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Development and functional characterization of new antioxidant dietary fibers from pomegranate, olive and artichoke by-products

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ABSTRACT

A novel ingredient acting as a slow digestible dietary fiber (DF) was developed by including native corn starch in calcium alginate microspheres (MS). In this study three types of antioxidant DF-rich ingredients were designed and developed by including in the MS, polyphenol-rich vegetable by-product extracts (obtained from pomegranate peels, olive leaves and artichoke leaves) and their potential functionality was assessed *in vitro*.

Specifically, the physico-chemical properties of the new MS were compared with those of six commercially available DF concentrates and with wheat and oat brans. To evaluate the potential efficacy to release PPs along the gastrointestinal tract (GiT), pomegranate peels-microspheres (PPe-MS) were subjected to *in vitro* simulated gastrointestinal digestion. Results showed that the newly developed MS had higher free antioxidant capacity (free-TAC) than commercial DF rich products, and the bound antioxidant capacity (bound-TAC) of PPe-MS was comparable to that of wheat bran and 4.4 folds higher than that of oat-bran. Furthermore, it was shown that the release of ellagitannins from cooked PPe-MS along *in vitro* simulated gastro-intestinal digestion decreased from the salivary to the small intestine phase whereas gallic acid, ellagic acid and its derivatives had an opposite trend. A certain amount of PPs was found in the spent pellet obtained from the *in vitro* digestion, which was mimicking the residue reaching the colon *in vivo*. In conclusion data showed that the new antioxidant MS have physical-chemical properties like those of wheat and oat brans, mainly including the bound antioxidant capacity. This open to new possibilities of functional utilization of vegetable by-products for obtaining valuable and healthy food ingredients.

1. Introduction

Polyphenols (PPs) and dietary fiber (DF) are two types of food components well-known for their beneficial effects on human health. PPs are secondary metabolites of plants and the most abundant antioxidant compounds in foods (Scalbert, Morand, Manach, & Rémésy, 2002). Whole grains, fruits and vegetables are the major dietary sources of PPs (Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004). Several *in vitro* and *in vivo* studies support the role of PPs in the prevention of non-communicable chronic diseases and obesity (Meydani & Hasan, 2010; Panickar, 2013; Scalbert, Manach, Morand, Rémésy, & Jiménez, 2005; Vauzour, Rodriguez-Mateos, Corona, Oruna-Concha, & Spencer, 2010; Visioli et al., 2011; Wang et al., 2014). Even though certain systemic health effects of PPs mirrored the extent of their absorption along the gastrointestinal tract (GiT) and post-absorptive metabolism, the gastrointestinal mucosa is the primary site that is associated to the beneficial effects of phytochemicals, because it is directly exposed to them after their release from the food matrix (Del Rio et al., 2013; Dryden, Song, & McClain, 2006; Halliwell, Zhao, & Whiteman, 2000; Scalbert et al., 2002).

In the intestinal lumen, PPs exert antioxidant and anti-inflammatory activity thus counteracting local subclinical oxidative stress and inflammation. Furthermore, PPs may affect the activity of key digestive enzymes thus influencing their bioavailability and the metabolism of carbohydrates and fats (Bahadoran, Mirmiran, & Azizi, 2013; Gonzalez et al., 2011; Hanhineva et al., 2010; McDougall et al., 2005; Romier,

Abbreviations: ALe, Artichoke leaves; ALe-MS, ALe-Microspheres; DF, Dietary fiber; EA, Ellagic acid; ETs, Ellagitannins; GA, Gallic acid; GiT, Gastrointestinal tract; IDF, Insoluble dietary fiber; MS, Microspheres; OHC, Oil holding capacity; OLe, Olive leaves; OLE-MS, OLe-Microspheres; PCA, Principal component analysis; PPe, Pomegranate peels; PPe-MS, PPe-Microspheres; PPs, Polyphenols; SDF, Soluble dietary fiber; SGP, Simulated gastric phase; SIP, Simulated intestinal phase; SOP, Simulated oral phase; TAC, Total antioxidant capacity; TDF, Total dietary fiber; TE, Trolox equivalents; WHC, Water-holding capacity

