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Insoluble dietary fibre scavenges reactive carbonyl species under simulated physiological conditions: The key role of fibre-bound polyphenols

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ABSTRACT

Polyphenols bound to insoluble fibre may scavenge reactive carbonyl species by surface chemical reactions. In the present study, this hypothesis was tested by investigating the ability of bound-polyphenol rich insoluble dietary fibre (BP-IDF) isolated from blackberry pomace, red cabbage, and wheat bran in scavenging carbonyl compounds. Three BP-IDF showed high scavenging efficacy for glyoxal, methylglyoxal, acrolein and malondialdehyde. Upon *in vitro* digestion, trapping capacity was retained by the insoluble fraction suggesting that carbonyl trapping activity and physiological relevance needs to be extended to undigestible materials. The removal of bound polyphenols from the polysaccharide backbones through alkaline and acidic treatment reduced by up to 90% of trapping capacity of BP-IDF. Moreover, methylglyoxal-polyphenol adducts were detected bound to blackberry pomace BP-IDF after hydrolysis. These findings demonstrated that polyphenols bound to IDF scavenged reactive carbonyl species and highlighted the physiological relevance of BP-IDF in limiting carbonyl stress along all the gastrointestinal tract.

1. Introduction

The gastrointestinal (GI) tract is prone to reactive carbonyl species (RCS) attack as it is constantly exposed to ingested foodstuffs vehicle of thermally and storage induced toxicants. The estimated exposure to unsaturated aldehydes in human is 5 mg/kg from the consumption of alcoholic beverages, bakery products and cigarette (Wang et al., 2008). Besides, the highly reactive environment of GI tract promotes lipid oxidation during digestion, which leads to the exposure of GI tract to a higher level of oxidation products than tissues and plasma (Wang et al., 2016). Human GI tract thus is repeatedly exposed to carbonyl oxidative threats, which is an important factor of GI inflammatory and neoplastic lesions: increasing evidence show that the abnormal accumulation of RCS, including glyoxal, methylglyoxal, acrolein and malondialdehyde, favours carbonyl stress, promoting modification of tissue protein and DNA, then leading to cell damage cycle in ageing and chronic diseases (Rabbani & Thornalley, 2015; Wang et al., 2016). Among RCS, glyoxal and methylglyoxal are dicarbonyl compounds constantly formed in the metabolism of carbohydrates and gut microbiota (Ferguson, Tötemeyer, MacLean, & Booth, 1998; Thornalley, 1985), while acrolein is the simplest α,β-unsaturated aldehyde and an ubiquitous pollutant existed in the environment and foods. Acrolein can be also generated endogenously through myeloperoxidase, copper-catalysed amine oxidation and gut microbial glycerol metabolism (Zhang, Sturla, Lacroix, & Schwab, 2018). Malondialdehyde, the major lipid peroxidation product of polyunsaturated fatty acid, is extensively used as biomarker of oxidative damage in several diseases including inflammatory bowel disease and colorectal cancer (Rašić, Rašić, Akšamija, & Radović, 2018). Therefore, scavenging RCS within GI tract by using polyphenols is crucial to counteract pathological conditions associated with reactive compounds.

Many papers investigated the carbonyl trapping capacity of free polyphenols and aqueous-organic extracts of the foods (Xie & Chen, 2013) and conjugated RCS metabolites of (−)-epigallocatechin-3-gallate in human faeces have been recently described by Zhang, Zhao, Ohland, Jobin, & Sang (2019). Furthermore, Jiang et al. (2019) reported that the toxicity of RCS toward Caco-2 cells is decreased after trapping.

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Melanoidins are protein-carbohydrates polymers formed during processing and they have RCS trapping activity due to the polyphenols moiety present in their structure as recently described by Zhang, Zhang, Troise, & Fogliano (2019). Although supramolecular organization of polyphenols in polysaccharides skeleton, the so-called "antioxidant dietary fibre" was extensively described (Saura-Calixto, 1998), the RCS trapping capacity of phenolics bound to insoluble food components, such as dietary fibre, remains unknown. Polyphenols associated to dietary fibre represent approximately 50% of total dietary antioxidants and are significant building blocks of dietary fibre, accounting for from 1.4 to 50.7% of the dry weight of insoluble dietary fibre (IDF) in plant foods (Goñi, Díaz-Rubio, Pérez-Jiménez, & Saura-Calixto, 2009; Pérez-Jiménez, Elena Díaz-Rubio, & Saura-Calixto, 2015). Antioxidant groups bound to the insoluble fractions have been proved to be still active to quench radicals present in the solvent through surface reaction (Gökmen, Serpen, & Fogliano, 2009). Other soluble antioxidant compounds can also regenerate this antioxidant capacity, preserving the functional properties of bound polyphenols (Çelik, Gökmen, & Fogliano, 2013). The whole picture clearly suggests that antioxidant dietary fibre can exert beneficial effects much longer than soluble polyphenols during their survival period in the human GI tract (Pérez-Jiménez, Díaz-Rubio, & Saura-Calixto, 2013; Vitaglione, Napolitano, & Fogliano, 2008). Considering a fibre intake recommendation from 19 up to 38 g per day (Quagliani & Felt-Gunderson, 2017), it is worth to investigate whether bound-polyphenol rich insoluble dietary fibre (BP-IDF) can efficiently act as an RCS scavenger and exert a physiological relevance modulating the level of RCS inside the gut lumen.

In this study, blackberry pomace, red cabbage and wheat bran, as the representative of fruit, vegetable and cereal, were used to prepare BP-IDF with different amounts of bound polyphenols. The carbonyl trapping capacity of these BP-IDF was investigated under simulated physiological conditions to evaluate their potential in controlling the RCS formation within the GI tract. In addition, the removal of bound polyphenols and the identification of putative bound RCS-polyphenol adducts were undertaken to elucidate the possible mechanisms behind trapping process.

2. Material and methods

2.1. Chemicals

Glyoxal (40% in water, w/v), methylglyoxal (40% in water, w/v), malondialdehyde tetrabutylammonium salt, quinoxaline, 2-methylquinoxaline, *o*-phenylenediamine (OPD), Folin-Ciocalteu reagent, salivary α-amylase (100 U/mg), porcine pepsin (2500 U/mg), porcine pancreatin (8 × USP), Protease E (≥3.5 U/mg solid) and Viscozyme L were purchased from Sigma-Aldrich (St. Louis, MO, USA). Thermostable α-amylase (50,000 U/mL), amyloglucosidase from *Aspergillus niger* (100,000 U/mL), protease (50, 000 U/g), quercetin, catechin, gallic acid, ferulic acid, caffeic acid, ellagic acid, acrolein (95%), 2,4-dinitrophenylhydrazine (DNPH), and acrolein-DNPH standard were purchased from Aladdin Reagents Database Inc. (Shanghai, China).

