW) basis. TDF (total dietary fibre), SDF (soluble dietary fibre), IDF (insoluble dietary fibre).

the ash content, our samples had a higher mean value (0.78%) than other berries (De Souza et al., 2014). The total carbohydrate content (11.9 g 100 g^{-1} FW) was similar to that in other reports (USDA, 2018) and corresponded to the sum of the soluble sugars (5.8 g 100 g^{-1} FW), mainly glucose, fructose and sucrose (Dincheva, Badjakov, Kondakova, & Batchvarova, 2013), and total dietary fibre (TDF). According to these results, raspberries are low-energy fruits composed mainly of carbohydrates and dietary fibre, principal components of a healthy and balanced diet (Lunn & Buttriss, 2007). During the last decade, a wide variety of genotypes of raspberry have been developed to obtain commercial cultivars with increased productivity, valuable nutrients and bioactive contents (Bobinaitė, Viškelis, & Venskutonis, 2016), however, different cultivars, environmental factors, processing and storage time may influence nutrient values (Zhang, Ahuja, & Burton-Freeman, 2019).

The raspberries were found to have a high content of TDF (6.1 g 100 g^{-1} FW), higher than in other berries - such as strawberry (1.3 g 100 g^{-1} FW), blueberry (1.9 g 100 g^{-1} FW) and blackberry (4.5 g 100 g^{-1} FW) - and cherry (2.1 g 100 g^{-1} FW) (De Souza et al., 2014), and fruits such as kiwi (3.0 g 100 g^{-1} FW), oranges (4.5 g 100 g^{-1} FW), apples (2.4 g 100 g^{-1} FW) and pears (3.1 g 100 g^{-1} FW) (USDA, 2018). Regarding the raspberry fibre fractions, the insoluble dietary fibre (IDF) represented 71% of the TDF (Table 1). The IDF fraction is mainly represented by cellulose, insoluble hemicellulose and lignin, which facilitate the gastrointestinal tract passage (Lattimer & Haub, 2010). The soluble dietary fibre (SDF) (30% of the TDF) is composed of soluble hemicellulose, pectins and gums, these being indigestible compounds fermented by the microbiota in the colon (Laroze, Díaz-Reinoso, Moure, Zúñiga, & Domínguez, 2010). The IDF:SDF ratio was 2.4, generally acceptable for the use of fibre fractions as ingredients in the food industry. This value is higher than those reported in papaya, mango and passion fruit, and lower than in guava, tomato peel, grapefruit, lemon, orange and apple (Figuerola, Hurtado, Estévez, Chiffelle, & Asenjo, 2005; Martínez et al., 2012; Navarro-González et al., 2011; Nieto Calvache, Cueto, Farroni, de Escalada Pla, & Gerschenson, 2016).

Taking into consideration the high content of dietary fibre of raspberries, these fruits, as well as their by-products from the processing industry, such as peels, seeds and pomace, may be used as ingredients for the development of innovative fibre containing foods, such as beverages and dairy or bakery products (Cho & Samuel, 2009; Shahidi, Vamadevan, Oh, & Peng, 2019), obtaining a revalorization of the super-plus, the waste or the by-products, being necessary to characterize its functional properties and chemical composition of the new ingredient.

3.2. Neutral sugars, cellulose and uronic acids

To know the composition of the dietary fibre fractions (TDF, IDF, SDF) isolated from raspberries, the individual neutral sugars profile, cellulose contents and the uronic acids content were determined (Table 2). Fibre is constituted by cellulose, uronic acids (composing pectins) and hemicellulose with different solubility properties. Hemicellulose is a heteropolysaccharide constituted by different sugars, which could be soluble or insoluble in water depending on its chemical composition. Besides glucose, sugar monomers in hemicellulose can include the five-carbon sugars, xylose and arabinose, the six-carbon sugars, mannose and galactose, and the six-carbon deoxy sugar rhamnose (Căpriță et al., 2010). The TDF and IDF showed similar profiles of neutral sugars, being glucose the main compound in the samples (47.76% and 53.65%, respectively), followed by xylose, arabinose, galactose and mannose. The SDF fraction had a contrasting profile of neutral sugars, being glucose (27.51%) and arabinose (34.04%), the most abundant monomers, followed by galactose, mannose and xylose. Hence, these results suggest that hemicellulose in the soluble fraction was mainly constituted by arabinose, mannose and galactose, whereas the hemicellulose in the insoluble fibre comprised mainly xylose, which was also found at higher concentrations in the TDF and IDF (4-fold

Table 2
Percentage of neutral sugars, and content of cellulose and uronic acids (g 100 g^{-1} DW) in raspberry dietary fibre fractions.

	Neutral sugars (%)					Cellulose	Uronic acids
	Arabinose	Xylose	Mannose	Galactose	Glucose		
TDF	13.53 ± 4.84*[b]	24.18 ± 5.84[a]	5.74 ± 0.92[b]	8.77 ± 0.59[b]	47.761 ± 0.51[a]	42.27 ± 7.44	11.04 ± 2.78[b]
IDF	6.20 ± 3.12[b]	29,44 ± 4.86[a]	4.18 ± 0.37[b]	5.52 ± 0.20[b]	53.65 ± 1.15[a]	45.77 ± 6.92	3.39 + 0.25[c]
SDF	34.04 ± 3.73[a]	8.89 ± 2.69[b]	10.21 ± 0.5[a]	19.32 ± 1.33[a]	27.51 ± 2.40[b]	–	35.43 + 2.00[a]

*Mean values of three determinations ± standard deviation (SD). Different letters within the same column indicate statistically significant differences among samples ($P < 0.05$). The results are expressed on a dry weight (DW) basis. TDF (total dietary fibre), IDF (insoluble dietary fibre), SDF (soluble dietary fibre).

higher than in the SDF), as the predominant component of the insoluble hemicellulose (Mudgil, 2017). Moreover, the xylose:arabinose ratio (≈4) in the present work may indicate the successful separation of hemicelluloses during the extraction of the SDF and IDF, as hemicelluloses with more side chains and complex structures (more insoluble), such as xylose, are obtained by precipitation (Peng, Nie, Li, Huang, & Li, 2019). Other component of the insoluble fraction of dietary fibre is the cellulose, an insoluble complex carbohydrate constituted by glucose monomers joined by β-1,4 linkages (Gray, 2007). In these samples, the contents of cellulose in TDF and IDF were 42 and 46 g 100 g^{-1} DW, respectively (Table 2). Regarding the uronic acids, higher values of these acid sugars were found in the SDF compared to the IDF and TDF (10- and 3-fold, respectively), representing the content of pectins. Pectins are heteropolysaccharides constituted by neutral sugars and uronic acids. Most of pectins have neutral sugars covalently linked to uronic acids as side chains, mainly arabinose and galactose, and to a lesser extent, xylose, rhamnose, and glucose (Căpriţă et al., 2010). For this reason, SDF fraction presented a higher proportion of arabinose and galactose, than TDF and IDF fractions. Similar values of neutral sugars and uronic acids were found by Ross et al. (2015) after analysing an aqueous extract of raspberry. In addition, the uronic acids present in the IDF may be formed from the insoluble hemicelluloses.

