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Potential bioaccessibility and functionality of polyphenols and cynaropicrin from breads enriched with artichoke stem

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ABSTRACT

In this study, an artichoke stem powder (ASP) was used at three concentrations (3%, 6% and 9%) in the formulation of new breads. The bioaccessibility of polyphenols and cynaropicrin from the ASP-enriched breads was evaluated *in vitro* by using a digestion model combined to high resolution mass spectrometry analysis. The overall total antioxidant capacity of the bioaccessible and unsolubilized fractions obtained during the intestinal steps and the potential ability to modulate α-glucosidase activity were tested. Data showed that 82% of totally bioaccessible polyphenols and 74% of cynaropicrin were released during the duodenal digestion whereas 88% of caffeic acid was released in the colon step. The antioxidant capacity and the α-glucosidase inhibitory activity of the duodenal extract correlated with the amount of ASP in the bread. Data demonstrated that ASP might be a valuable functional ingredient to create a reducing environment in the intestine and to partially modulate glucose metabolism.

1. Introduction

Globe artichoke (*Cynara cardunculus* L. var. *scolymus*) is a widely cultivated crop in the Mediterranean regions. Italy is the first world producer (451,461 tons/year) followed by Egypt (266,196 tons/year) and Spain (234,091 tons/year) (FAO, 2014). The immature inflorescence (capitula or head) represents the edible part of the plant and is consumed as fresh, frozen, or canned food (de Falco, Incerti, Amato, & Lanzotti, 2015). Stems and external leaves represent about 80–85% of the total fresh weight of the plant and are the most abundant by-products from industrial processing of artichoke (Lattanzio, Kroon, Linsalata, & Cardinali, 2009).

Both edible fractions and artichoke by-products are well known sources of dietary fiber (DF) and polyphenols (PPs), mainly including caffeoylquinic acids (CQA), di-caffeoylquinic acids (DCQA) and flavones (Femenia, Robertson, Waldron, & Selvendran, 1998; Lattanzio et al., 2009; Pandino, Lombardo, & Mauromicale, 2013). Chlorogenic acid (5-*O*-caffeoylquinic acid), 1,5-di-*O*-caffeoylquinic acid, 3,4-di-*O*-caffeoylquinic acid and 3,5-di-*O*-caffeoylquinic acid are the most abundant phenolic acids in artichoke tissues, whereas luteolin and apigenin glycosides and rutinosides derivatives are the most abundant flavones (Lattanzio et al., 2009). These molecules exert *in vitro* antioxidant, antimicrobial and anticancer activities and act as natural inhibitors of key enzymes involved in metabolic syndrome development (de Falco et al., 2015; Matsui, Ogunwande, Abesundara, & Matsumoto, 2006; Villiger, Sala, Suter, & Butterweck, 2015). Several human studies demonstrated that the health benefits associated with the intake of artichoke bioactive compounds, mainly include antioxidative, hypolipidemic, hypoglycemic, anti-inflammatory, and anti-obesity effects (Rondanelli, Giacosa, Orsini, Opizzi, & Villani, 2011; Rondanelli, Monteferrario, Perna, Faliva, & Opizzi, 2015; Rondanelli et al., 2014). Those effects are due to the ability of artichoke phenolics to modulate cellular antioxidant defence systems and some crucial enzymatic pathways.

Sesquiterpene lactones (guaianolides) are another abundant class of compounds present in artichoke, mainly in external leaves. They are associated with several biological properties, such as anti-hyperlipidemic, anti-inflammatory and anti-photoaging activities (de Falco et al., 2015). Cynaropicrin is the most abundant guaianolide, accounting for the 80% of the typical bitter taste of artichoke (Cravotto, Nano, Binello, Spagliardi, & Seu, 2005). At very low concentrations, cynaropicrin and grosheimin can activate *in vitro* the human bitter taste receptor hTAS2R46 (Brockhoff, Behrens, Massarotti, Appendino, & Meyerhof, 2007). This property may contribute to the metabolic benefits mostly associated to the consumption of artichoke DF and PPs. Indeed, mounting evidence shows that bitter compounds modulate neurohormonal response behind gastrointestinal motility, glucose homoeostasis and appetite control through the activation of bitter taste receptors located along the gastrointestinal tract (GiT) (Mennella et al., 2016).

