Supplementary Material

Influence of *in vitro* digestion and colonic fermentation on carbonyls scavenging capacity of fiber-bound polyphenols from quinoa

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2.8 Quantification of phenolic compounds by UPLC-MS/MS

The polyphenols composition and content were determined using an UPLC-MS/MS system (Waters, Milford, MA) following the protocol described in our previously published work.[1] The column was a 100 mm ×3.0 mm, 2.7 μm Poroshell 120 SB-AQ C18 column (Agilent) operating at 35 °C with a flow rate of 0.4 mL/min, and the injection volume was 5 μL. The mobile phase was consisted of a 0.1% aqueous formic acid (A) and 0.1% formic acid in acetonitrile (B) with the gradient program: 0-10 min, B 5-10%; 10-20 min, B 15-30%; 20-30 min, B 30-55%; 30-35 min, B 55-90%; 35-50 min, B 90-5%. The mass spectrometry conditions were set as follows: ionization mode, negative; spray voltage, 3.5 kV; cone voltage, 30 V; collision energy, 25-30 eV; ion source temperature, 120 °C; cone gas, 50 L/h. Spectra were scanned in the mass range from 50-1500 m/z. The polyphenols were identified by comparing their UV-Vis, retention times and mass spectra with these of corresponding commercial standards. The individual polyphenols were quantified according to corresponding calibration curve. All analyses were performed in triplicate.

2.9 Determination carbonyls scavenging capacity

The supernatants were derivatized using an OPD solution (for MGO and GO) or DNPH solution (for ACR and MDA) to determine the concentration of carbonyl compounds. MGO or GO was derivatized by adding 200 μL OPD solution (0.2%, w/v) for 3 h at room temperature in dark.[2] ACR or MDA was derivatized by adding 20 μL of HCl (6 M) and 50 μL DNPH solution (2 mg/mL in acetonitrile/HCl 9:1, v/v) for 5 h at room temperature in dark.[3] The carbonyl-OPD/DNPH derivatives were

determined using an LC-20A HPLC system (Shimadzu, Japan) following the protocol described in a previous work.[3] Separation was chromatographed on an Agilent Poroshell 120 SB-AQ C18 column (100 mm ×3.0 mm, 2.7μm) at a column temperature of 35 °C. The mobile phase consisted of (A) 0.1% aqueous formic acid and (B) 0.1% formic acid in acetonitrile. The following gradient program was used: 0 min, 10% B;10 min, 23% B; 20 min, 45% B; 30 min, 70% B; 35 min, 90% B; The injection volume was 5 μL and the flow rate was 0.4 mL/min.

References

1 J. X. Li, H. Zhang, X. J. Yang, L. Zhu, G. C. Wu, X. G. Qi and H. Zhang, Trapping of reactive carbonyl species by fiber-bound polyphenols from whole grains under simulated physiological conditions, *Food Res Int*., 2022, **156**, 111142.

2 H. Zhang, H. Zhang, A. D. Troise and V. Fogliano, Melanoidins from coffee, cocoa, and bread are able to scavenge α-dicarbonyl compounds under simulated physiological conditions, *J. Agric. Food Chem*., 2019, **67**, 10921-10929.

3 H. Zhang, A. D. Troise, Y. J. Qi, G. C. Wu, H. Zhang and V. Fogliano, Insoluble dietary fibre scavenges reactive carbonyl species under simulated physiological conditions: The key role of fibre-bound polyphenols, *Food Chem*., 2021, **349**, 129018.

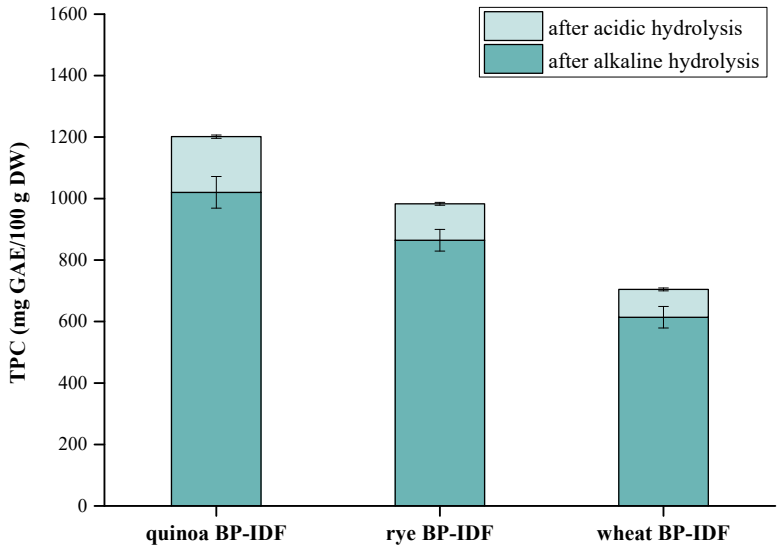

Fig. S1 The total contents of bound phenolic compounds released by alkaline and acidic hydrolysis from the three whole grains BP-IDF (mg GAE/100 g DW)