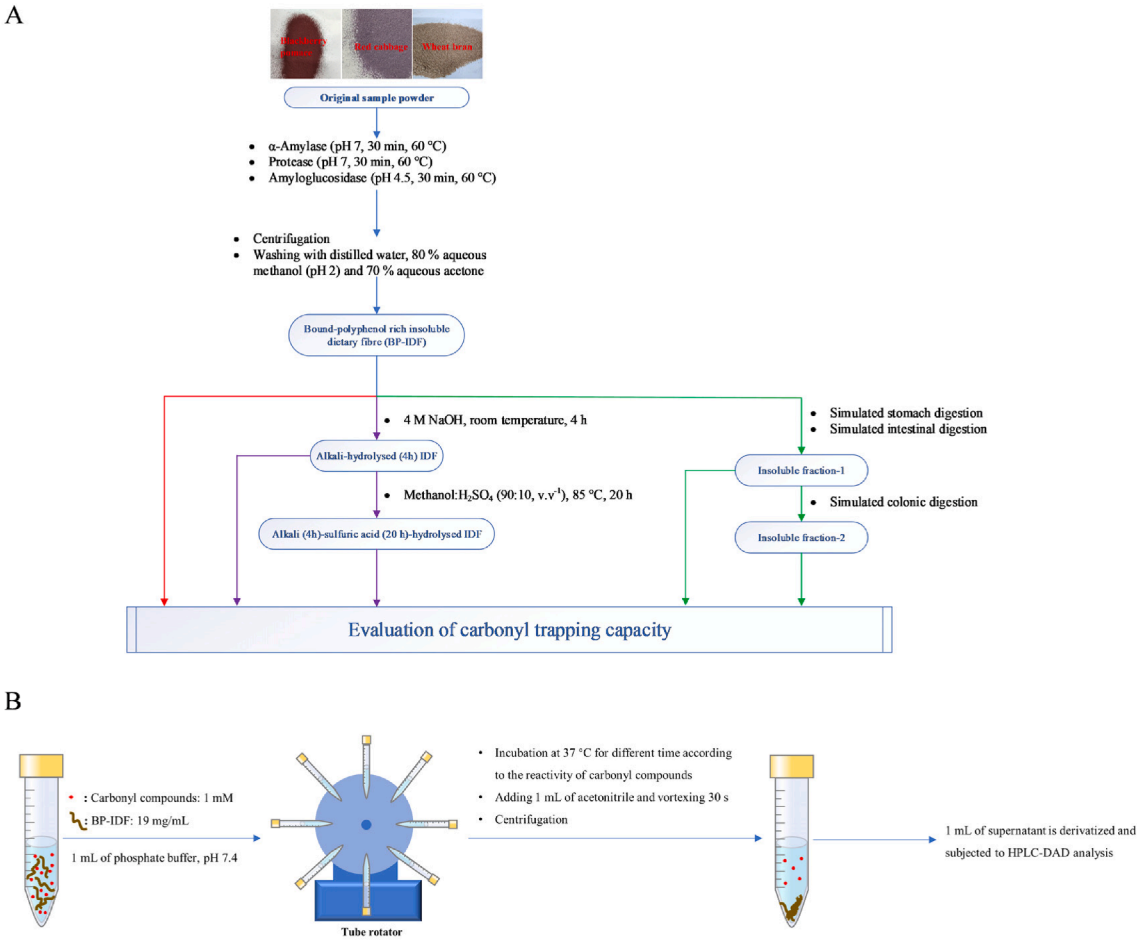

Fig. 1. A: Scheme of preparation of bound-polyphenol rich insoluble dietary fibre (BP-IDF), hydrolysed IDF and the steps of the simulated *in vitro* digestion process. Red, purple and green lines correspond to Sections 2.3–2.5, respectively. B: Procedure of evaluation of carbonyl trapping capacity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.2. Preparation of blackberry pomace, red cabbage and wheat bran BP-IDF

Red cabbage and blackberry were purchased from a local market. Wheat bran was obtained from Binzhou Zhongyu Food Company Limited (Binzhou, China) and washed to remove physical contaminants. Fresh blackberry was transferred into a cold press juicer to obtain blackberry pomace. After freeze-drying, all of materials were ground in a mill and passed through a 150 μm sieve to give original sample powders. BP-IDF was prepared using an established method (Liu et al., 2019) with some modifications (Fig. 1**A**). Briefly, 200 g of original sample powder was defatted two times using 2 L of *n*-hexane. After air-dried overnight, the defatted samples were subjected to sequential enzymatic digestion with 0.3% α-amylase (pH 7), 0.5% protease (pH 7.5) and 0.2% amyloglucosidase (pH 4.5) at 60 °C for 30 min, respectively. After centrifugation (3000 × *g*, 10 min), the substrate was washed with distilled water, 80% aqueous methanol (pH 2.0 adjusted by HCl) and 70% aqueous acetone. The resulting solid residues were freeze-dried and ground to pass through a 150 μm sieve to give blackberry pomace, red cabbage and wheat bran BP-IDF, respectively. The yield of blackberry pomace, red cabbage and wheat bran BP-IDF in this experiment was around 31%, 33% and 64%, respectively.

2.3. Scavenging efficacy studies of RCS by BP-IDF under simulated physiological conditions

A model reaction system was set up as illustrated in Fig. 1**B** based on the fibre intake recommendation (38 g per day) (Quagliani & Felt-Gunderson, 2017) and assumed volume of isotonic chyme (2L) reaching the colon each day for healthy people (Hammer, Pruckmayer, Bergmann, Kletter, & Gangl, 1997). The concentrations of carbonyl compounds used in this model system were selected by taking the estimated daily intake of unsaturated aldehydes and dicarbonyl compounds into account (Degen, Hellwig, & Henle, 2012; Wang et al., 2008). Briefly, glyoxal (1.0 mM), methylglyoxal (1.0 mM), acrolein (1.0 mM) or malondialdehyde (0.2 mM) were incubated with 19 mg of different BP-IDF or cellulose as blank in phosphate buffer (0.1 M, pH 7.4, final volume 1 mL) at 37 °C. The incubation was conducted under a nitrogen atmosphere and dark within a rotating wheel mixer (50 rpm) up to 12 h for glyoxal, methylglyoxal and malondialdehyde, and 2 h for acrolein. Each reaction model was incubated in triplicate. After incubation, 1 mL of acetonitrile was added, and the mixture was vigorously vortexed for 30 s to extract non-covalent trapped carbonyl compounds and precipitate macromolecules followed by centrifugation at 13,000 × *g* for 10 min. To detect the remaining glyoxal and methylglyoxal, 1 mL of supernatant with glyoxal or methylglyoxal was derivatized by 200 μL of 0.2% OPD solution according to our previous method (Zhang et al., 2019) and analysed by HPLC-DAD (Section 2.6). To detect the remaining acrolein and malondialdehyde, 1 mL of supernatant with acrolein or malondialdehyde was derivatized by adding 20 μL of 6 M HCl and 50 μL DNPH solution (2 mg/mL in acetonitrile/HCl 9:1, v/v) at room temperature for 5 h and analysed by HPLC-DAD (Section 2.6).

2.4. Release of bound polyphenols from blackberry pomace, red cabbage and wheat bran BP-IDF

2.4.1. Alkaline hydrolysis

The alkaline treatment of BP-IDF was based on the method by Nardini et al. (2002). Two grams of BP-IDF were treated with 40 mL of 4 M NaOH solution containing 10 mM EDTA and 1% ascorbic acid under a nitrogen atmosphere for 4 h to release insoluble-bound polyphenols through cleaving ester and ether bonds (Fig. 1**A**). After hydrolysis and centrifugation, the residue was washed to neutral pH using distilled water and freeze-dried to give alkali-hydrolysed IDF. The supernatant was acidified to pH 2 with HCl (6 M) and extracted with 20 mL of ethyl acetate for six times. The combined extract was dried using a rotary evaporator under vacuum (35 °C) and the dry residue was reconstituted in 4 mL of 80% aqueous methanol for the characterization of bound polyphenols (Section 2.7).

2.4.2. Acidic hydrolysis

Alkali-hydrolysed IDF was subjected to acidic hydrolysis for the further release of bound polyphenols through cleaving glycosidic bonds according to Arranz & Saura Calixto (2010). An aliquot of obtained alkali-hydrolysed IDF was treated by 10 mL of methanol: sulfuric acid solution (90:10, v/v) at 85 °C up to 20 h. The mixture was centrifuged and the residue was washed to neutral pH followed by freeze-drying to give alkali-sulfuric acid-hydrolysed IDF. Both resulting IDF were subjected to evaluation of carbonyl scavenging capacity as described above (Section 2.3).