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Schneider, Larondelle, & During, 2009; Savini, Catani, Evangelista, Gasperi, & Avigliano, 2013; Sergent, Vanderstraeten, Winand, Beguin, & Schneider, 2012). For these reasons, several technological strategies have been developed aiming to obtain new functional foods and ingredients for the targeted delivery of PPs along the GiT (Fang & Bhandari, 2010; Munin & Edwards-Lévy, 2011). In fruits, vegetables and whole grains, PPs are present in a free form (mainly as glycosides) or covalently bound to cell wall structural components (Acosta-Estrada, Gutiérrez-Uribe, & Serna-Saldívar, 2014). Additionally, PPs may be found physically entrapped or linked to main food macronutrients (*e.g.* starch, proteins and lipids) mainly through non-covalent interactions (Parada & Aguilera, 2007; Zhang et al., 2014). Free and non-covalently bound PPs can be dissolved in the upper GiT directly or after the action of digestive enzymes on the food matrix. Conversely, PPs covalently bound to DF, pass unmodified through the upper intestine and reach the colon. In this site, bound PPs are released by the action of microbial enzymes, thus creating a reducing environment and being available for absorption in their original chemical forms or as microbial metabolites (Saura-Calixto, 1998; Saura-Calixto, 2010; Vitaglione et al., 2015; Vitaglione, Napolitano, & Fogliano, 2008). In the colon, PPs may also positively modify the composition of the microbiota and, in turn, the host physiology (Etxeberria et al., 2013; Selma, Espin, & Tomas-Barberan, 2009).

Therefore from a physiological perspective, plant foods having PPs bound to DF can be considered as natural carriers of PPs along the GiT. It is also reported that the reduced incidence of several non-communicable-chronic diseases found by epidemiological studies associated to a regular consumption of fruits and vegetables and whole grains, is linked to their content of both DF and micronutrients, including PPs and minerals (Slavin, 2013). As a consequence a minimum daily intake of 25 g of DF was recommended by EFSA, FAO and WHO (Jones, 2014), and the development and marketing of functional foods enriched with PPs and DF is always an important topic discussed by both food scientists and industry (Nehir El & Simsek, 2012; Quirós-Sauceda et al., 2014; Siro, Kapolna, Kapolna, & Lugasi, 2008). In this direction, the recent inclusion, by the CODEX Alimentarius Commission, among the definitions of DF as "*synthetic carbohydrate polymers showing health benefits*" along with the classical definitions of DF as "*edible carbohydrate polymers naturally occurring in foods*" and "*carbohydrate polymers, obtained from raw foods by physiological, enzymatic or chemical means and showing health benefits*" (Jones, 2014; Phillips & Cui, 2011) is opening new opportunities in the development of functional ingredients.

Venkatachalam, Zhang, Kushnick, and Hamaker (2007) developed a new source of DF named starch-entrapped microspheres (MS) constituted by a spherical electrostatically cross-linked alginate matrix forming a web-like structure that is filled with native corn starch. The basic structure of MS, *i.e.* corn starch entrapped in the alginate matrix, was previously demonstrated to limit the extent of starch digestion in the small intestine *in vitro* thus allowing its fermentation in the colon (Kaur, Rose, Rumpagaporn, Patterson, & Hamaker, 2011; Rose et al., 2009; Rose, Venema, Keshavarzian, & Hamaker, 2010). Moreover, starch-based MS attenuated the postprandial glycemic and insulinemic response and improved bowel habit (Rasmussen et al., 2017; Venkatachalam, Kushnick, Zhang, & Hamaker, 2009). This innovative source of DF was patented by Hamaker and coworkers (Hamaker, Venktachalam, Zhang, Keshavarzian, & Rose, 2013).

Some plant by-products from agricultural processes and the food industry are rich and inexpensive sources of PPs and DF. These materials can be dehydrated and subjected to solvent extraction for the recovery of PPs-rich extracts, or directly used in their whole form as functional ingredients for the formulation of new foods (Colantuono, Ferracane, & Vitaglione, 2016; Ferreira et al., 2015; O'Shea, Arendt, & Gallagher, 2012; Schieber, Stintzing, & Carle, 2001; Sharma et al., 2016; Troise et al., 2014).