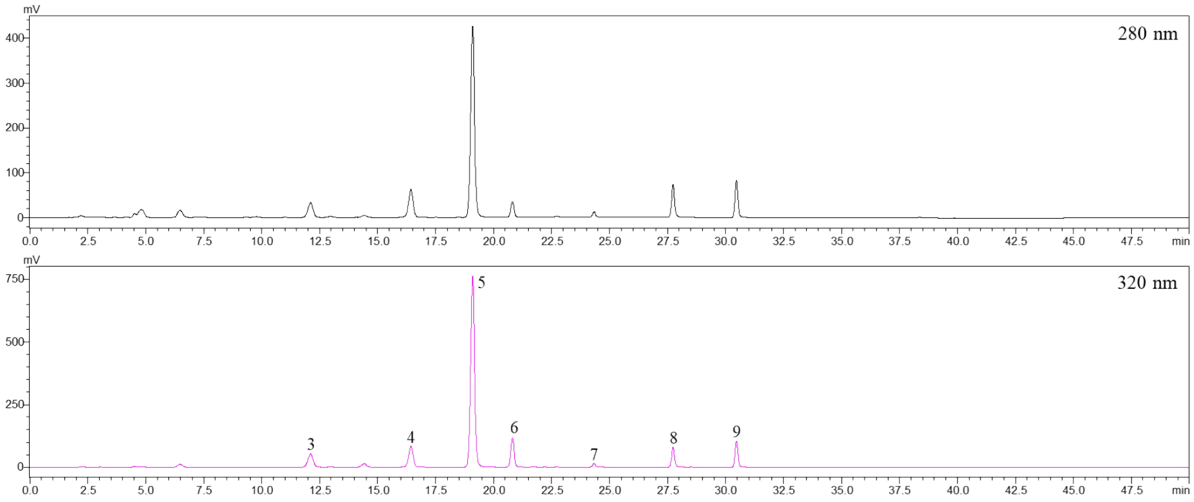

Fig. S2 UPLC chromatogram (280 nm and 320 nm) of alkaline hydrolysate of quinoa BP-IDF. (3) caffeic acid, (4) *p*-coumaric acid, (5) ferulic acid, (6) sinapic acid, (7) myricetin, (8) quercetin, (9) kaempferol.