2.5. In vitro digestion of blackberry pomace, red cabbage and wheat bran BP-IDF

To investigate the effect of gastrointestinal digestion on the carbonyl scavenging ability of BP-IDF, they were subjected to the *in vitro* digestion process. The *in vitro* digestion procedure was conducted following the published method (Hamzalıoğlu & Gökmen, 2016) with some modifications. Simulated salivary fluid (SSF), gastric fluid (SGF) and intestinal fluid (SIF) were prepared to mimic the conditions of the gastrointestinal tract and the compositions of digestion fluids were shown in Table S1. Five grams of BP-IDF were mixed with 10 mL of SSF and incubated in a water bath with shaking at 37 °C for 2 min. For stomach digestion, 10 mL of SGF was added to the mixture and the pH was adjusted to 3.0 by 6 M HCl. Then 5 mL of pepsin solution (1.25 mg/mL) was added to the acidified mixture and shaken in the water bath at 200 rpm and 37 °C for 2 h. After the gastric phase the pH was brought up to 7.0 using 4 M NaOH, and then 20 mL of SIF containing bile salts (100 mg/mL) and 5 mL of pancreatin solution (5 mg/mL) were added to the gastric chime solution. After saturation with nitrogen to remove oxygen, the mixture was incubated at 37 °C with shaking for 2 h to simulate the duodenal phase. Finally, the mixture was first treated with 5 mL of Protease E solution (1.0 mg/mL, pH 8.0) at 37 °C for 1 h, and then with 150 μL of Viscozyme L at 37 °C for 16 h (pH 4.0) under stirring to simulate colonic digestion process. To obtain insoluble fractions (Fig. 1A), the digested meal after duodenal and colon phase was collected, respectively, and filtered through a paper filter (Whatman 595). The solid residue was washed several times using distilled water and freeze-dried. The resultant insoluble fractions were submitted to the evaluation of carbonyl scavenging capacity as described above (Section 2.3). Each sample was prepared in triplicate. To compare the carbonyl trapping ability of BP-IDF after digestion process with other dietary food components, catechin (0.2 mg/mL) and caffeic acid (0.3 mg/mL) were selected as the representative of free flavonoids and phenolic acids. The doses used for these components were based on the ratio of their estimated dietary intake to that of fibre (Bo' et al., 2019).

2.6. Determination of OPD/DNPH-Derivatives by HPLC-DAD

Determination of OPD/DNPH derivatives was performed on a Shimadzu Prominence LC-20A Modular HPLC system (Shimadzu, Kyoto, Japan). Separation was achieved using an Agilent Poroshell 120 SB-AQ C18 column (100 mm × 3.0 mm, 2.7 μm) at a flow rate of 0.4 mL/min and 40 °C. A gradient elution program was used as follows (A, 0.1% aqueous formic acid; B, 0.1% formic acid in acetonitrile): 0 min, 10% B; 10 min, 23% B; 20 min, 45% B; 30 min, 70% B; 35 min, 90% B; followed by a re-equilibration step. The injection volume was 5 μL and chromatograms were recorded at 315 nm for quinoxaline derivatives, 310 nm for malondialdehyde-DNPH derivative and 370 nm for acrolein-DNPH derivative. The remaining glyoxal, methylglyoxal and acrolein in different samples was quantified based on their corresponding standard curve (0.01 mM to 1.0 mM) with linearity higher than 0.99 for all

the investigated compounds. Working solutions of malondialdehyde in the concentration range between 0.01 mM and 0.2 mM were derivatized as described above to establish its external calibration curve. Trapping capacity for each RCS was calculated using the equation (Eq. (1)) as below:

$$\text{Trapping capacity}(\%) = \frac{\text{amount of RCS in blank} - \text{amount of RCS in samples}}{\text{amount of RCS in blank}} \times 100\% \quad (1)$$

2.7. Characterization of fibre-bound polyphenols by UPLC-PDA-QTOF-MS/MS analysis

Bound polyphenols of IDF extracted by alkaline hydrolysis was characterized using a Waters Acquity ultraperformance liquid chromatography (UPLC) system coupled to a photodiode array detector and a Xevo-G2-S quadrupole time-of-flight mass spectrometer (QTOF-MS, Waters, Milford, MA). The separation was performed on a Poroshell 120 SB-AQ C18 column (Agilent) using 0.1% aqueous formic acid (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The gradient elution was used at a flow rate of 0.4 mL/min: 0 min, 5% B; 10 min, 15% B; 20 min, 30% B; 30 min, 70% B; 35 min, 90% B. The column temperature was 35 °C and the injection volume was 5 μL. UV–vis signals were recorded on-line with the wavelength range of 200–600 nm. The major operating parameters for the Q-TOF-MS were set as follows: negative ion mode; capillary voltage 3.5 kV; cone voltage 30 V; source temperature 120 °C; desolvation temperature 400 °C; desolvation gas and cone gas were set to 700 and 50 L/h. Centroid mode data were acquired using a multiplexed MS/MS acquisition with alternating low and high energy acquisition (MS^E), from *m/z* 50 to 1500. MS/MS experiments were performed by using argon as a collision gas and product ions were screened by using collision energy ranging from 25 to 30 eV. Mass chromatogram and mass spectral data were collected and processed by MassLynx version 4.1 (Waters). Caffeic acid, sinapic acid, ferulic acid, gallic acid, ellagic acid, catechin and quercetin were identified by comparison of the retention times and $[M-H]^-$ ion mass with their corresponding commercial standards. Other polyphenols were identified by comparison of their $[M-H]^-$ ion mass, MS^2 spectra, UV–visible spectrum and retention times with previous reports, especially in blackberry, red cabbage and wheat bran (Arranz & Saura Calixto, 2010; Ayoub, De Camargo, & Shahidi, 2016; Mizgier et al., 2016).

UV–vis signals were used for relative quantification based on the calibration curve of standards for each class of polyphenols (Pérez-Ramírez, Reynoso-Camacho, Saura-Calixto, & Pérez-Jiménez, 2018). Flavanols and hydroxybenzoic acids were monitored at 280 nm and quantitated with catechin and gallic acid, respectively; hydroxycinnamic acids and ellagitannins were monitored at 320 nm and quantitated with ferulic acid and ellagic acid, respectively; flavonols were monitored at 365 nm and quantitated with quercetin.

2.8. Analysis of the bound methylglyoxal-polyphenol adducts in the blackberry pomace BP-IDF-methylglyoxal system by UPLC-MS/MS

To obtain mass spectrometry information of fibre-bound adduct of methylglyoxal-polyphenol, 100 mg of blackberry pomace IDF was incubated with methylglyoxal (1.0 mM) in 10 mL of phosphate buffer (pH 7.4, 0.1 M) at 37 °C for 12 h. After incubation, the sample was mixed thoroughly with 10 mL of acetonitrile and centrifuged at 10,000 × *g* for 15 min. The resultant residue was subjected to alkaline hydrolysis for 2 h as described above. The hydrolysate was acidified to pH 2.0 and extracted with ethyl acetate for six times. The combined extract was dried using a rotary evaporator under vacuum (35 °C) and the dry residue was reconstituted in 4 mL of 80% aqueous methanol for UPLC-MC/MS analysis. To prepare the conjugated samples of methylglyoxal with the major bound polyphenols of blackberry pomace BP-IDF, gallic acid, quercetin and catechin (0.5 mM) was incubated with methylglyoxal (0.5 mM) in phosphate buffer (pH 7.4, 0.1 M) at 37 °C for 4 h, respectively. After incubation, the adducts were directly characterized by UPLC-MS/MS.

