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Polyphenol release from protein and polysaccharide embedded plant extracts during in vitro digestion ☆

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ABSTRACT

Encapsulated phenol extracts from industrial processing residues of onions and apples are discussed as health promoting food additives. Prerequisite for their use in human/animal nutrition is the quantitative liberation of the phenols during digestion. In the present in vitro study we, thus, determined the release rate of a variety of spray dried phenol extracts embedded in different carrier molecules. Onion and apple extracts encapsulated with either lupine/gum acacia (ratio extract to wall material: 1:2), gum acacia (1:3), pea protein isolate (1:4), or modified starch (1:3) were screened employing a two-step (gastric: pepsin, pH 2.0; intestinal: pancreatin, pH 7.5) in vitro model (pH stat). Total phenol release in the incubation solutions (solid phase extraction) was followed spectrophotometrically (Folin–Ciocalteu method); quercetin-4′-*O*-glucoside (onion) and phloretin-2′-*O*-glucoside (apple) were analysed with HPLC. Extracts spray dried in the presence of carbohydrates alone (gum acacia, modified starch) or in combination (lupine/gum acacia) exhibited an immediate, non-enzymatic phenol release (80%) due to instability in aqueous solutions. Pepsin catalysed enzymatic hydrolysis of apple extracts embedded with pea protein isolate resulted in a time-depending total phenol (max. 70% of embedded material) and phloretin-2′-*O*-glucoside (max. 20%) liberation; pancreatin showed no effect. Phenols were stable in gastric fluid; in the presence of pea protein isolate, phenols partly decomposed at basic pH. These results demonstrate that only spray dried phenol extracts embedded in the presence of pea protein isolate effectively release phenols under in vitro digestion conditions.

1. Introduction

Aromatic phenols with one or more hydroxyl groups are characteristic compounds of onions (*Allium cepa* L.) and apples (*Malus domestica*) (Balasundram, Sundram, & Samman, 2006). In onions, the predominant phenols are phenolic acids and flavonoids especially flavonol glycosides such as rutin, kaempferol-3-*O*-glucoside, quercetin-4′-*O*-glucoside and quercetin-3,4′–*O*-diglucoside among others (Albishi, John, Al-Khalifa, & Shahidi, 2013; Riggi, Avola, Siracusa, & Ruberto, 2013; Simin et al., 2013). The major phenols of apple pomace comprise benzoic acids, hydroxycinnamic acids, flavanols, flavonols and dihydrochalcones including compounds like phloretin-2′-O-glucoside, chlorogenic acid and quercetin (Grigoras, Destandau, Fougére, & Elfakir, 2013; Rana, Rana, Gulati, & Bhushan, 2013; Rupasinghe & Kean, 2008). In vitro, polyphenols exhibit antioxidant and anticarcinogenic properties (Dudonne, Vitrac, Coutiere, Woillez, & Merillon, 2009; Ramos, 2008) and may, thus, be pivotal for health-promoting effects of onion and apple consumption observed in recent human studies (Boyer & Liu, 2004; Griffiths, Trueman, Crowther, Thomas, & Smith, 2002).

During industrial processing, e.g. by peeling of onion bulbs or during apple juice production, large amounts of onion and apple wastes (in the EU between 1999 and 2009: 480,000 and 700,000 tonnes, respectively) are produced (FAO Statistics, 2009). These residues still contain several nutritionally relevant compounds, e.g. the majority of the naturally occurring phenols. Keeping in mind their health supporting properties, polyphenol extracts from onion and apple residues may be used as food additives to improve the nutritive value of certain food products (Larrosa, Llorach, Espin, & Tomas-Barberan, 2002; Llorach, Tomas-Barberan, & Ferreres, 2005; Rupasinghe, Wang, Huber, & Pitts, 2008). The use of phenolic extracts as value-added ingredients is, however, limited by their bitterness and astringency. A promising way to mask this unpleasant taste is the encapsulation or spray drying of phenols with carriers like proteins and/or polysaccharides before adding to the

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food products (Fang & Bhandari, 2010; Lesschaeve & Noble, 2005). Currently, Rocha-Guzman et al. (2010) were successful to encapsulate phenolic-rich extract of *Quercus resinosa* leaves with lactose-sodium caseinate mixtures by simple spray drying. A successful masking of penetrating smell of the functional extracts of Makoni tea was realized by encapsulation with maltodextrin–pectin matrix (Sansone et al., 2011).