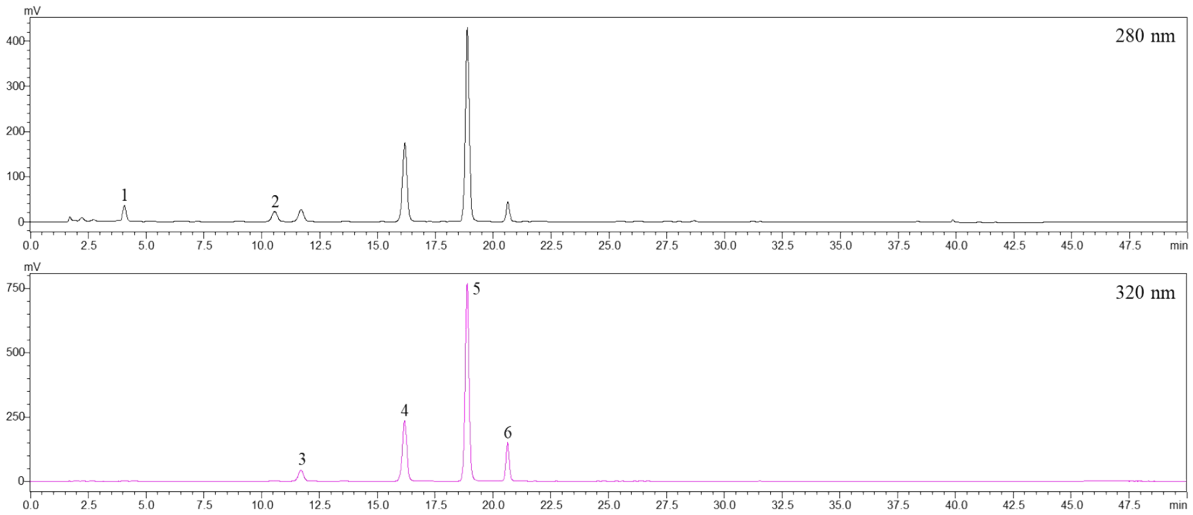

Fig. S3 UPLC chromatogram (280 nm and 320 nm) of alkaline hydrolysate of rye BP-IDF. (1) protocatechuic acid, (2) vanillic acid, (3) caffeic acid, (4) *p*-coumaric acid, (5) ferulic acid, (6) sinapic acid.

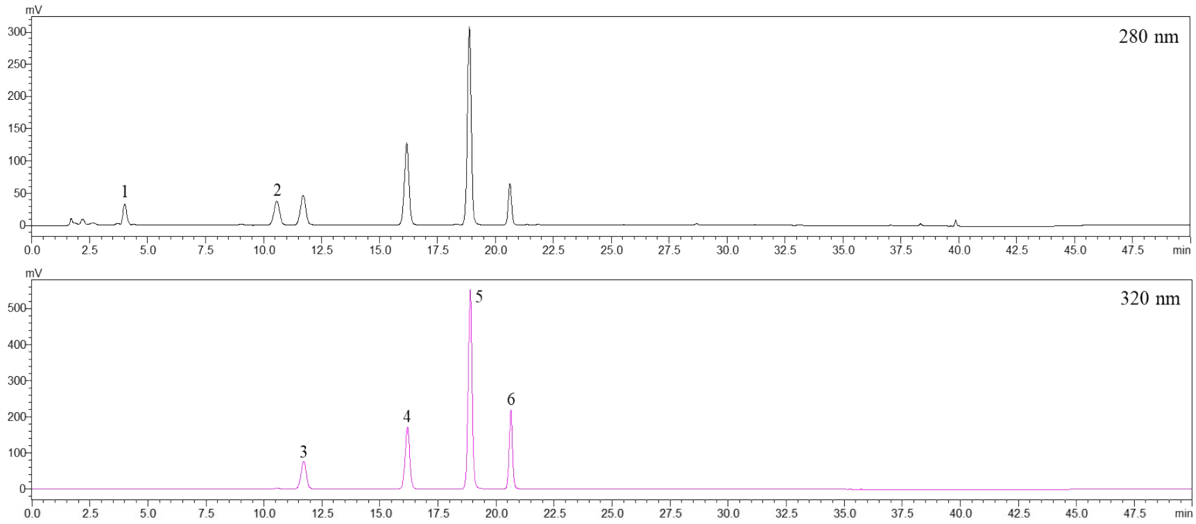

Fig. S4 UPLC chromatogram (280 nm and 320 nm) of alkaline hydrolysate of wheat BP-IDF. (1) protocatechuic acid, (2) vanillic acid, (3) caffeic acid, (4) *p*-coumaric acid, (5) ferulic acid, (6) sinapic acid.