The methylglyoxal-polyphenol adducts were identified under the same chromatographic conditions with those of phenolic qualitative analysis but using a TQD triple quadrupole mass spectrometer as detector (Waters, Milford, MA). Negative electrospray ionization was used with the capillary voltage at 3.0 kV. Desolvation gas and cone gas were set at 600 and 50 L/h, respectively. The molecular ions of possible methylglyoxal-polyphenol adducts formed in chemical reaction models were identified in preliminary trials; different channels were used under selected ion monitoring (SIM) mode: *m/z* 169 for gallic acid, *m/z* 241 for mono-methylglyoxal-gallic acid, *m/z* 289 for catechin, *m/z* 361 for mono-methylglyoxal-catechin, *m/z* 433 for di-methylglyoxal-catechin, *m/z* 301 for quercetin, *m/z* 373 for mono-methylglyoxal-quercetin, *m/z* 455 for di-methylglyoxal-quercetin. Structural information on three polyphenols and their major methylglyoxal adducts was obtained by product ion scan with 25 eV collision energy. The mass range was set from *m/z* 100–500, and data were acquired and processed with MassLynx version 4.1 (Waters).

2.9. Statistical analysis

All analyses were performed in triplicate. Tukey's HSD test was used to determine significant differences between means at the level of $p < 0.05$ using the IBM SPSS Statistics 23 (IBM Corp., NY, USA).

3. Results and discussion

3.1. Bound phenolic compounds profile of BP-IDF by UPLC-PDA-QTOF analysis

Fibre-bound phenolic compounds extracted by alkaline hydrolysis were characterized and quantified as shown in Table 1 and the typical UPLC chromatograms at 280, 320 and 365 nm are presented in Figures S1–S3. The total amount of bound phenolic compounds in alkaline extract of blackberry pomace BP-IDF was 1044.0 mg/100 g DW (dry weight) with 12 polyphenols identified. Ellagitannins accounted for about 43% of the bound polyphenols in alkaline extract followed by phenolic acids (32.2%) and flavonoids (24.8%). Ellagitannins fraction consisted of ellagic acid and sanguisorbic acid dilactone, while gallic acid and caffeic acid gave the major phenolic acids contribution. Regarding flavonoids, five compounds were identified in the alkaline hydrolysate of blackberry pomace BP-IDF, mainly catechin and quercetin. The bound polyphenol composition of blackberry pomace BP-IDF is in line with previous findings reported by Ayoub, De Camargo, & Shahidi (2016): the most abundant bound phenolic compounds in blackberry seed meals are gallic acid, catechin, quercetin and their derivatives. Additionally, it is important to mention that the alkali hydrolysis required for the release of bound polyphenols from macromolecules may also degrade some of the original phenolic structures. Indeed, sanguisorbic acid dilactone and ellagic acid were generated from the degradation of blackberries ellagitannins through strong chemical hydrolysis (Macierzyński, Sójka, Kosmala, & Karlińska, 2020).

In red cabbage BP-IDF, a total of 9 phenolic acids and one ellagitannin were found in its alkaline extract. The most abundant phenolic acid was sinapic acid (372.1 mg/100 g DW), followed by *p*-coumaric acid (88.5 mg/100 g DW) and ferulic acid (74.8 mg/100 g DW), while the contents of other phenolic acids ranged from 3.5 to 11.6 mg/100 g DW. Mizgier et al. (2016) also found that hydroxycinnamic acid derivatives are the dominant bound phenolic compounds in red cabbage and mainly include residues of sinapic, *p*-coumaric acid and ferulic acid. Low amount of hydroxycinnamic acid derivatives detected in present study could be a consequence of the strongly alkaline treatment.

Looking at wheat bran BP-IDF, a total of nine different compounds

Table 1
UPLC-ESI-QTOF-MS/MS profile and contents (mg/100 g DW)[a] of bound phenolic compounds of blackberry, red cabbage and wheat bran bound-polyphenol rich insoluble dietary fibre (BP-IDF) extracted by alkaline hydrolysis.[b.]

Peak no.	Proposed compound	t_R (min)	$[M-H]^-$ (*m/z*)	MS/MS ion (*m/z*)	Blackberry pomace	Red cabbage	Wheat bran
Phenolic acids							
1	Gallic acid	2.65	169.0083	125	174.9 ± 6.3	5.3 ± 0.2	n.d.
2	*p*-Coumaric acid glucoside	3.32	325.0963	163, 119	n.d.	3.5 ± 0.1	n.d.
3	Protocatechuic acid	3.80	153.0347	109	25.7 ± 1.1	5.2 ± 0.3	6.9 ± 0.3
4	Ferulic acid 4-*O*-glucoside	5.52	355.1050	193, 134	n.d.	6.7 ± 0.2	n.d.
5	Salicylic acid	5.68	137.0201	93	n.d.	n.d.	1.3 ± 0.1
6	Vanillic acid	7.17	167.0617	108	n.d.	n.d.	3.6 ± 0.2
7	Caffeic acid	7.65	179.0352	135	119.7 ± 4.8	8.1 ± 0.2	11.5 ± 0.4
8	Sinapoyl D-glucoside	7.94	385.1145	223	n.d.	7.4 ± 0.1	n.d.
9	5-methoxysalicylic acid	8.73	167.1201	108	n.d.	n.d.	3.4 ± 0.2
10	3,4-dihydroxyphenylaceticacid	9.66	167.0216	123	n.d.	11.6 ± 0.5	n.d.
11	*p*-coumaric acid	10.53	163.0378	119	4.3 ± 0.1	84.5 ± 2.1	12.2 ± 0.3
12	Ferulic acid	12.18	193.0498	178, 134	11.8 ± 0.4	74.8 ± 1.2	412.3 ± 8.4
13	Sinapic acid	13.25	233.0594	208, 164, 149	n.d.	372.1 ± 7.8	16.4 ± 0.2
Flavonoids							
14	Catechin	6.74	289.0732	245, 205, 115	152.6 ± 3.9	n.d.	2.5 ± 0.2
15	Quercetin 3-*O*-glucuronide	8.38	477.0792	301	8.3 ± 0.3	n.d.	n.d.
16	Epicatechin	8.60	289.0716	245, 205	21.5 ± 1.6	n.d.	n.d.
17	Rutin	15.85	609.1463	301	3.6 ± 0.2	n.d.	n.d.
18	Quercetin	19.62	301.0461	179, 151, 119	72.2 ± 1.4	n.d.	n.d.
Ellagitannins							
19	Sanguisorbic acid dilactone	12.89	469.005	315, 301	173.2 ± 2.7	n.d.	n.d.
20	Ellagic acid	14.54	300.9901	229, 185	276.5 ± 5.5	44.1 ± 2.3	n.d.

[a] DW, dry weight of dietary fibre; n.d., not detected.
[b] Results are expressed as mean ± SD (n = 3).

were identified in its alkaline hydrolysate, reaching 469.9 mg/100 g DW. Phenolic acids accounted for 99% of the bound phenolic compounds released by alkaline treatment from wheat bran BP-IDF. Ferulic acid, which accounted for 88.2% of total phenolic acids, was the most representative among the eight identified phenolic acids. This result confirms the previous study carried out by Verma, Hucl, & Chibbar (2009).