To be health effective, encapsulated phenols have to be effectively released and subsequently taken up in the digestive tract. Consequently, release kinetics of polyphenols embedded has to be investigated before their use in food production.

In general, in vitro systems modelling in vivo gastrointestinal (GI)[1] conditions are used to screen encapsulated material in this respect. Currently, the in vitro digestion of encapsulated bilberry extract with whey protein revealed an almost complete anthocyanin release within the first 10 min of the gastric digestion (Oidtmann et al., 2012). The shellac coating of the bilberry extract with different formulations of the pore former HPMC enables by subjecting to a three step simulated digestion a retarded anthocyanin release until the colon simulation (Oehme, Valotis, Krammer, Zimmermann, & Schreier, 2011).

The aim of the present in vitro work was, thus, to assess the extent of phenol release from spray dried onion and apple extracts embedded in proteins and/or polysaccharides following simulated GI digestion. In addition, stability of target molecules in gastric and intestinal fluids was investigated.

2. Materials and methods

2.1. Materials

Formic acid, caffeic acid, phloretin-2′-*O*-glucoside and quercetin-4′-*O*-glucoside were supplied by Roth (Karlsruhe, Germany). Folin & Ciocalteu's phenol reagent (F9252), metaphosphoric acid and pepsin (P7012; porcine) were purchased from Sigma-Aldrich (Steinheim, Germany). Pancreatin NB (porcine) and sodium carbonate were supplied by Serva (Heidelberg, Germany). Propan-2-ol and trifluoroacetic acid (TFA) were obtained from Fisher Scientific (Loughborough, UK). Acetonitrile (ACN) and CHROMABOND polyamide 6 cartridges (500 mg adsorbent weight) for solid phase extraction (SPE) were supplied by VWR (Leuven, Belgium) and Machery-Nagel (Düren, Germany), respectively. All other chemical reagents were from Merck (Darmstadt, Germany).

Embedded extracts of onion processing residues (apical trimmings, outer dry/semi-dry layers, rejected bulbs; Düpmann GmbH & Co. KG, Marienfeld, Germany) and apple pomace (Herbstreith & Fox KG, Neuenbürg, Germany) were provided by the Department of Food Technology and Biotechnology, University Bonn. For this purpose onion material and apple pomace were ground to a particle size of 10 and 2 mm, respectively, and afterwards the polyphenols were extracted. Extracts with maximal polyphenol content were achieved for onion material by 65 °C, 72% (v/v) aqueous isopropanole and 799 g onion material/l, and for apple pomace by 47 °C, 48% (v/v) aqueous isopropanole and 200 g apple pomace/l. Extraction was conducted without stirring for 1 h in an Erlenmeyer flask sealed with aluminium foil and cooled with ice. Isopropanole content of the extracts was removed by rotary evaporation (220 mPa, 65 °C, 18 min). Onion extract was embedded by spray drying with either using a mixture of lupine protein (L, Lupidor® HP; Georg Breuer GmbH, Königstein, Germany) and gum acacia (GA, Spray-dried 396 A; kindly provided by Rudolf Wild GmbH & Co. KG, Heidelberg, Germany) (onion-LGA; 1:4) or pure GA (onion-GA) as carrier materials. Embedding of apple extract was carried out with pea protein isolate (PPI; pisane; Georg Breuer GmbH, Königstein, Germany) (apple-PPI) or modified starch (MS; Capsule®; National Starch, New Jersey, USA) (apple-MS). Pure liquid onion and apple extracts were freeze dried (Virtis Sentry™ 8EL, Virtis, New York, USA) to gain unprocessed extracts. Composition and phenol content of the protein and/or polysaccharide embedded as well as unprocessed extracts used in this study are summarized in Table 1. The morphologies of the embedded extracts, as seen under an electron microscope, are shown in Fig. 1.