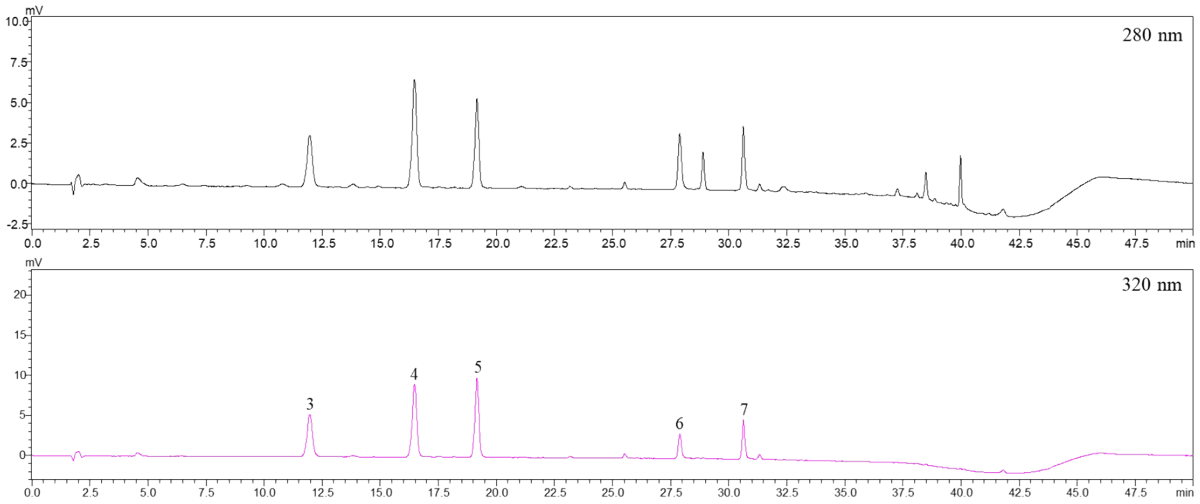

Fig. S5 UPLC chromatogram (280 nm and 320 nm) of released bound polyphenols from quinoa BP-IDF after simulated gastrointestinal digestion. ((3) caffeic acid, (4) *p*-coumaric acid, (5) ferulic acid, (6) quercetin, (7) kaempferol.

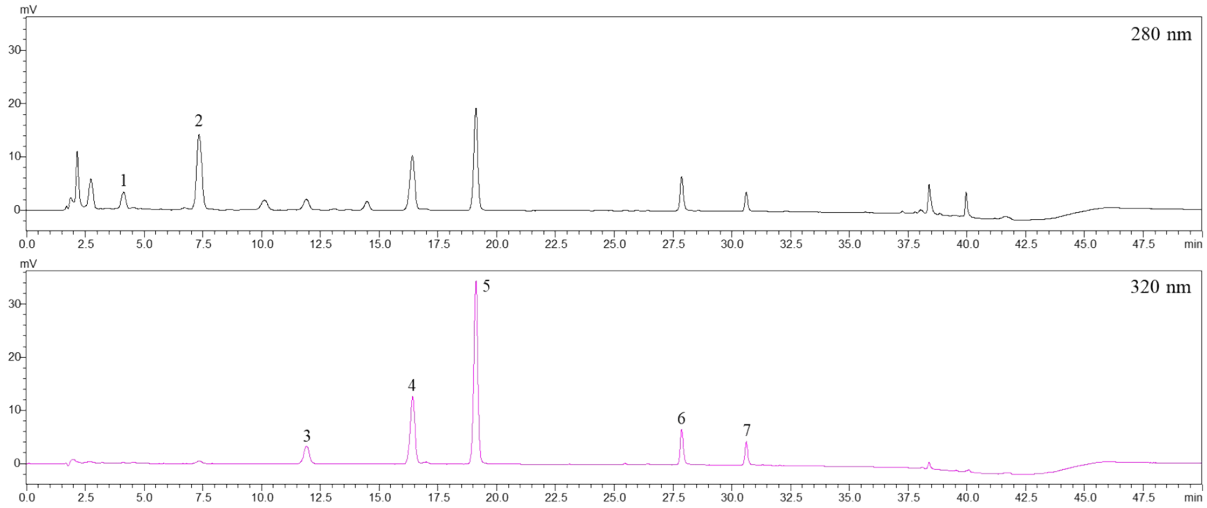

Fig. S6 UPLC chromatogram (280 nm and 320 nm) of released bound polyphenols from quinoa BP-IDF at 6 h of colonic fermentation. (1) protocatechuic acid, (2) *p*-hydroxybenzoic acid, (3) caffeic acid, (4) *p*-coumaric acid, (5) ferulic acid, (6) quercetin, (7) kaempferol.

