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Influence of heat treatment on antioxidant capacity and (poly)phenolic compounds of selected vegetables

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ABSTRACT

The impact of cooking heat treatments (frying in olive oil, frying in sunflower oil and griddled) on the antioxidant capacity and (poly)phenolic compounds of onion, green pepper and cardoon, was evaluated. The main compounds were quercetin and isorhamnetin derivates in onion, quercetin and luteolin derivates in green pepper samples, and chlorogenic acids in cardoon. All heat treatments tended to increase the concentration of phenolic compounds in vegetables suggesting a thermal destruction of cell walls and sub cellular compartments during the cooking process that favor the release of these compounds. This increase, specially that observed for chlorogenic acids, was significantly correlated with an increase in the antioxidant capacity measured by DPPH ($r = 0.70$). Griddled vegetables, because of the higher temperature applied during treatment in comparison with frying processes, showed the highest amounts of phenolic compounds with increments of 57.35%, 25.55% and 203.06% compared to raw onion, pepper and cardoon, respectively.

1. Introduction

The Mediterranean diet is characterized by the high consumption of fruit and vegetables. The European Union produces a broad range of fruits and vegetables thanks to its varied climatic and topographic conditions and it is one of the main global producers of some vegetables such as tomatoes, carrots and onions. Specifically, 5.7 million tonnes of onions were produced in 2013 in Europe, and Spain was one of the main producer countries with around 21% of total onion production (Eurostat, 2014). This high vegetables production favors their high consumption. Recent data indicate that the consumption of fresh vegetables in Spain in 2014 was 260.96 g/capita/day (MAGRAMA, 2014). This consumption was increased around 31% since 2011 when the intake of fresh vegetables was 179.17 g/capita/day (AECOSAN, 2011). Onion and pepper are two of the most consumed vegetables in Spain, however there are lots of local vegetables as cardoon, chard or borage which have also a high acceptability among population depending on the region (AECOSAN, 2011). In any case, plant foods are the main source of dietary antioxidants, including phenolic compounds. (Poly)phenols rich foods have been reported to exhibit a wide range of biological effects such as protective effects against cardiovascular diseases, neurodegenerative diseases and cancer, probably due to their ability to protect against oxidative damage in cells (Del Rio et al., 2013; Rodríguez-Mateos et al., 2014).

Many dietary vegetables are usually eaten both crude or after cooking in different ways. Culinary processes induce significant changes in foods such as water loss, changes in the total fat content and in the fatty acid profile, degradation of thermolabile compounds, and formation of others due to heat-induced chemical reactions (Miglio, Chiavaro, Visconti, Fogliano, & Pellegrini, 2008; Miranda et al., 2010). (Poly)phenolic compounds can also be affected by thermal processes and, consequently the antioxidant capacity of consumed vegetables too (Ramírez-Anaya, Samaniego-Sánchez, Castañeda-Saucedo, Villalón-Mir, & de la Serrana, 2015). There are some studies that report the effect of heat treatment on antioxidant activity and (poly)phenolic compounds in vegetables. While boiling is the most investigated cooking method, few studies are about frying process, both deep frying and pan frying (Palermo, Pellegrini, & Fogliano, 2014) and as far as we know, only one study was found about the effect of griddling on the antioxidant capacity of vegetables, (Jiménez-Monreal, García-Diz, Martínez-Tomé, Mariscal, & Murcia, 2009), but none on the (poly)phenols profile. However, results reported on the effect of heat treatment on the (poly)phenolic compounds are

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not clear cut. Onion is one of the most studied vegetables, nevertheless both losses and gains in (poly)phenolic compounds after heat treatment are reported in the literature (Crozier, Lean, McDonald, & Black, 1997; Ewald, Fjelkner-Modig, Johansson, Sjöholm, & Åkesson, 1999; Harris, Brunton, Tiwari, & Cummins, 2015; Lombard, Peffley, Geoffriau, Thompson, & Herring, 2005; Price, Bacon, & Rhodes, 1997; Rodrigues, Pérez-Gregorio, García-Falcón, & Simal-Gándara, 2009; Rohn, Buchner, Driemel, Rauser, & Kroh, 2007). The studies found in literature about effect of heat treatment on green pepper are focused on antioxidant activity, but not on (poly)phenolic compound profiles changes (Jiménez-Monreal et al., 2009) and up to our knowledge, this is the first time where the influence of heat treatment on antioxidant capacity and (poly)phenol compounds of cardoon stalks (*Cynara cardunculus* L.) has been studied. Therefore, the aim of this work was to study the impact of three cooking heat treatments (frying in olive oil, frying in sunflower oil and griddled) on the antioxidant capacity and (poly)phenolic compound profiles of onion, green pepper and cardoon, commonly consumed as crude in salads and cooked in several ways in the mediterranean diet.

2. Material and methods

2.1. Chemical and reagents

Yellow onion (*Allium cepa*), sweet Italian green pepper (*Capsicum annuum*), cardoon stalks (*C. cardunculus L*), olive oil and sunflower oil were obtained from local stores.

The methanol and ethanol were of analytical grade from Panreac (Barcelona, Spain). The methanol (HPLC grade) was purchased from Panreac (Barcelona, Spain). Trolox (6-hydroxy-2, 5,7,8-tetramethylchroman-2-carboxylic acid), 2,2′-azinobis (3-eth ylbenzothiazonile-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), as well as the standards used for identification and quantification of phenolic compounds (quercetin, luteolin, isorhamnetin, 5-caffeoylquinic acid and caffeic acid), were purchased from Sigma–Aldrich (Steinheim, Germany).