Within this framework, the main objective of this study was to develop new antioxidant DF-ingredients by using agricultural by-products as natural sources of PPs. For this purpose, three PPs-rich extracts were obtained from pomegranate peels (PPe), olive leaves (OLe) and artichoke leaves (ALe), and were used as a functional filling for the development of new starch-alginate based MS. The physico-chemical properties of the new ingredients were compared to those of six commercially available DF concentrates, as well as to wheat and oat brans. PPe-microspheres (PPe-MS) were subjected to *in vitro* simulated gastrointestinal digestion to evaluate their potential efficacy to deliver PPs and to promote antioxidant activity along the GiT.

2. Materials and methods

2.1. Chemicals

HPLC-grade methanol, acetonitrile and water were purchased from Merck (Darmstadt, Germany). Ethanol, formic acid, ethyl acetate and acetone were obtained from VWR international (Fontenay-sous-Bois, France). Total DF assay kit was purchased from Megazyme International (Wicklow, Ireland). Calcium chloride, celite, hydrochloric acid (HCl), 4-morpholineethanesulfonic acid (MES), 2,2-diphenyl-1-picrylhydrazyl, 95% (DPPH), tris(hydroxymethyl)-aminomethane hydrochloride (Tris-HCl), sodium alginate, sodium hydroxide and 6-hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid were purchased from Sigma-Aldrich (St. Louis, MO). Standards of punicalagin, ellagic acid (EA), gallic acid (GA), oleuropein, chlorogenic acid, caffeic acid, cynarin, ferulic acid, p-cumaric acid, rutin, apigenin, hydroxytyrosol, luteolin and protocatechuic acid were purchased from Sigma-Aldrich (St. Louis, MO). The cynaropicrin standard was obtained from Extrasynthese (Lyon, France). The luteolin-4-O-glucoside standard was obtained from Indofine Chemical Company (Somerville, NJ). Corn starch was obtained from Tate & Lyle (Decatur, IL). Organic wheat and oat brans (Fior di loto, Orbassano, Italy) were purchased in a local market. A barley β-glucans concentrate (Glucagel) was obtained from DKSH (Miribel Cedex, France). Potato DF, pea cortical DF, citrus DF, carrot DF, bamboo DF were purchased from ITALI (Reggio Emilia, Italy). PPe powder was obtained from Detrade UG (Bremen, Germany). ALe and OLe dry powders were purchased in a local herbalist's shop. PPe, ALe and OLe were pure raw materials.

2.2. Preparation of plant by-products extracts

The preparation of the antioxidant extracts from PPe powder, OLe powder, and ALe powder was performed by adding to 250 g of each powder 1 L of a solution ethanol/water (50/50, v/v) acidified with 0.5% formic acid, or 1 L of a solution ethanol/water (70/30, v/v), or 1.8 L of a solution ethanol/water (75/25, v/v) acidified with 0.1% formic acid, respectively. The mixtures were subjected to ultrasound assisted extraction for 30 min and subsequently filtered through a filter paper (VWR international, qualitative filter paper 600). The extracts recovered after filtration (590 mL for PPe, 490 mL for OLe and 1149 mL for ALe) were concentrated under vacuum in a rotary evaporator ($T < 30$ °C) and then freeze-dried. Prior to the preparation of PPe-MS, OLe-MS and Ale-MS the dry extracts were dissolved in 590 mL, 490 mL and 670 mL of distilled water, respectively.

2.3. Preparation of functional starch-based MS

Starch-alginate based MS filled with the PPs rich extracts were produced using the protocol previously described by Rose et al., (2009) with few modifications. PPe, ALe and OLe extracts prepared as described above, were separately mixed at room temperature with distilled water, native corn starch, and sodium alginate. Three suspensions with composition: 69% of distilled water (552 g, w/w), 20% of PPs-rich extract (160 g, w/w), 9% of native corn starch (72 g, w/w) and 2% of sodium alginate (16 g, w/w), were obtained. In parallel, a suspension with composition: 89% of distilled water (712 g, w/w), 9% of native corn starch (72 g, w/w) and 2% of sodium alginate (16 g, w/w), was