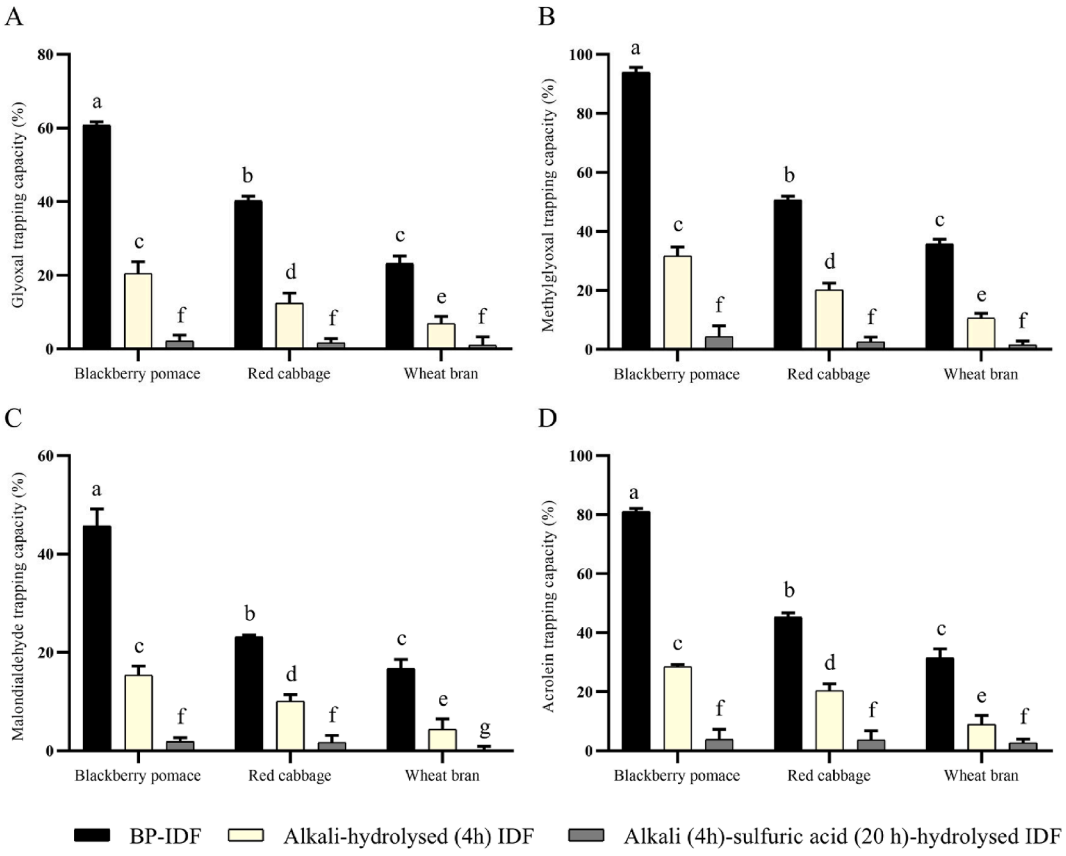

Fig. 2. Carbonyl trapping capacity of bound-polyphenol rich insoluble dietary fibre (BP-IDF) and hydrolysed IDF. Results are expressed as mean ± SD for n = 3. Bars with the different letters indicate significant difference at $p < 0.05$.

3.2. Evaluation of the carbonyl trapping capacity of blackberry pomace, red cabbage and wheat bran BP-IDF and the major role of bound polyphenols

To investigate the physiological relevance of BP-IDF through carbonyl trapping capacity, BP-IDF was prepared at a concentration in line with the recommended daily intake of fibre, in the range of 25–38 g for adults (Quagliani & Felt-Gunderson, 2017). Similarly, concentrations of the carbonyl compounds were based on the estimated dietary intake of unsaturated aldehyde and dicarbonyl compounds (Degen et al., 2012; Wang et al., 2008). As shown in Fig. 2, all the four tested carbonyls were effectively scavenged by blackberry pomace, red cabbage and wheat bran BP-IDF. The scavenging efficacy of three BP-IDF ranged from 16.7% to 45.7% for malondialdehyde, from 23.2% to 60.8% for glyoxal, and from 35.8% to 93.9% for methylglyoxal. Regarding acrolein, more than 81.2%, 45.3% and 31.5% of total amount were eliminated within 2 h by blackberry pomace, red cabbage and wheat bran BP-IDF, respectively. Concerning the contribution of BP-IDF to total carbonyl trapping capacity of plant foods, wheat bran BP-IDF accounts for more than 63.2% carbonyl trapping capacity of wheat bran whereas blackberry pomace and red cabbage BP-IDF showed relative low contribution, reaching 27.4 and 29.1%, respectively (Fig. S4). After hydrolysis of BP-IDF, the hydrolysed IDF showed much lower carbonyl trapping capacity compared to BP-IDF (Fig. 2). Alkaline treatment reduced more than 65%, 54% and 70% of scavenging efficacy for blackberry pomace, red cabbage and wheat bran BP-IDF, respectively, and nearly no carbonyl trapping capacity was retained after the further acidic hydrolysis. This behaviour is in agreement with our previous results showing that alkaline hydrolysis significantly reduces dicarbonyl trapping capacity of soluble coffee melanoidins (Zhang et al., 2019). In BP-IDF, bound polyphenols are linked to the cellular matrix by ester, ether as well as glycosidic bonds, which can be broken through alkaline and acidic hydrolysis, leading to a structural difference between BP-IDF and IDF (Pérez-Jiménez & Torres, 2011). The decline of carbonyl trapping capacity can be attributed to the removal of bound phenolic compounds from BP-IDF (Fig. S5), indicating that the insoluble-bound polyphenols significantly contributed to the carbonyl trapping capacity of dietary fibre.

BP-IDF showed the highest trapping capacity for acrolein, followed by methylglyoxal, glyoxal and malondialdehyde. The reasons may be related to the different reactivity of carbonyl compounds towards polyphenols and to their arrangement in aqueous solution. The predominant form of malondialdehyde is enolate anion at pH 7.4, which inhibits the nucleophilic reaction between malondialdehyde and bound polyphenols (Esterbauer, Schaur, & Zollner, 1991). While in aqueous solution glyoxal exists mainly as the hydrated monomer, dimer and trimer, which may slow down the reaction rate of the trapping process for glyoxal by polyphenols (Zhu, Poojary, Andersen, & Lund, 2019).

Differences observed in the trapping behaviour of BP-IDF may rise from the nature of bound polyphenols and the microstructure of dietary fibre. On one hand, blackberry pomace BP-IDF had a high amount of bound polyphenols including flavonoids, which are proven to be more reactive towards carbonyls compared to phenolic acids (Xie & Chen, 2013). On the other hand, some of bound polyphenols are not located on the surface of fibre structure, which may strongly influence the interaction with free carbonyl compounds. The ability of bound polyphenols to scavenge carbonyl compounds paralleled previously published studies on antioxidant activity of dietary fibre: the antioxidant groups associated to insoluble fractions can quench free radicals present in aqueous solution through surface reactions, which are influenced by total surface area of the reacting solid (Gökmen et al., 2009). Dietary fibre remains up to about 24 h in the GI tract, in particular, in the lower gut lumen: this can increase the antioxidant activity of caecal content and the expression of endogenous antioxidant systems, thus reducing the tumour-promoting reactive oxygen species present in the colon (Pérez-Jiménez et al., 2013; Vitaglione et al., 2008). According to the concept of "antioxidant dietary fibre", our result indicated that BP-IDF from plant foods may help in eliminating RCS that are constantly formed in the GI tract.