The differing compositions of the TDF, SDF and IDF may determine their beneficial physiological effects. Thus, a high amount of uronic acids in SDF, representing the pectin content which is soluble in water, indicates that this fraction is likely to be fermented by microbiota, producing the formation of SCFAs, which exert several beneficial effects for human health (Koh, De Vadder, Kovatcheva, & Bäckhed, 2016; Tan et al., 2016). On the contrary, the IDF fractions did not form gels and its fermentation will be severely limited (Lattimer & Haub, 2010). The content of insoluble hemicellulose in the IDF has been associated with a lower postprandial glucose response in humans, which could be related to a faster bowel transit and reduced glucose absorption (Mudgil, 2017).

3.3. Phenolic compounds and antioxidant capacity of raspberry and its fibre fractions

Phenolic compounds are the predominant bioactive compounds in raspberry - among them, anthocyanins and ellagitannins are the main groups present in this fruit (Bobinaitė et al., 2016; González-Barrio, Borges, Mullen, & Crozier, 2010). Generally, the evaluation of these compounds reported in the literature refers to the EPC analysed by aqueous-organic solvents extraction. Nevertheless, a significant non-extractable amount of these bioactive compounds, the HPC, remains in the sample and should be extracted by acidic hydrolysis of the extraction residues (Arranz et al., 2009). These dietary HPC are not significantly released from the food matrix by digestion; therefore, they are bioaccessible in the colon and are metabolized by the microbiota, thus, allowing the absorption of derived metabolites and providing beneficial effects on gastrointestinal health (Pérez-Jiménez et al., 2008).

In the present work, phenolic compounds from the extractable and hydrolysable fractions of raspberry and dietary fibre fractions (TDF, SDF, IDF) were analysed by HPLC-DAD. The total content of phenolic compounds in the samples were calculated as the sum of the individual compounds. Among the EPC, anthocyanins and ellagitannins were found

as the main groups of (poly)phenols in raspberry, being 38.01 mg g^{-1} DW and 4.61 mg g^{-1} DW, respectively, followed by ellagic acid derivatives (0.18 mg g^{-1} DW), flavonols (0.11 mg g^{-1} DW) and caffeic acid (0.01 mg g^{-1} DW) (Table 3). Differences in the contents of phenolic compounds reported in raspberries are quite remarkable maybe because the existing differences among analytical methods, cultivars, conventional and organic farming, harvested time and processing and manipulation (Ponder & Hallmann, 2019; Stamenković et al., 2019; Hidalgo & Almajano, 2017). However, total contents of phenolic compounds reported in this work were higher compared to other research works (11–37 mg g^{-1} DW) (Bobinaitė et al., 2016; Pantelidis, Vasilakakis, Manganaris, & Diamantidis, 2007; Zhao, 2007).

Compared to the total, soluble and insoluble dietary fibre fractions, raspberry showed the highest content of anthocyanins and ellagitannins (Table 3). These results suggest that these phenolic compounds are mostly solubilized in the organic solvents after disruption of the cells during the isolation of the fibres, since their potential to be attached to the polysaccharides of the plant cell wall is lower (Padayachee et al., 2012).

Regarding the HPC in raspberry, ellagic acid derivatives were also found (0.06 mg g^{-1} DW), being these compounds released from the ellagitannins associated with the cell wall polysaccharides after hydrolysis (Arranz, Silván, & Saura-Calixto, 2010; Ross, Gordon, McDougall, & Stewart, 2007). Thus, in addition to the ellagic acid from extractable fraction reported before, raspberry contained a total amount of 0.24 mg g^{-1} DW of ellagic acid derivatives. This value is within the range of the very varied data found in the literature (0.006–5 mg g^{-1} DW). This variation is may be due to the difficult extraction of free ellagic acid from the fruit, which requires an acid hydrolysis of the ellagitannins present in the samples (De Ancos, González, & Cano, 2000; Mullen et al.,

Table 3
Extractable and hydrolysable phenolic compounds by HPLC-DAD and antioxidant capacity of dietary fiber fraction isolated from raspberry (mg g^{-1} DW).

	Raspberry	TDF	SDF	IDF
Extractable phenolic compounds (EPC)				
Anthocyanins	38.01 ± 4.39*[a]	0.06 ± 0.005[b]	0.10 ± 0.011[b]	0.26 ± 0.04[b]
Ellagitannins	4.06 ± 0.33[a]	0.06 ± 0.008[c]	0.04 ± 0.005[c]	0.35 ± 0.05[b]
Ellagic acid derivatives	0.18 ± 0.02[b]	0.25 ± 0.04[b]	0.05 ± 0.02[c]	0.95 ± 0.20[a]
Flavonols	0.11 ± 0.02	–	–	–
Caffeic acid	0.01 ± 0.001			
Hydrolyzable phenolic compounds (HPC)				
Ellagic acid derivatives	0.06 ± 0.01[d]	7.45 ± 0.18[a]	1.81 ± 0.13[c]	5.95 ± 0.90[b]
Total phenolic compounds	42.43 ± 0.31[a]	7.83 ± 0.20[b]	2.00 ± 0.18[c]	7.52 ± 0.95[b]
Antioxidant capacity (mg TE g^{-1})				
FRAP	32.63 ± 0.15[a]	3.87 ± 0.09[c]	0.60 ± 0.02[d]	9.40 ± 0.21b
ORAC	69.08 ± 5.25[a]	10.68 ± 0.70[c]	7.76 ± 0.78[c]	20.15 ± 0.70[b]

*Mean values of three determinations ± standard deviation (SD). Different letters within the same row indicate statistically significant differences among samples ($P < 0.05$). The results are expressed on a dry weight (DW) basis.

2002; Rothwell et al., 2013).

On the contrary, dietary fibre fractions (TDF, SDF, IDF) contained higher contents of phenolic compounds in the hydrolysable fraction (HPC) compared to the extractable compounds (EPC), representing a 95%, 90% and 80% of the total content for the TDF, SDF and IDF, respectively (Table 3). These HPC were represented by ellagic acid derivatives, as it was found in raspberry. These findings indicate the presence of ellagitannins attached to the polysaccharides of the raspberry fibre fractions, which after resisting the intestinal digestion, reach the colon, being also relevant to the health benefits related to fibre consumption (Macagnan, da Silva, & Hecktheuer, 2016; Quirós-Sauceda et al., 2014). Ellagic acid derivatives are of great interest due to their health effects related to gastric mucosa protection through anti-inflammatory and antioxidant activities (Sangiovanni et al., 2013). These compounds have also been reported in other fruit by-products containing fibre, such as pomegranate husk, tea production residues, oak acorns and orange peels (Sepúlveda et al., 2020). These results point out the relevance of the extraction, not only for the EPC using aqueous-organic solvents, but also for the HPC fraction.