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The multitude of bioactive compounds in artichoke by-products make them a good source of ingredients useful to enhance the antioxidant activity and the functional properties of wheat bread, as already proposed by some researchers (Boubaker, Damergi, Marzouk, Blecker, & Bouzouita, 2016; Frutos, Guilabert-Antón, Tomás-Bellido, & Hernández-Herrero, 2008). Despite the undisputable social value to valorize agricultural by-products, their use as new functional food ingredients needs a bio-efficacy validation inside the food. In fact, food processing as well as food structure influence the chemical composition and the bioavailability of bioactive compounds from the enriched-food products (Dziki, Różyło, Gawlik-Dziki, & Świeca, 2014).

The bioaccessibility, the bioavailability and the potential antioxidant effects along the GiT of PPs from artichoke heads, were previously investigated both *in vivo* and *in vitro* (Azzini et al., 2007; D'Antuono, Garbetta, Linsalata, Minervini, & Cardinali, 2015). Scientific literature is still lacking on the bioaccessibility and potential bioefficacy of bioactive compounds from foods enriched with artichoke by-products.

In this study, artichoke stems were selected among artichoke by-products and added at 3%, 6% and 9% in the formulation of new types of bread. Potential bioaccessibility of PPs and cynaropicrin from the breads as well as overall total antioxidant capacity (TAC) and potential ability to modulate α-glucosidase activity in the GiT were assessed.

2. Materials and methods

2.1. Chemicals

Water, methanol and acetonitrile were obtained from Merck (Darmstadt, Germany). Ethanol and formic acid were purchased from VWR international (Fontenay-sous-Bois, France). Cellulose powder was obtained from Fluka (Buchs, Switzerland). Total dietary fiber assay kit was purchased from Megazyme International (Wicklow, Ireland). Calcium chloride, potassium chloride, sodium chloride, potassium phosphate monobasic, magnesium chloride hexahydrate, ammonium carbonate, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl, 95% (DPPH), sodium bicarbonate, sodium hydroxide, hydrochloric acid, Celite, tris(hydroxymethyl)-aminomethane hydrochloride, 4-morpholinoethanesulfonic acid (MES), *para*-nitrophenyl-α-d-glucopyranoside, sodium phosphate dibasic heptahydrate (≥ 99,99% trace metals basis) were purchased from Sigma-Aldrich (St. Louis, MO). α-amylase from porcine pancreas (type VI-B ≥ 10 U/mg), α-glucosidase from *Saccharomyces cerevisiae* (Type I ≥ 10 U/mg), protease from *Streptomyces griseus*, (Pronase E, Type XIV ≥ 3.5 U/mg), cell wall degrading enzyme complex from *Aspergillus* sp. (Viscozyme L), pancreatin from porcine pancreas (4 X USP) and pepsin from porcine gastric mucosa (≥ 250 U/mg) were purchased from Sigma-Aldrich (St. Louis, MO). Analytical standards of cynarin, chlorogenic acid, and luteolin 7-*O*-glucoside were purchased from Sigma-Aldrich (St. Louis, MO). The analytical standard of cynaropicrin was purchased from Extrasynthese (Lyon, France). The calibration solutions for HRMS analysis were obtained from Thermo Fisher Scientific (Bremen, Germany). The ingredients for the preparation of white-bread and enriched breads were purchased from a local market.