3.3. Influence of in vitro digestion on the carbonyl trapping capacity of BP-IDF

To get insights into the effects of gastrointestinal digestion on the carbonyl trapping capacity, BP-IDF were subjected to the *in vitro* digestion process. Data summarized in Fig. 3, showed that after gastrointestinal digestion, three BP-IDF still exerted considerable carbonyl trapping capacity for all tested RCS, although a significant decrease was observed after16 h of simulated colon digestion process, which is probably related to the release of bound phenolic compounds from polysaccharides (Papillo, Vitaglione, Graziani, Gokmen, & Fogliano, 2014). The activity of BP-IDF was compared to free phenolic acids and flavonoids as caffeic acid and catechin according to their estimated dietary intake (600 mg/day and 400 mg/day, respectively) (Bo' et al., 2019). The concentration of catechin used in this model system was close to that of total phenolic compounds in the alkaline extract of blackberry pomace BP-IDF (Table 1). The carbonyl scavenging of free catechin was lower than that of blackberry pomace BP-IDF but higher than that of red cabbage BP-IDF, while caffeic acid showed a similar carbonyl scavenging efficacy to wheat bran BP-IDF. The high carbonyl scavenging activity of blackberry pomace BP-IDF could be explained by the incomplete release of bound phenolic compounds by alkaline hydrolysis and the possible additive effects from different polyphenols (Pérez-Jiménez & Torres, 2011; Shao et al., 2014). Although dietary polyphenols intake and their compositions are strongly affected by the diversity of eating habits among different geographical areas and populations, ranging from 60 to 600 mg/day for flavonoids and from 100 to 729 mg/day for phenolic acids, our results suggested that BP-IDF considerably contributed to the carbonyl scavenging within the GI tract compared to the dietary free polyphenols.

Food composition and physicochemical aspects of digesta and reactions occurring during digestion can further support the protective role of BP-IDF. Different dietary food components are digested together and some of them can sequester carbonyl compounds including free amino acids and small peptides (Jiang et al., 2019; Zhang et al., 2019). Most of digested proteins and some of free polyphenols are absorbed in small intestine whereas BP-IDF reach the colon with bound-polyphenols being nearly intact (Pérez-Jiménez et al., 2013). Moreover, antioxidant compounds bound to BP-IDF can be regenerated by free polyphenols present in the meal through synergistic antioxidant mechanism as observed for cereal products (Çelik et al., 2013). Regeneration mechanism underpins the potential beneficial effect of carbonyl scavenging capacity by BP-IDF on GI health: functional hydroxyl groups of BP-IDF are preserved and regenerated in the digestive system, thus maintaining an effective defence against reactive carbonyl electrophiles during their transit in the lower gut lumen. This evidence also opens a possibility for the utilization of BP-IDF in the design of functional foods with targeted prebiotic properties, supplying substrates for gut microbiota and protecting them against exogenous and endogenous carbonyl compounds. Recently, Brighina et al. (2021) reported that considerable amounts of 1,2-dicarbonyl compounds can pass through the *in vitro* gastrointestinal digestion phases and reduce probiotic species under the *in vitro* colonic fermentation conditions, promoting negative effects on the intestinal bacterial population and unwanted consequences on the digestion. In this respect the presence of polyphenols can be a valuable strategy in minimizing the toxicity of RCS for the gut microbiota, but further studies on the metabolites produced are needed to have a complete picture of the interconnection between polyphenols bound to dietary fibre and gut microbiota metabolism.

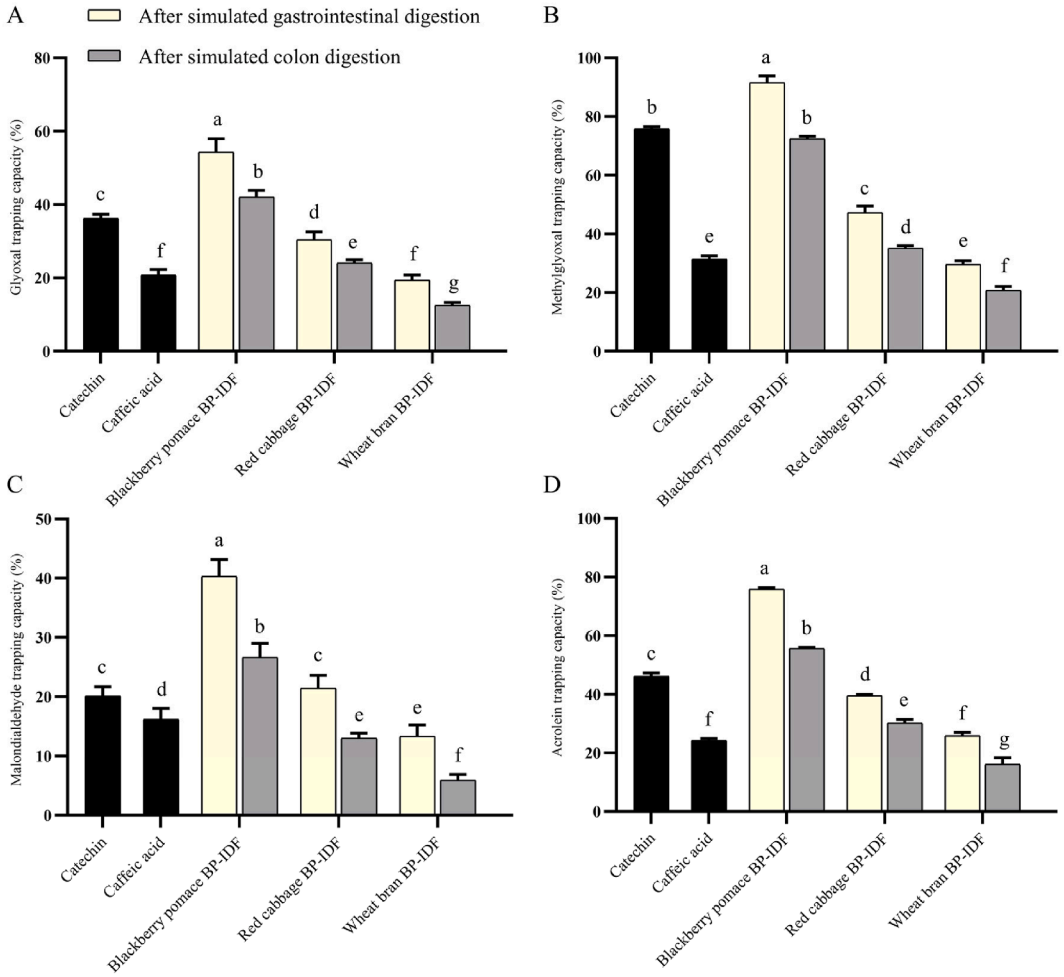

Fig. 3. Carbonyl trapping capacity of the three bound-polyphenol rich insoluble dietary fibre (BP-IDF) after *in vitro* digestion. Results are expressed as mean ± SD for n = 3. Bars with the different letters indicate significant difference at $p < 0.05$.