Related to the differences in the (poly)phenols profile among the three fibre fractions, TDF and IDF showed a similar content of (poly) phenols, being the HPC significantly higher in TDF (7.45 mg g^{-1} DW) than in IDF (5.95 mg g^{-1} DW) (Table 3). By contrast, the contents of EPC (anthocyanins, ellagitannins and ellagic acid derivatives) were higher in the IDF than in the TDF, may be due to the different isolation procedure of these fibre fractions, being ethanol not added for the IDF extraction (Fig. 1), avoiding the solubilization of a considerable part of the EPC. Curiously, the highest content of extractable ellagic acid derivatives were found in the IDF (0.95 mg g^{-1} DW), maybe due to the release of a portion of these compounds contained in the insoluble fibre with the hydromethanolic extraction of EPC.

On the other hand, the SDF fraction showed the lowest content of phenolic compounds, suggesting that these compounds are mainly solubilized by ethanol during the extraction procedure of this soluble fraction. In addition, the SDF does not contain cellulose, where the (poly)phenols are bound in the dietary fibre (Căpriță et al., 2010).

With respect to the *in vitro* antioxidant capacity of the samples, it was evaluated by two different methods, the ORAC assay, which involves a hydrogen atom transfer from the antioxidant to peroxyl radicals, reflecting physiological relevant perturbations (Floegel, Kim, Chung, Koo, & Chun, 2011), and the FRAP assay to reduce the Fe(III)/tripyr-idyltriazine complex, based on an electron transfer from the antioxidant, which has showed high correlation with total phenolic contents (Thaipong et al., 2006). Raspberry showed the highest antioxidant capacity (69 and 33 mg TE g^{-1} DW, for ORAC and FRAP, respectively), which is highly related to the high content of extractable anthocyanins and ellagitannins, in agreement with the scientific literature (Moore, Perkins-Veazie, Weber, & Howard, 2008; Pantelidis et al., 2007; Wang & Lin, 2000; Zhao, 2007). In spite of the similar content of phenolic compounds in the TDF and IDF, higher antioxidant capacity was found in the IDF, suggesting that EPC might be largely responsible for the antioxidant activity in the fibre, as the extraction procedure in the antioxidant capacity assays did not break down the (poly)phenols attached to the cell walls (corresponding to > 80% of the phenolic compounds in the fibre fractions). The antioxidant capacity of the IDF from raspberry (33–69 mg TE g^{-1} DW) was higher than that of other fibre extracts, such as papaya fibre (2.5–6 mg TE g^{-1} DW) (Nieto Calvache et al., 2016) or mango, passion fruit, pineapple and guava (0.7–5 mg TE g^{-1} DW) (Martínez et al., 2012). Therefore, the fibre fractions of raspberry had high contents of EPC with their potential free radical scavenging activity and antioxidant capacity *in vivo*, being the EPC absorbed and metabolized in the small intestine, increasing the antioxidant nature of the environment (Arranz et al., 2010).

3.4. Functional properties of dietary fibre fractions

In addition to the chemical composition, the functional properties of the fibre fractions should be evaluated to ascertain their potential physiological functions related to homeostatic and therapeutic effects in the gastrointestinal system. In addition, these properties should be taken into consideration when selecting a fibre source as ingredient, due to its possible individual responses to processing conditions. In this work, the functionality of dietary fibres was determined by the study of its physical and hydration properties, such as the WRC, SWC, FAC, GDRI and osmotic pressure (Table 4). The hydration properties of dietary fibres, evaluated as WRC and SWC, described the ability of the fibre matrix to retain water, which may affect the pattern of nutrient absorption, postprandial satiety, intestinal motility in the upper intestine, and increase the stool weight (Navarro-González et al., 2011; Tan, Wei, Zhao, Xu, & Peng, 2017). The TDF fraction showed a WRC of 5.3 g g^{-1}, the value of this parameter being higher in the SDF (10.4 g g^{-1}) and very low in the IDF fraction (0.6 g g^{-1}). These values are in agreement with the SWC, which was also greater in the soluble fraction (2.0 g g^{-1}), whereas the low value of SWC in the raspberry TDF (0.8 g g^{-1}) might be related to the low content of SDF (31% of TDF, Table 1). The soluble polysaccharides (mainly pectins and soluble hemicellulose) are the structures capable of absorbing water (swelling) (Lattimer & Haub, 2010), being the first step of polysaccharide solubilization, which may slow down gastric emptying and increase satiety (Tan et al., 2016; Thebaudin, Lefebvre, Harrington, & Bourgeois, 1997). In accordance with our results, other plant materials containing around 70% IDF showed similar hydration properties, such as tomato peel (Navarro-González et al., 2011), artichoke (López et al., 1996) or seaweeds (Gómez-Ordóñez, Jiménez-Escrig, & Rupérez, 2010). Despite the importance of the hydrophilic properties of the distinct fractions, the water affinity of fibre is affected by other physical characteristics, such as structure, viscosity and particle size, as well as by the extraction method (Chau, Wang, & Wen, 2007).

Regarding FAC, the ability of fibre to adsorb fat or oil, the TDF showed a significantly higher value (3.9 g oil g^{-1}) compared to the SDF and IDF fractions (1.6 and 2.0 g oil g^{-1}, respectively). This functional property is an important characteristic of ingredients to be used in the food industry, to avoid fat loss from foods during cooking, stabilise emulsions and improve shelf-life (Elleuch et al., 2011). The raspberry TDF and its fractions showed FAC values higher than those of other vegetable matter, such as tomato peels, grapefruits, lemon and apple fibre concentrates (Figuerola et al., 2005; Navarro-González et al., 2011), but similar to those reported in orange peels (De Moraes Crizel, Jablonski, de Oliveira Rios, Rech, & Flôres, 2013) and pomegranate peel (Hasnaoui, Wathelet, & Jiménez-Araujo, 2014). Thus, the TDF from raspberry could be useful in the formulation of fibre-enriched foods that require emulsifying properties or even as fat removers in low-calorie products, as have been described for other fibres. These differences are due to the diverse chemical composition of the dietary vegetable fibres, with regard to the surface properties, particle size, overall charge

Table 4
Functional properties of raspberry dietary fibre fractions.