2.2. Plant material and extraction of bioactive compounds

Fresh globe artichokes from the varietal type “Tondo di Paestum” were used. External leaves and floral stems were separated from the artichoke heads (including outer bracts, inner bracts and the receptacle). The three fractions were washed, freeze-dried and then minced with a Grindomix knife mill (10,000 rpm for 30 s) to obtain three dry powders. The extraction of PPs and cynaropicrin from artichoke powders was carried out using a method adapted by Menin et al. (2012). Ten milliliters of a solution methanol/water (75:25, v/v) acidified with 0.1% of formic acid were added to 500 mg of dry sample and an ultrasound assisted extraction for 20 min was performed. Then, samples were centrifuged for 10 min at 4000 rpm at 4 °C and 2 mL of supernatant were collected and a further ultra-centrifuged for 10 min at 14,800 rpm at 4 °C before the analysis. The identification and quantification of PPs and cynaropicrin was carried out using an U-HPLC system coupled to an Exactive mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Santonin was used as internal standard for the evaluation of cynaropicrin extraction recovery.

2.3. Total phenols and antioxidant capacity of artichoke fractions

One milliliter of the extracts obtained as reported above was dried under nitrogen flow and re-dissolved in 1 mL of methanol, that was appropriately diluted before the analysis. The amount of total phenols was measured using the Folin–Ciocalteu assay as described by Ferracane, Graziani, Gallo, Fogliano, and Ritieni (2010). The results were expressed as mg of gallic acid equivalents (GAE)/g of dry weight (DW). The antioxidant capacity (AC) of the extracts was measured by using the DPPH assay as reported by Papillo, Vitaglione, Graziani, Gokmen, and Fogliano (2014). Results were expressed as μmol Trolox equivalents (TE)/g of DW.

2.4. Insoluble, soluble and total dietary fiber of artichoke by-products

Soluble dietary fiber (SDF), insoluble dietary fiber (IDF) and total dietary fiber (TDF) of artichoke dry powders, were measured according to the protocol described by Prosky, Asp, Schweizer, DeVries, and Furda (1988). Results were reported as mg/g of DW and the IDF/SDF ratio for each sample was calculated.

2.5. Food preparation

Three types of artichoke stem powder (ASP) enriched bread were formulated by using a traditional bread recipe and replacing wheat flour with ASP so that its final amount in the dough resulted 3%, 6% and 9% by weight. Control bread was prepared by mixing 50 g of water with 100 g of wheat flour, 1 g of salt and 1.2 g of dry yeast. In 3%, 6% and 9% ASP-enriched breads, 4.6 g, 9.1 g and 13.7 g of ASP replaced the same amount of wheat flour, respectively. The ingredients were mixed for 12 min and portions (150 g) of dough were left to rise at room temperature for one hour before baking (200 °C for 20 min and 185 °C for 15 min) in a convection oven.

Total lipids (method 935.38), proteins (method 950.36) and ashes (method 930.22) in control bread and ASP-enriched breads were determined according to standard AOAC procedures (2016). The content of total dietary fiber was measured according to the protocol described by Prosky, Asp, Schweizer, DeVries, and Furda (1988). Total carbohydrates were assessed by difference: 100 − (weight in grams of proteins + lipids + water + ash + dietary fiber for 100 g of food).

2.6. In-vitro simulated gastrointestinal digestion

The gastrointestinal digestion of the ASP-enriched breads was performed by using the method described by Minekus et al. (2014) and briefly modified. For the oral phase 2.5 g of freeze-dried sample were minced and mixed for 2 min at 37 °C in a thermostatic shaking bath with 1.75 mL of simulated salivary fluid (SSF), 0.25 mL of α-amylase from porcine pancreas (1500 U/mL), 12.5 μl of 0.3 M $CaCl_2$ and 488 μl of water. For the simulated gastric phase, oral bolus was mixed with 3.75 mL of SGF, 2.5 μl of 0.3 M $CaCl_2$ and 0.8 mL of a solution of porcine pepsin (25,000 U/mL) made up in simulated gastric fluid (SGF). The pH was adjusted to 3.0 with 1 M HCl, the volume was filled up to 10 mL with distilled water and the samples were incubated for 2 h at 37 °C in a thermostatic shaking bath. For the simulated duodenal phase (SDP) the gastric chyme was mixed with 5.5 mL of simulated intestinal fluid (SIF), 2.5 mL of pancreatin solution (800 U/mL), 1.25 mL of a bile