3.4. Methylglyoxal adducts of gallic acid, catechin and quercetin bound to blackberry pomace BP-IDF

Our group recently described the formation of melanoidins-bound caffeic acid-methylglyoxal adduct (Zhang et al., 2019). Therefore, we hypothesized that similarly to melanoidins, the formation of fibre-bound polyphenol-methylglyoxal adducts can occur also in the blackberry pomace BP-IDF which proved to be extremely effective in methylglyoxal scavenging (Fig. 2 panel B). We screened the methylglyoxal conjugates of main bound polyphenols, gallic acid, quercetin and catechin, in the alkaline extract of methylglyoxal-treated blackberry pomace BP-IDF by using tandem mass spectrometry. Furthermore, gallic acid, quercetin and catechin were also reacted with methylglyoxal in model systems to putatively identify gallic acid-, quercetin- and catechin-methylglyoxal adducts released from methylglyoxal-treated blackberry pomace BP-IDF upon alkaline treatment. As highlighted in Fig. 4, five peaks with *m/z* 241 $[M-H]^-$, *m/z* 373 $[M-H]^-$, *m/z* 455 $[M-H]^-$, *m/z* 361 $[M-H]^-$, and *m/z* 433 $[M-H]^-$, respectively, were detected in blackberry pomace BP-IDF-methylglyoxal system after alkaline hydrolysis in SIM mode; each of them showed the same retention times with those from the model system of gallic acid, quercetin and catechin with methylglyoxal under simulated physiological conditions. The peak at 2.32 min exhibited the molecular ion at *m/z* 241 $[M-H]^-$ and the fragment ions at *m/z* 169 and *m/z* 125, indicating the loss of methylglyoxal moiety and carbonyl group, respectively (Fig. 5A). This was in line with the fragmentation profile of mono-methylglyoxal-gallic acid conjugate formed in chemical reaction system of gallic acid and methylglyoxal, suggesting the release of mono-methylglyoxal-gallic acid adduct from methylglyoxal-treated blackberry pomace BP-IDF (de Falco et al., 2020).

The two peaks at 15.09 and 16.81 min in the chromatogram of blackberry pomace BP-IDF-methylglyoxal system shared the same retention time and fragmentation pattern with the mono-methylglyoxal-quercetin at *m/z* 373 $[M-H]^-$ and di-methylglyoxal-quercetin at *m/z* 455 $[M-H]^-$, in the chemical reaction system of quercetin and methylglyoxal (Fig. 5**B**). The fragment ions with *m/z* 301 $[M-72-H]^-$ and *m/z* 205 $[M-18-150-H]^-$ from the mono-methylglyoxal-quercetin adduct could match with a loss of one methylglyoxal and a loss of water molecule plus the typical breakdown of the C ring (*m/z* 150), which was in agreement with the previous observation on the conjugation of methylglyoxal with quercetin at the C-6 or C-8 position of A ring (Li, Zheng, Sang, & Lv, 2014). Similarly, the MS^2 spectra of di-methylglyoxal-quercetin adduct had the same precursor ion having the daughter ion *m/z* 277 $[M-18-150-H]^-$. Using a similar approach, we detected several products generated in the reaction of catechin and methylglyoxal. The two major peaks at 5.78 and 6.30 min were also observed in the alkaline extract of methylglyoxal-treated blackberry pomace BP-IDF, and they had the same retention time and MS^2 spectrum (Fig. 5**C**). The peak with molecular ion *m/z* 361 $[M-H]^-$ had the daughter ion of *m/z* 289 $[M-72-H]^-$, formed upon the loss of the methylglyoxal moiety, and the daughter ion of *m/z* 181 $[M-180-H]^-$, which was in line with the most abundant fragment ion of catechin (*m/z* 109 $[M-180-H]^-$) and could match with the typical cleavage of catechin on B ring (Yuzuak, Ballington, & Xie, 2018). Therefore, it was

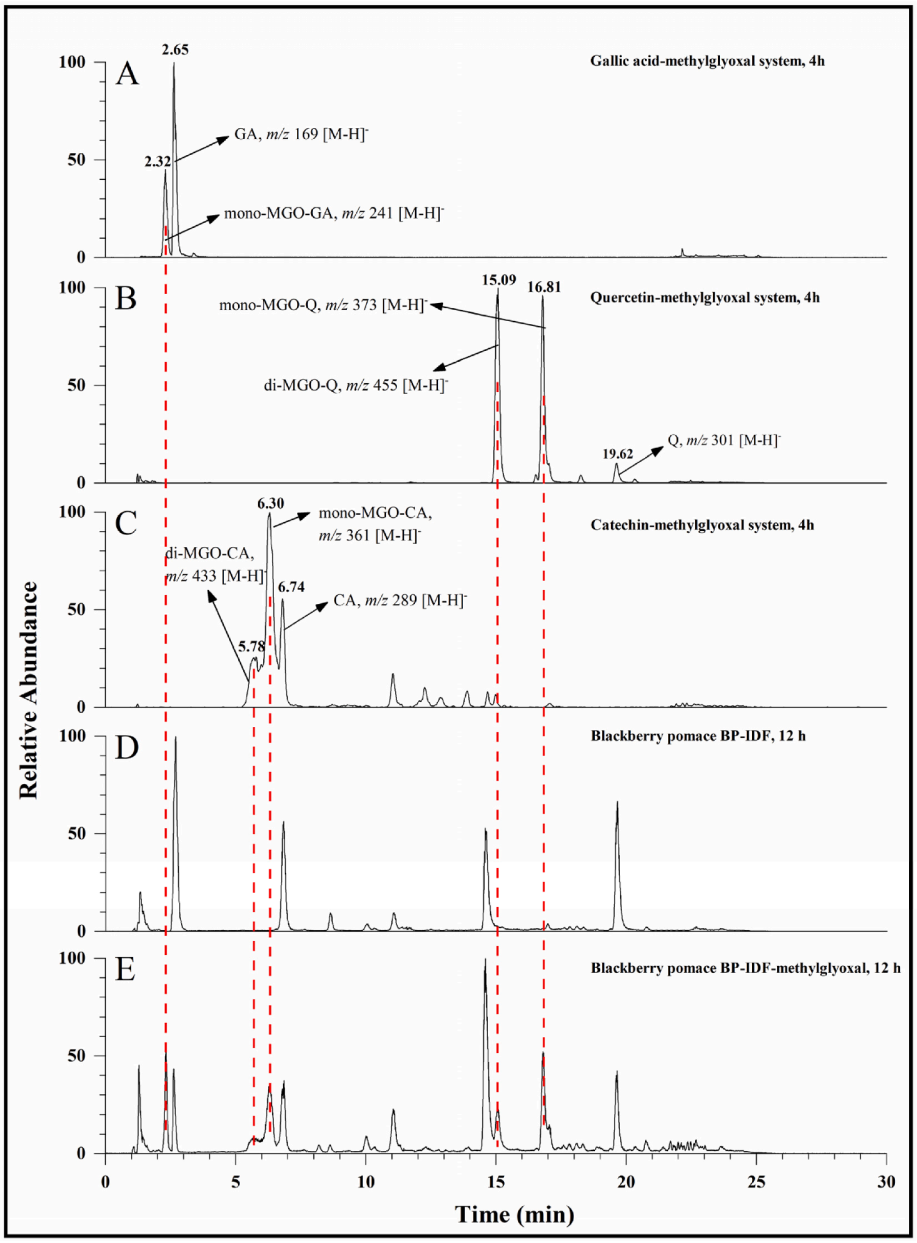

Fig. 4. The formation of methylglyoxal adducts of gallic acid, quercetin, catechin associated to blackberry pomace bound-polyphenol rich insoluble dietary fibre (BP-IDF). Total ion chromatograms of the samples collected from gallic acid- (A), quercetin- (B) and catechin-methylglyoxal (C) chemical reaction systems after incubation at 37 °C, and the samples collected from the alkaline extracts of blackberry pomace BP-IDF after the incubation with phosphate buffer (D, as negative control) or methylglyoxal (E) at 37 °C.

identified as the mono-methylglyoxal-catechin adduct. In the MS^2 spectra of *m/z* 433 $[M-H]^-$, fragment ions at *m/z* 361, *m/z* 289, and *m/z* 181 suggested the structure of mono-methylglyoxal-catechin, indicating that the peak at 5.78 min was the di-methylglyoxal-catechin adduct.