	TDF	SDF	IDF
WRC (g water g^{-1})	5.3 ± 0.3*^{b}	10.4 ± 0.4^a	0.6 ± 0.0 ^{c}
SWC (mL water g^{-1})	0.8 ± 0.0 ^{b}	2.0 ± 0.0^a	0.5 ± 0.0^c
FAC (g oil g^{-1})	3.9 ± 0.5^a	1.6 ± 0.1^b	2.1 ± 0.1 ^{b}
GDRI 30 min (%)	6.1 ± 0.5 ^{b}	8.9 ± 0.7^a	0.0 ± 0.0
60 min (%)	7.0 ± 0.6 ^{a}	3.0 ± 0.5^b	3.12 ± 0.9^b
Osmotic pressure (mmol kg^{-1} NaCl)	302 ± 15 ^{b}	342 ± 3 ^{a}	321 ± 2^{ab}

*Mean values of three determinations ± standard deviation (SD). Different letters within the same row indicate statistically significant differences among samples ($P < 0.05$). The results are expressed on a dry weight (DW) basis. WRC (water retention capacity), SWC (swelling water capacity), FAC (fat absorption capacity), GDRI (glucose diffusion retardation index).

and grade of lipophilicity of the components (Karaman, Yılmaz, & Tuncel, 2017).

The GDRI of dietary fibre fractions predicts the ability of fibre components to retain glucose and, therefore, to delay glucose absorption by the intestine. The results show that after 30 min of dialysis the SDF had the highest GDRI value (8.9%), while the IDF did not retain glucose, allowing the dialysis of glucose through the membrane. In contrast, the two fibre fractions showed similar glucose retention values (around 3%) at 60 min. This behavior can be explained because the SDF had stronger properties of hydration, which reduced the absorption of glucose more quickly, whereas the IDF required a longer hydration time to reduce the glucose dialysis. For the TDF, similar behavior along the study was observed, with a dialysis trend almost constant in time (Table 4). We can assert that the soluble and insoluble fractions acted at different times due to their differences in composition. However, the TDF showed a constant capacity to retard glucose diffusion at different times, which could be explained by the mix of the fibre components with different solubilities. This property is important to determine the functional effect of fibres in relation to the glycaemic index of foods. At 60 min, the GDRI of cocoa TDF was lower (4.4%) than for our samples (Lecumberri et al., 2007), while the value obtained with tomato peel dietary fibre at 60 min was higher (39%) (Navarro-González et al., 2011). The osmotic pressure of the raspberry fibre fractions was evaluated and compared to the control (300 ± 2 mmol kg^{-1} NaCl in the physiological state) (data not shown). The results show that the IDF and SDF may slightly increase the intestinal osmotic pressure; however, these values are not expected to induce diarrhoea after consumption.

3.5. Evaluation of the prebiotic-like effect of raspberry fractions by in vitro fermentation

In vitro batch cultures are usually used for evaluation of the fermentation characteristics and products of prebiotic substrates, providing a rapid first-pass screening of prebiotic foods and ingredients (Parkar et al., 2019). In the present work, the prebiotic effects of the freeze-dried digested raspberries (200 mg), their corresponding dietary fibre fractions - TDF (94 mg), SDF (28 mg), IDF (67 mg) - and extracted phenolic compounds (~2.8 mg) were studied separately to evaluate

their effects on the intestinal microbiota metabolic activity. For this aim, the increases in major (acetic, propionic and butyric acids) and minor (isobutyric, isovaleric, valeric, isocaproic, caproic and heptanoic acids) SCFAs produced by the human faecal microbiota were analysed after 4, 6, 24 and 48 h of *in vitro* fermentations with raspberry and its different fractions, the data being normalised by the subtraction of the baseline. The presence and increase in end-product metabolites, such as SCFAs, are broadly recognised as markers of a healthy gut microbiome (Flint et al., 2012). In general terms, the production of SCFAs was increased upon fermentation (Fig. 2), maintaining a general proportion of 60–70% acetic acid, as the most relevant contributor to the total SCFAs, 12% propionic acid and 10–13% butyric acid, as has been found commonly in humans (Den Besten et al., 2013; Hijová & Chmelarova, 2007). Among the different samples and substrates, the raspberry fruits led to the highest increases in acetic and propionic acids over time (Fig. 2), while the increments in butyric acid and minor SCFAs were statistically significant only after 24 and 48 h of fermentation. These results may reflect a strong prebiotic effect of the raspberry as a complete food matrix, since it contains bioactive compounds, fibre and simple sugars, among other constituents, that can lead to higher production of metabolites by the microbiota. The production of SCFAs is interesting from a nutritional point of view, since they are involved in several metabolic functions. Particularly, the increase in acetic and propionic acids may affect the functions of peripheral organs (e.g. liver, pancreas, brain, muscle), while butyric acid is a substrate of the energy metabolism of colonocytes (Poeker et al., 2018). Regarding the effect of the phenolic extract (56 µg mL^{-1}), containing extractable and hydrolysable compounds, there was a tendency for it to be higher than that of the fibre fractions, and statistically significant increases were found for acetic acid after 4 and 48 h of fermentation, propionic acid after 24 h, and butyric acid and minor SCFAs after 48 h. These results agree with those of Parkar, Trower, and Stevenson (2013), who observed an increase in SCFAs, mainly propionic and butyric acids, and higher growth of Bifidobacteria after treatments with (poly)phenols (10 µg mL^{-1}) (Parkar et al., 2013). Slight differences were found among the three fibre fractions. The soluble fraction (SDF) usually led to higher values of the main SCFAs than the other fractions (TDF and IDF), as can be seen for acetic and butyric acids, but statistically significant differences were only found for propionic acid after 24 h
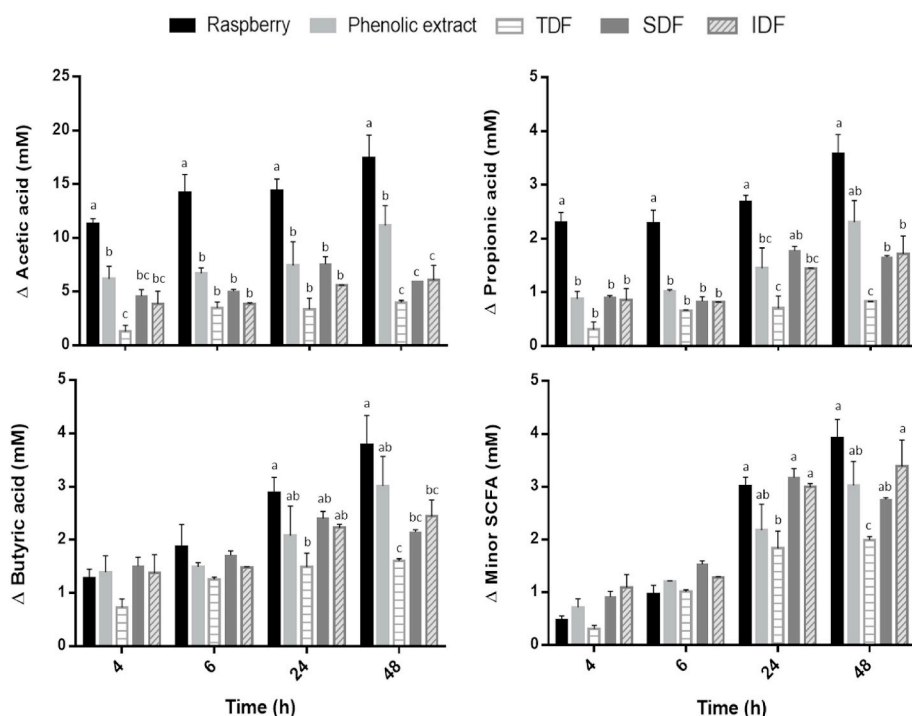
Fig. 2. Increase (Δ) in the main short chain fatty acids (SCFAs) (acetic, propionic and butyric acids) and minor SCFAs (valeric, isovaleric, isobutyric and caproic acids) produced by human faecal microbiota after 4, 6, 24 and 48 h of *in vitro* fermentations with different treatments: raspberry, phenolic extract, total dietary fibre (TDF), insoluble dietary fibre (IDF) and soluble dietary fibre (SDF). The values are expressed as means ± SEM. Letters (a–c) indicate differences (P < 0.05) among samples at a given time.