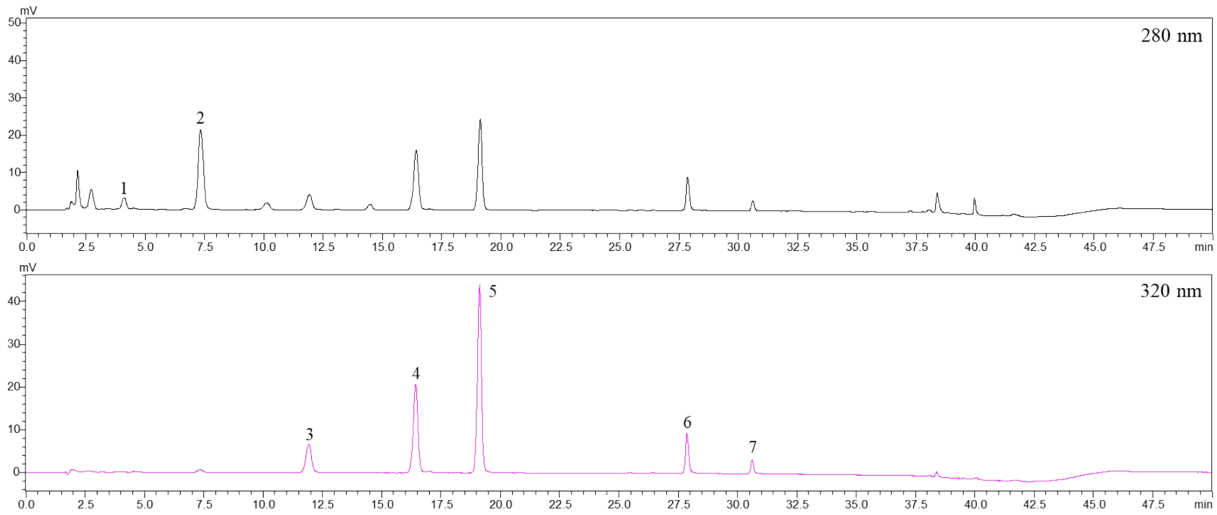

Fig. S7 UPLC chromatogram (280 nm and 320 nm) of released bound polyphenols from quinoa BP-IDF at 12 h of colonic fermentation. (1) protocatechuic acid, (2) *p*-hydroxybenzoic acid, (3) caffeic acid, (4) *p*-coumaric acid, (5) ferulic acid, (6) quercetin, (7) kaempferol.

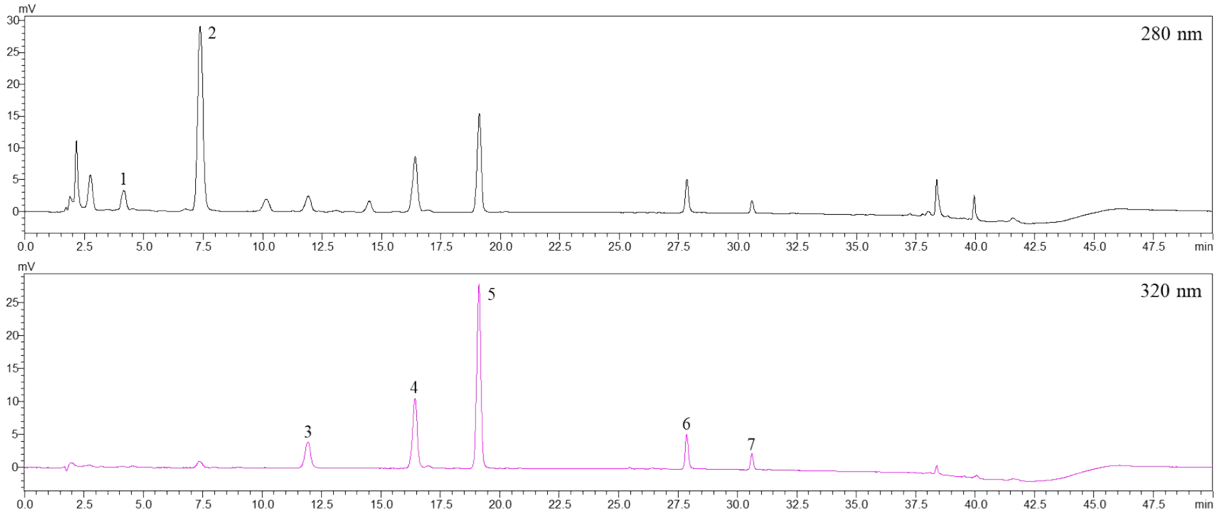

Fig. S8 UPLC chromatogram (280 nm and 320 nm) of released bound polyphenols from quinoa BP-IDF at 24 h of colonic fermentation. (1) protocatechuic acid, (2) *p*-hydroxybenzoic acid, (3) caffeic acid, (4) *p*-coumaric acid, (5) ferulic acid, (6) quercetin, (7) kaempferol.

Table S1 The pH values of different fermentation groups during colonic fermentation

	6 h	12 h	24 h
MGO+quinoa BP-IDF	5.5	5.0	5.6
GO+quinoa BP-IDF	6.2	5.8	6.0
ACR+quinoa BP-IDF	5.2	5.0	5.5
MDA+quinoa BP-IDF	6.5	6.2	6.8