Along with methylglyoxal, glyoxal, acrolein and malondialdehyde adducts of gallic acid, quercetin and catechin were also investigated in the chemical reaction systems. Among all the model systems, mono-glyoxal-quercetin, mono-glyoxal-catechin, mono-acrolein-gallic acid, mono-acrolein-quercetin and mono-acrolein-catechin were tentatively identified according to their molecular ions and MS^2 spectra (Figures S6 and S7). The malondialdehyde adducts of three polyphenols were not detected although they showed considerable trapping capacity for malondialdehyde suggesting the primary adduct is not stable undergoing a quick degradation or intramolecular cyclization in our conditions.

Mass spectrometry results confirmed the formation of methylglyoxal adducts of polyphenols after the incubation of blackberry pomace BP-IDF and methylglyoxal. Indeed, active sites of IDF-bound polyphenols can trap RCS through a nucleophilic reaction, as previously described for free polyphenols (Shao et al., 2014). Concerning BP-IDF as source of various types of polyphenols, different fibre-bound phenolic structures could enhance carbonyl trapping efficacy of each other because of additive and regeneration effects. This evidence indicated that insoluble-bound polyphenols can alternatively act as antioxidants and carbonyl scavengers in the gut.

4. Conclusion

Blackberry pomace, red cabbage and wheat bran BP-IDF effectively scavenged all the four RCS tested, namely glyoxal, methylglyoxal, malondialdehyde and acrolein. BP-IDF contributed to more than 21.9%, 24.7% and 63.2% of total carbonyl trapping capacity of plant foods in the case of blackberry pomace, red cabbage and wheat bran, respectively. Our data showed that after *in vitro* digestion, there were still considerable carbonyl trapping capacity retained for all of three BP-IDF. The removal of fibre-bound polyphenols by alkaline and acidic hydrolysis reduced by up to 90% of carbonyl trapping capacity of BP-IDF. Finally, we detected methylglyoxal conjugates of polyphenols bound to blackberry pomace BP-IDF and the mono-acrolein adducts of gallic

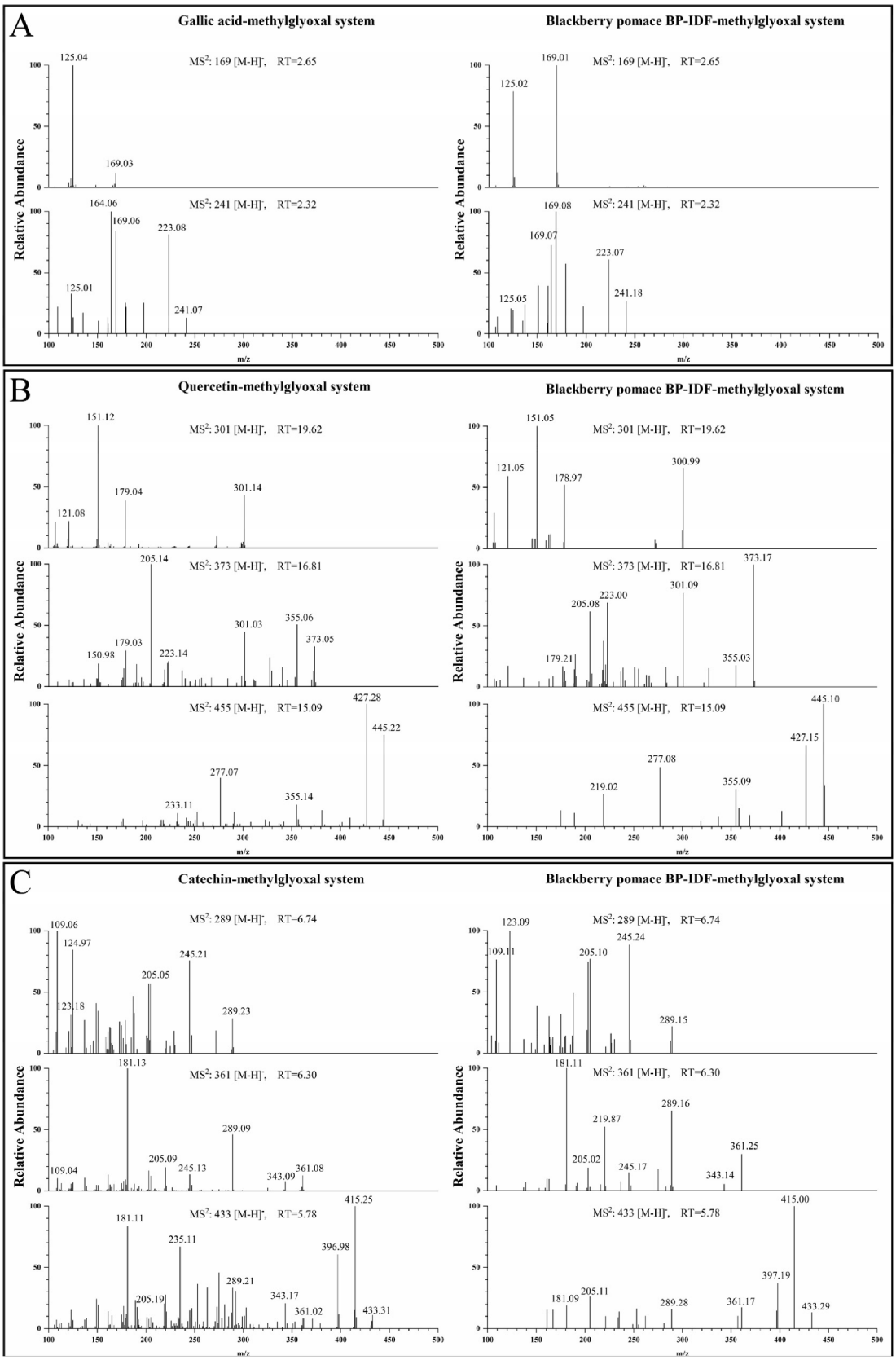

Fig. 5. A: ESI-MS^2 spectra of gallic acid (*m/z* 169 [M−H$^-$]) and mono-methylglyoxal-gallic acid (*m/z* 241 [M−H$^-$]); B: ESI-MS^2 spectra of quercetin (*m/z* 301 [M−H$^-$]), mono-methylglyoxal-quercetin (*m/z* 373 [M−H$^-$]) and di-methylglyoxal-quercetin (*m/z* 455 [M−H$^-$]); C: ESI-MS^2 spectra of catechin (*m/z* 289 [M−H$^-$]), mono-methylglyoxal-catechin (*m/z* 361 [M−H$^-$]) and di-methylglyoxal-catechin (*m/z* 433 [M−H$^-$]).

acid, quercetin and catechin, suggesting the ability of insoluble-bound polyphenols to trap RCS under simulated physiological conditions. Looking at these results with a wider physiological prospective, the carbonyl trapping capacity of BP-IDF should be considered for its potential benefits inside the GI tract. The quantification of the polyphenol-RCS adduct can be used to have indications about the role of dietary intervention in the reduction of oxidative stress in the colon.

CRediT authorship contribution statement

Hao Zhang: Conceptualization, Investigation, Methodology, Formal analysis, Data curation, Resources, Visualization, Writing - original draft. **Antonio Dario Troise:** Methodology, Formal analysis, Visualization, Supervision, Writing - review & editing. **Yajing Qi:** Methodology, Formal analysis, Visualization, Resources. **Gangcheng Wu:** Formal analysis, Software. **Hui Zhang:** Project administration, Conceptualization, Supervision, Funding acquisition, Writing - review & editing. **Vincenzo Fogliano:** Conceptualization, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2021.129018.

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