of fermentation. Even though we expected a higher prebiotic effect of the SDF compared to the TDF and IDF, due to the presence of fermentable fibres such as pectins and hemicelluloses, we did not see a high effect. The similar prebiotic effects of the IDF and SDF could be related to the presence in the former of the (poly)phenols fraction attached to polysaccharides. Additionally, the TDF of raspberry generally showed the lowest prebiotic effect, maybe because when both fractions are present together the substances with potential prebiotic effects are less accessible. In this sense, the poorer access of the microbiota to the soluble compounds of the SDF and to the (poly)phenols attached to polysaccharides of the insoluble fraction could reduce the prebiotic activity instead of producing a synergistic effect in the TDF. Thus, the prebiotic effect of raspberry found in this work appears to be associated more with the (poly)phenols content than with fibre fractions, even though the phenolic compounds present in the fibre could be released after microbial activity in the colon. Moreover, regarding the evaluation of SCFAs, further experiments for the identification and quantification of microbiota populations would highlight the effects of different raspberry dietary fractions in the gut by establishing a relationship between the species of bacteria present and SCFAs production. The *in vivo* activities of dietary fibre and (poly)phenolic compounds are still poorly understood in relation to the prevention of oxidative stress-related diseases.

4. Conclusions

Red raspberry is a rich source of fibre, mainly insoluble components, and (poly)phenolic compounds. Its dietary fibre fractions (total, soluble and insoluble) have great functional properties (GDRI and fat and water retention capacities) and a high amount of associated phenolic compounds, which predominate over the extractable ones. We must emphasise that the content of bioactive compounds depended on the type of fibre, since the insoluble fraction contained a higher amount of (poly)phenols, giving it a high antioxidant capacity. Taking into consideration its functional properties, dietary fibre from raspberry constitutes a potential ingredient to improve the nutritional characteristics of fibre-rich innovative products, providing a wide spectrum of antioxidant compounds and a potential prebiotic-like effect.

CRediT authorship contribution statement

Nieves Baenas: Writing - original draft. **Vanesa Nuñez-Gómez:** Investigation, Formal analysis. **Inmaculada Navarro-González:** Investigation, Formal analysis. **Lorena Sánchez-Martínez:** Investigation, Formal analysis. **Javier García-Alonso:** Writing - review & editing. **Mª Jesús Periago:** Conceptualization, Funding acquisition, Writing - review & editing. **Rocío González-Barrio:** Conceptualization, Methodology, Writing - review & editing.

Declaration of competing interest

None.

References

Anson, N. M., Havenaar, R., Vaes, W., Coulier, L., Venema, K., Selinheimo, E., et al. (2011). Effect of bioprocessing of wheat bran in wholemeal wheat breads on the colonic SCFA production in vitro and postprandial plasma concentrations in men. *Food Chemistry, 128*(2), 404–409. https://doi.org/10.1016/j.foodchem.2011.03.043.

AOAC. (2016). *Official methods of analysis of AOAC INTERNATIONAL, 20th edition, volume II*.

Arranz, S., Saura-Calixto, F., Shaha, S., & Kroon, P. A. (2009). High contents of nonextractable polyphenols in fruits suggest that polyphenol contents of plant foods have been underestimated. *Journal of Agricultural and Food Chemistry, 57*(16), 7298–7303. https://doi.org/10.1021/jf9016652.

Arranz, S., Silván, J. M., & Saura-Calixto, F. (2010). Nonextractable polyphenols, usually ignored, are the major part of dietary polyphenols: A study on the Spanish diet. *Molecular Nutrition & Food Research, 54*(11), 1646–1658. https://doi.org/10.1002/mnfr.200900580.

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry, 239*(1), 70–76. https://doi.org/10.1006/abio.1996.0292.

Bobinaitė, R., Viškelis, P., & Venskutonis, P. R. (2016). Chemical composition of raspberry (*Rubus* spp.) cultivars. *Nutritional Composition of Fruit Cultivars*, 713–731. https://doi.org/10.1016/B978-0-12-408117-8.00029-5.

Chau, C., Wang, Y., & Wen, Y. (2007). Different micronization methods significantly improve the functionality of carrot insoluble fibre. *Food Chemistry, 100*(4), 1402–1408. https://doi.org/10.1016/j.foodchem.2005.11.034.

Cho, S., & Samuel, P. (2009). In S. Sungsoo Cho, & P. Samuel (Eds.), *Fiber ingredients ;: Food applications and health benefits*. CRC Press. ISBN: 9781420043853.

Căpriță, A., Căpriță, R., Simulescu, V. O. G., & Drehe, R.-M. (2010). Dietary fiber: Chemical and functional properties. *Journal of Agroalimentary Processes and Technologies, 16*(4), 406–416.

De Ancos, González, & Cano. (2000). Ellagic acid, vitamin C, and total phenolic contents and radical scavenging capacity affected by freezing and frozen storage in raspberry fruit. *Journal of Agricultural and Food Chemistry, 48*(10), 4565–4570. https://doi.org/10.1021/JF0001684.