2.2. Simulated in vitro GI digestion

Simulated GI digestion (TitraLab® system comprising a VIT90 Video Titrator, an ABU93 Triburette, and a SAM90 Sample Station (Radiometer Copenhagen, Copenhagen, Denmark)) was performed in pH-stat mode according to Tagliazucchi, Verzelloni, Bertolini, and Angela (2010) in two consecutive steps mimicking first gastric digestion followed by intestinal digestion. Briefly, a homogenous solution of 3 g of spray dried material in 15 mL of pepsin-free simulated gastric fluid (SGF; 2 g NaCl and 7 mL 32% HCl were added to 1 L distilled water (USP, 2009)) was placed in a heated water bath (37 °C). 306 units/mL pepsin were added in 15 mL SGF solution and pH adjusted to 2.0 with 5 M HCl. The gastric incubation was then performed for 2 h under continuous stirring; pH was kept constant by titration with 0.1 M HCl. The intestinal phase was then started after increasing the pH to 7.5 (drop-wise addition of 5 M NaOH) and subsequently adding 0.8 mg/mL pancreatin in gastric digesta. Incubations of capsules with bile salts revealed a diminished recovery of phlorizin; thus, routine incubations were performed without adding bile salts. The intestinal mixture was incubated for another 2 h at 37 °C with continuous stirring and constant pH.

Samples from the incubation mixture (500 μL) were taken immediately as well as 60 and 120 min after starting gastric and intestinal digestion, respectively. The samples were deproteinized by the addition of 2 M metaphosphoric acid; samples of onion particles were additionally treated with dimethyl sulfoxide (DMSO; concentration in the final sample was 20%) to ensure quercetin-4′-*O*-glucoside solubility. After centrifugation of the samples (16,000 ×*g* at 5 °C for 10 min) the supernatants were stored at +3 °C for SPE on the same day.

To check possible interference of the phenol analysis with the Folin reagent by the digestive enzymes and solution, an in vitro GI digestion alone with pepsin and pancreatin was conducted as control. Unprocessed onion and apple extracts as well as pure quercetin-4′-*O*-glucoside and phloretin-2′-*O*-glucoside were also subjected to the GI digestion to analyse their stability under GI conditions. All incubations were performed in duplicate.

Table 1
Composition and phenol content of the embedded and unprocessed extracts used in the in vitro GI digestion[a].

	Extract: wall material	Total phenols[b] (mg CAE/g)	Quercetin-4′-*O*-glucoside[c] (mg/g)	Phloretin-2′-*O*-glucoside[c] (mg/g)
Onion extract	–	40.4 ± 0.7	22.1 ± 0.7	n.d.
Onion-LGA	1: 2	9.1 ± 0.5	4.6 ± 0.0	n.d.
Efficiency[d]		68%	63%	
Onion-GA	1: 3	9.1 ± 0.1	4.6 ± 0.1	n.d.
Efficiency		90%	84%	
Apple extract	–	15.1 ± 0.6	n.d.	3.2 ± 0.1
Apple-PPI	1: 4	2.1 ± 0.0	n.d.	0.6 ± 0.0
Efficiency		70%		90%
Apple-MS	1: 3	3.7 ± 0.1	n.d.	0.7 ± 0.0
Efficiency		99%		91%

[a]Data are presented as mean ± SD (n = 2). L, lupine; GA, gum acacia; L:GA = 1:4; PPI, pea protein isolate; MS, modified starch; n.d., not detectable. [b]Total phenols were investigated by the Folin–Ciocalteu method and expressed as mg caffeic acid equivalent (CAE). [c]Quercetin-4′-*O*-glucoside and phloretin-2′-*O*-glucoside were analysed by HPLC. [d]Encapsulation efficiency (%): ratio of the phenol content from extracts after spray drying with carrier materials to that of the unprocessed extracts.

[1] Abbreviations: ACN, acetonitrile; CAE, caffeic acid equivalent; DMSO, dimethyl sulfoxide; GA, gum acacia; GI, gastrointestinal; L, lupine protein; LGA, lupine gum acacia; LOD, limit of detection; LOQ, limit of quantification; MS, modified starch; PPI, pea protein isolate; SGF, simulated gastric fluid; SPE, solid phase extraction; TFA, trifluoroacetic acid.