2.2. Samples preparation

Chopped vegetables (yellow onion, green pepper, and cardoon stalks) (300 g) were fried with olive or sunflower oils (30 mL) at 115 °C for 10 min in a non-stick frying pan. Then, temperature was decreased to 108 °C for 5 min. Chopped vegetables were also submitted to heating at 150 °C for 10 min and then at 110 °C for 5 min in a non-stick griddle without oil addition. Then, raw and cooked vegetables were lyophilized in a freeze dryer Cryodos-80 (Telstar, Terrasa, Spain), and stored at −18 °C until analysis.

2.3. Vegetables extracts

Vegetables extracts were prepared according to Siddiq, Roidoung, Sogi, and Dolan (2013) with some modifications. Briefly, thirty mL of ethanol/water (80/20) was added to 2 g of lyophilized vegetables. The content was mixed on a mechanical shaker for 1 h at room temperature and then centrifuged at 4000 rpm for 10 min. Supernatant was collected and residues were re-extracted twice using 10 mL of ethanol 80% by vortexing (1 min) and centrifuged at 4000 rpm for 5 min. All three supernatants were combined and frozen at −18 °C for antioxidant capacity and UHPLC–PDA–H R–MS analysis.

2.4. Antioxidant capacity by ABTS assay

The ABTS antioxidant capacity was performed according to the method of Re et al. (1999). The radicals $ABTS^{\cdot+}$ were generated by the addition of 0.36 mM potassium persulfate to a 0.9 mM ABTS solution prepared in phosphate buffered saline (PBS) (pH 7.4), and the $ABTS^{\cdot+}$ solution was stored in darkness for 12 h. The $ABTS^{\bullet+}$ solution was adjusted with PBS to an absorbance of 0.700 (±0.020) at 734 nm in a 3 mL capacity cuvette (1 cm length) at 25 °C (Lambda 25 UV–VIS spectrophotometer, Perkin-Elmer Instruments, Madrid, Spain). An aliquot of 100 μL of each vegetable extract sample properly diluted in demineralized water, was added to 2 mL of $ABTS^{\cdot+}$ solution. The absorbance was measured spectrophotometrically at 734 nm after exactly 18 min. Calibration was performed with Trolox solution (a water-soluble vitamin E analog), and the antioxidant capacity was expressed as micromoles of Trolox equivalent per gram of dry matter sample (μmol Trolox/g dm).

2.5. Antioxidant capacity by DPPH assay

The antioxidant capacity was also measured using 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$) decolorization assay (Brand-Williams, Cuvelier, & Berset, 1995) with some modifications. A 6.1×10^{-5} M $DPPH^{\cdot}$ methanolic solution was prepared immediately before use. The $DPPH^{\cdot}$ solution was adjusted with methanol to an absorbance of 0.700 (±0.020) at 515 nm in a 3 mL capacity cuvette (1 cm length) at 25 °C (Lambda 25 UV–VIS spectrophotometer, Perkin-Elmer Instruments, Madrid, Spain). Vegetable extracts were properly diluted in demineralized water prior to analysis. Samples (50 μL) were added to 1.95 mL of the $DPPH^{\cdot}$ solution. After mixing, the absorbance was measured at 515 nm after exactly 18 min. Calibration was performed with Trolox solution (a water-soluble vitamin E analog). The antioxidant capacity was expressed as micromoles of Trolox equivalent per gram of dry matter sample (μmol Trolox/g dm).

2.6. (Poly)phenolic compounds by UHPLC–PDA–HR–MS

(Poly)phenolic compounds were analysed using an UPLC with a PDA detector scanning from 200–600 nm, equipped with an autosampler cooled at 4 °C (Dionex Ultimate 3000 RS, Thermo Corporation) and an Exactive™ Orbitrap mass spectrometer fitted with a heated electrospray ionization probe (HESI) (Thermo Fisher Scientific, San José, USA). Chromatographic separation was performed at 40 °C on a Kinetex 5 μm RP 250 × 4.6 mm reversed phase column (Phenomenex, Macclesfield, UK). Ten microliter of each ethanolic extract was analysed using an 80 min 5–50% gradient of acetonitrile in 0.1% aqueous formic acid at a constant flow rate of 1 mL/min. After passing the PDA flow cell, the eluate was split and 0.2 mL/min was directed to the mass spectrometer with the HESI operating in negative ionization mode. Analysis was carried out in full-scan (100–800 m/z) and full-scan with In-Source Collision-induced dissociation (CID) (100–800 m/z; CID 25.0 eV). Capillary temperature was 300 °C; sheath gas and auxiliary gas were 60 and 20 units/min, respectively; source voltage was 4.0 kV. Identification was achieved by comparing the exact mass and retention time with pure reference standards. In absence of standards, compounds were tentatively identified by comparing the theoretical exact mass of the molecular ion with the experimentally measured accurate mass of the molecular ion. In addition identification was confirmed by the appearance of typical fragments produced from the molecular ion. Quantification was performed by PDA at 325 nm for caffeic acid glucosides and chlorogenic acids, and at 360 nm for quercetin-, isorhamnetin-, luteolin-, and apigenin derivatives. Typical UPLC–PDA chro-