De Moraes Crizel, T., Jablonski, A., de Oliveira Rios, A., Rech, R., & Flôres, S. H. (2013). Dietary fiber from orange byproducts as a potential fat replacer. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 53*(1), 9–14. https://doi.org/10.1016/J.LWT.2013.02.002.

De Souza, V. R., Pereira, P. A. P., da Silva, T. L. T., de Oliveira Lima, L. C., Pio, R., & Queiroz, F. (2014). Determination of the bioactive compounds, antioxidant activity and chemical composition of Brazilian blackberry, red raspberry, strawberry, blueberry and sweet cherry fruits. *Food Chemistry, 156*, 362–368. https://doi.org/10.1016/J.FOODCHEM.2014.01.125.

Den Besten, G., Van Eunen, K., Groen, A. K., Venema, K., Reijngoud, D. J., & Bakker, B. M. (2013, September). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *Journal of Lipid Research, 54*, 2325–2340. https://doi.org/10.1194/jlr.R036012.

Dincheva, I., Badjakov, I., Kondakova, V., & Batchvarova, R. (2013). Metabolic profiling of red raspberry (Rubus idaeus) during fruit development and Ripening. *International Journal of Agricultural Science and Research, 3*(2), 71–78. https://www.academia.edu/3311743/Metabolic_Profiling_of_Red_Raspberry_Rubus_Idaeus_During_Fruit_Development_and_Ripening.

Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C., & Attia, H. (2011). Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: A review. *Food Chemistry, 124*(2), 411–421. https://doi.org/10.1016/J.FOODCHEM.2010.06.077.

Englyst, H. N., Quigley, M. E., Hudson, G. J., & Cummings, J. H. (1992). Determination of dietary fibre as non-starch polysaccharides by gas–liquid chromatography. *The Analyst, 117*(11), 1707–1714. https://doi.org/10.1039/AN9921701707.

Espín, J. C., González-Sarrías, A., & Tomás-Barberán, F. A. (2017). The gut microbiota: A key factor in the therapeutic effects of (poly)phenols. *Biochemical Pharmacology*. https://doi.org/10.1016/j.bcp.2017.04.033.

Figuerola, F., Hurtado, M. L., Estévez, A. M., Chiffelle, I., & Asenjo, F. (2005). Fibre concentrates from apple pomace and citrus peel as potential fibre sources for food enrichment. *Food Chemistry, 91*(3), 395–401. https://doi.org/10.1016/J.FOODCHEM.2004.04.036.

Flint, H. J., Flint, H. J., Scott, K. P., Louis, P., Duncan, S. H., Flint, H. J., et al. (2012). The role of the gut microbiota in nutrition and health. *Nature Reviews | Gastroenterology & Hepatology, 9*, 577–589. https://doi.org/10.1038/nrgastro.2012.156.

Floegel, A., Kim, D. O., Chung, S. J., Koo, S. I., & Chun, O. K. (2011). Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *Journal of Food Composition and Analysis, 24*, 1043–1048. https://doi.org/10.1016/j.jfca.2011.01.008.

Gibson, G. R., Scott, K. P., Rastall, R. A., Tuohy, K. M., Hotchkiss, A., Dubert-Ferrandon, A., et al. (2010). Dietary prebiotics: Current status and new definition. *IFIS, Food Science and Technology Bulletin*. Retrieved from http://centaur.reading.ac.uk/17730/.

Gómez-Ordóñez, E., Jiménez-Escrig, A., & Rupérez, P. (2010). Dietary fibre and physicochemical properties of several edible seaweeds from the northwestern Spanish coast. *Food Research International, 43*(9), 2289–2294. https://doi.org/10.1016/J.FOODRES.2010.08.005.

Gonzalez-Barrio, R., Edwards, C. A., & Crozier, A. (2011). Colonic catabolism of ellagitannins, ellagic acid, and raspberry anthocyanins: *In vivo* and *in vitro* studies. *Drug Metabolism and Disposition, 39*(9), 1680–1688. https://doi.org/10.1124/dmd.111.039651.

González-Barrio, R., Periago, M. J., Luna-Recio, C., García-Alonso, F. J., & Navarro-González, I. (2018). Chemocal composition of the edible flowers, pansy (Viola wittrockiana) and snapdragon (Antirrhinum majus) as new sources of bioactive compounds. *Food Chemistry, 252*, 373–380. https://doi.org/10.1016/j.foodchem.2018.01.102.

González-Barrio, R., Borges, G., Mullen, W., & Crozier, A. (2010). Bioavailability of anthocyanins and ellagitannins following consumption of raspberries by healthy humans and subjects with an ileostomy [†]. *Journal of Agricultural and Food Chemistry, 58*(7), 3933–3939. https://doi.org/10.1021/jf100315d.

Gray, K. A. (2007). International sugar journal. *International Sugar Journal, 109*. https://www.cabdirect.org/cabdirect/abstract/20073053467.

Hasnaoui, N., Wathelet, B., & Jiménez-Araujo, A. (2014). Valorization of pomegranate peel from 12 cultivars: Dietary fibre composition, antioxidant capacity and functional properties. *Food Chemistry, 160*, 196–203. https://doi.org/10.1016/j.foodchem.2014.03.089.

Hidalgo, G.-I., & Almajano, M. (2017). Red fruits: Extraction of antioxidants, phenolic content, and radical scavenging determination: A review. *Antioxidants, 6*(1), 7. https://doi.org/10.3390/antiox6010007.

Hijová, E., & Chmelarova, A. (2007). Short chain fatty acids and colonic health. *Bratislava Medical Journal, 108*(8), 354–358. https://www.ncbi.nlm.nih.gov/pubmed/18203540.

Jean-Gilles, D., Li, L., Ma, H., Yuan, T., Chichester, C. O., & Seeram, N. P. (2012). Anti-inflammatory effects of polyphenolic-enriched red raspberry extract in an antigen-induced arthritis rat model. *Journal of Agricultural and Food Chemistry, 60*(23), 5755–5762. https://doi.org/10.1021/jf203456w.

Karaman, E., Yılmaz, E., & Tuncel, N. B. (2017). Physicochemical, microstructural and functional characterization of dietary fibers extracted from lemon, orange and grapefruit seeds press meals. *Bioactive Carbohydrates and Dietary Fibre, 11*, 9–17. https://doi.org/10.1016/j.bcdf.2017.06.001.

Koh, A., De Vadder, F., Kovatcheva, P., & Bäckhed, F. (2016). From dietary fiber to host physiology: Short-chain fatty acids as key bacterial metanolites. *Cell, 165*(2), 1332–1345. https://doi.org/10.1016/j.cell.2016.05.041.

Krauze-Baranowska, M., Majdan, M., Hałasa, R., Głód, D., Kula, M., Fecka, I., et al. (2014). The antimicrobial activity of fruits from some cultivar varieties of Rubus idaeus and Rubus occidentalis. *Food and Function, 5*(10), 2536–2541. https://doi.org/10.1039/c4fo00129j.

Kshatriya, D., Li, X., Giunta, G. M., Yuan, B., Zhao, D., Simon, J. E., et al. (2019). Phenolic-enriched raspberry fruit extract (Rubus idaeus) resulted in lower weight gain, increased ambulatory activity, and elevated hepatic lipoprotein lipase and heme oxygenase-1 expression in male mice fed a high-fat diet. *Nutrition Research, 68*, 19–33. https://doi.org/10.1016/J.NUTRES.2019.05.005.

Laínez, M., & Periago, M. J. (2019). The bioeconomy: An opportunity for the Spanish economy. In *bioeconomy*. IntechOpen. https://doi.org/10.5772/intechopen.84917.

Laroze, L. E., Díaz-Reinoso, B., Moure, A., Zúñiga, M. E., & Domínguez, H. (2010). Extraction of antioxidants from several berries pressing wastes using conventional and supercritical solvents. *European Food Research and Technology, 231*(5), 669–677. https://doi.org/10.1007/s00217-010-1320-9.

Lattimer, J. M., & Haub, M. D. (2010). Effects of dietary fiber and its components on metabolic health. *Nutrients, 2*(12), 1266–1289. https://doi.org/10.3390/nu2121266.

Lecumberri, E., Mateos, R., Izquierdo-Pulido, M., Rupérez, P., Goya, L., & Bravo, L. (2007). Dietary fibre composition, antioxidant capacity and physico-chemical properties of a fibre-rich product from cocoa (Theobroma cacao L.). *Food Chemistry, 104*(3), 948–954. https://doi.org/10.1016/J.FOODCHEM.2006.12.054.

López, G., Ros, G., Rincón, F., Periago, M. J., Martínez, M. C., & Ortuño, J. (1996). Relationship between physical and hydration properties of soluble and insoluble fiber of artichoke. *Journal of Agricultural and Food Chemistry, 44*(9), 2773–2778. https://doi.org/10.1021/jf9507699.

Lunn, J., & Buttriss, J. L. (2007). Carbohydrates and dietary fibre. *Nutrition Bulletin, 32*(1), 21–64. https://doi.org/10.1111/j.1467-3010.2007.00616.x.

Macagnan, F. T., da Silva, L. P., & Hecktheuer, L. H. (2016). Dietary fibre: The scientific search for an ideal definition and methodology of analysis, and its physiological importance as a carrier of bioactive compounds. *Food Research International, 85*, 144–154. https://doi.org/10.1016/J.FOODRES.2016.04.032.

Martínez, R., Torres, P., Meneses, M. A., Figueroa, J. G., Pérez-Álvarez, J. A., & Viuda-Martos, M. (2012). Chemical, technological and in vitro antioxidant properties of mango, guava, pineapple and passion fruit dietary fibre concentrate. *Food Chemistry, 135*(3), 1520–1526. https://doi.org/10.1016/J.FOODCHEM.2012.05.057.

Moore, P. P., Perkins-Veazie, P., Weber, C. A., & Howard4, L. (2008). Environmental effect on antioxidant content of ten raspberry cultivars. In P. Bañados, & A. Dale (Eds.), *Proc. IX int. Rubus and rives symp*. Acta Horticulturae 777, ISHS 2008 https://naldc.nal.usda.gov/download/20943/PDF.

Mudgil, D. (2017). The interaction between insoluble and soluble fiber. *Dietary Fiber for the Prevention of Cardiovascular Disease*, 35–59. https://doi.org/10.1016/B978-0-12-805130-6.00003-3.

Mullen, W., Stewart, A. J., Lean, M. E. J., Gardner, P., Duthie, G. G., & Crozier, A. (2002). Effect of freezing and storage on the phenolics, ellagitannins, flavonoids, and antioxidant capacity of red raspberries. *Journal of Agricultural and Food Chemistry, 50*(18), 5197–5201. https://doi.org/10.1021/jf020141f.

Navarro-González, I., García-Valverde, V., García-Alonso, J., & Periago, M. J. (2011). Chemical profile, functional and antioxidant properties of tomato peel fiber. *Food Research International, 44*(5), 1528–1535. https://doi.org/10.1016/J.FOODRES.2011.04.005.

Nieto Calvache, J., Cueto, M., Farroni, A., de Escalada Pla, M., & Gerschenson, L. N. (2016). Antioxidant characterization of new dietary fiber concentrates from papaya

pulp and peel (Carica papaya L.). *Journal of Functional Foods, 27*, 319–328. https://doi.org/10.1016/J.JFF.2016.09.012.

Ou, B., Hampsch-Woodill, M., & Prior, R. L. (2001). Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of Agricultural and Food Chemistry, 49*(10), 4619–4626. http://www.ncbi.nlm.nih.gov/pubmed/11599998.

Padayachee, A., Netzel, G., Netzel, M., Day, L., Zabaras, D., Mikkelsen, D., et al. (2012). Binding of polyphenols to plant cell wall analogues - Part 1: Anthocyanins. *Food Chemistry, 134*(1), 155–161. https://doi.org/10.1016/j.foodchem.2012.02.082.

Pantelidis, G. E., Vasilakakis, M., Manganaris, G. A., & Diamantidis, G. (2007). Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and Cornelian cherries. *Food Chemistry, 102*(3), 777–783. https://doi.org/10.1016/J.FOODCHEM.2006.06.021.

Parkar, S. G., Davis, P., Trower, T. M., Hedderley, D. I., Sutton, K. H., & Ingram, J. R. (2019). Factors affecting microbial metabolism in a human fecal fermentation model to evaluate prebiotics. *Journal of Nutrition & Intermediary Metabolism, 15*, 55–57. https://doi.org/10.1016/J.JNIM.2018.12.003.

Parkar, S. G., Trower, T. M., & Stevenson, D. E. (2013). Fecal microbial metabolism of polyphenols and its effects on human gut microbiota. *Anaerobe, 23*, 12–19. https://doi.org/10.1016/j.anaerobe.2013.07.009.

Peng, X., Nie, S., Li, X., Huang, X., & Li, Q. (2019). Characteristics of the water- and alkali-soluble hemicelluloses fractionated by sequential acidification and graded-ethanol from sweet maize stems. *Molecules, 24*(1), 212. https://doi.org/10.3390/molecules24010212.

Pérez-Jiménez, J., Arranz, S., Tabernero, M., Díaz- Rubio, M. E., Serrano, J., Goñi, I., et al. (2008). Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: Extraction, measurement and expression of results. *Food Research International, 41*(3), 274–285. https://doi.org/10.1016/J.FOODRES.2007.12.004.

Peris, P. G., Lesmes, I. B., Cuerda, C. De, & Álvarez, M. C. (2002). Metabolismo colónico de la fibra. *Nutrición Hospitalaria, XVII* (2), 11–16. http://www.nutricionhospitalaria.com/pdf/3360.pdf.

Poeker, S. A., Geirnaert, A., Berchtold, L., Greppi, A., Krych, L., Steinert, R. E., et al. (2018). Understanding the prebiotic potential of different dietary fibers using an in vitro continuous adult fermentation model (PolyFermS). *Scientific Reports, 8*(1). https://doi.org/10.1038/s41598-018-22438-y.

Ponder, A., & Hallmann, E. (2019). The effects of organic and conventional farm management and harvest time on the polyphenol content in different rapsberry cultivars. *Food Chemistry, 301*, 125295. https://doi.org/10.1016/j.foodchem.2019.125295.

Probst, Y. (2015). A review of the nutrient composition of selected *Rubus* berries. *Nutrition & Food Science, 45*(2), 242–254. https://doi.org/10.1108/NFS-07-2014-0063.

Prosky, L., Asp, N. G., Schweizer, T. F., DeVries, J. W., & Furda, I. (1988). Determination of insoluble, soluble, and total dietary fiber in foods and food products: Interlaboratory study. *Journal - Association of Official Analytical Chemists, 71*(5), 1017–1023. http://www.ncbi.nlm.nih.gov/pubmed/2853153.

Quirós-Sauceda, A. E., Palafox-Carlos, H., Sáyago-Ayerdi, S. G., Ayala-Zavala, J. F., Bello-Perez, L. A., Alvarez-Parrilla, E., et al. (2014). Dietary fiber and phenolic compounds as functional ingredients: Interaction and possible effect after ingestion. *Food & Function, 5*(6), 1063–1072. https://doi.org/10.1039/c4fo00073k.

Ross, H. A., Gordon, J., McDougall, J., & Stewart, D. (2007). Antiproliferative activity is predominantly associated with ellagitannins in raspberry extracts. *Phytochemistry, 68*, 218–228.

Ross, K., Siow, Y., Brown, D., Isaak, C., Fukumoto, L., & Godfrey, D. (2015). Characterization of water extractable crude polysaccharides from cherry, raspberry, and ginseng berry fruits: Chemical composition and bioactivity. *International Journal of Food Properties, 18*(3), 670–689. https://doi.org/10.1080/10942912.2013.837066.

Rothwell, J. A., Perez-Jimenez, J., Neveu, V., Medina-Remón, A., M'Hiri, N., García-Lobato, P., et al. (2013). Phenol-explorer 3.0: A major update of the phenol-explorer database to incorporate data on the effects of food processing on polyphenol content. *Database*. https://doi.org/10.1093/database/bat070.

Sangiovanni, E., Vrhovsek, U., Rossoni, G., Colombo, E., Brunelli, C., Brembati, L., et al. (2013). Ellagitannins from Rubus berries for the control of gastric inflammation: *In vitro* and *in vivo* studies. *PLoS One, 8*(8), e71762. https://doi.org/10.1371/journal.pone.0071762.

Scott, R. W. (1979). Colorimetric determination of hexuronic acids in plant materials. *Analytical Chemistry, 51*(7), 936–941. https://doi.org/10.1021/ac50043a036.

Sepúlveda, L., Laredo-Alcalá, E., Buenrostro-Figueroa, J., Ascacio-Valdés, J. A., Genisheva, Z., Aguilar, C., et al. (2020). Ellagic acid production using polyphenols from orange peel waste by submerged fermentation. *Electronic Journal of Biotechnology, 43*, 1–7. https://doi.org/10.1016/j.ejbt.2019.11.002.

Shahidi, F., Vamadevan, V., Oh, W. Y., & Peng, H. (2019). Phenolic compounds in agri-food by-products, their bioavailability and health effects. *International Society for Nutraceuticals and Functional Foods, 5*, 57–119. https://doi.org/10.31665/JFB.2019.5178.

Simmonds, M. S. J., & Preedy, V. R. (2015). In M. Simmonds, & V. R. Preedy (Eds.), *Nutritional composition of fruit cultivars*. Academic Press. ISBN: 9780124080645.

Stamenković, Z., Pavkov, I., Radojčin, M., Tepić Horecki, A., Kešelj, K., Bursać Kovačević, D., et al. (2019). Convective drying of fresh and frozen raspberries and change of their physical and nutritive properties. *Foods, 11*(7), 251. https://doi.org/10.3390/foods8070251.

Tan, C., Wei, H., Zhao, X., Xu, C., & Peng, J. (2017). Effects of dietary fibers with high water-binding capacity and swelling capacity on gastrointestinal functions, food

intake and body weight in male rats. *Food & Nutrition Research, 61*, 1–8. https://doi.org/10.1080/16546628.2017.1308118.

Tan, C., Wei, H., Zhao, X., Xu, C., Zhou, Y., & Peng, J. (2016). Soluble fiber with high water-binding capacity and swelling capacity, and fermentability reduces food intake by promoting satiety rather than satiation in rats. *Nutrients, 8*, 1–15. https://doi.org/10.3390/nu8100615.

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., & Hawkins Byrne, D. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis, 19*(6–7), 669–675.

Thebaudin, J. Y., Lefebvre, A. C., Harrington, M., & Bourgeois, C. M. (1997). Dietary fibres: Nutritional and technological interest. *Trends in Food Science & Technology, 8* (2), 41–48. https://doi.org/10.1016/S0924-2244(97)01007-8.

USDA. (2018). National nutrient database for standard reference. Retrieved June 12, 2019, from report 09302, raspberries. United states department of agriculture.

website https://ndb.nal.usda.gov/ndb/foods/show/09302?fgcd=&manu=&format=&count=&max=25&offset=&sort=default&order=asc&qlookup=raspberry&ds=&qt=&qp=&qa=&qn=&q=&ing=.

Wang, S. Y., & Lin, H. S. (2000). Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. *Journal of Agricultural and Food Chemistry, 48*(2), 140–146. https://doi.org/10.1021/jf9908345.

Zhang, X., Ahuja, J. K. C., & Burton-Freeman, B. M. (2019). Characterization of the nutrient profile of processed red raspberries for use in nutrition labeling and promoting healthy food choices. *Nutrition and Healthy Aging, 5*(3), 225–236. https://doi.org/10.3233/NHA-190072.

Zhao, Y. (2007). In Y. Zhao (Ed.), *Berry Fruit: Value-added products for health promotion.* CRC Press. https://doi.org/10.1021/acs.jafc.5b02062. ISBN: 9780849358029.