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Phytochemical and microbiological stability of spent espresso coffee grounds in capsules

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ABSTRACT

Wet spent coffee grounds (SCGs) from espresso capsules, a post-consumer organic solid residue produced worldwide, were analysed to determine their chemical and microbiological stability during storage. In particular, the changes in the total phenolic content and antioxidant capacity (based on two free radical scavenging assays and one oxygen radical absorbance assay) were determined on espresso SCG stored in capsules for up to one month at room temperature in a container open to the air. Phenolic compounds were also identified and quantified using high performance liquid chromatography coupled with diode array and mass detectors. Microbiological analysis was performed in parallel on the same stored SCG to determine the total counts and quantify the main microbial groups present during the storage. The total phenolic content, antioxidant capacity and the most important bioactive compounds, such as the total caffeoylquinic acids, were significantly stable during storage for up to one month, while overall microbial stability was observed for up to two weeks of storage. Overall, the recovery of espresso coffee capsules within 15 days could guarantee the maintenance of microbiological stability as well as the content of valuable antioxidant compounds.

1. Introduction

The need to reduce waste production as well as to minimise its economic and environmental impacts has prompted researchers to optimise extraction techniques with the goal of obtaining bioactive compounds from plant-derived residues (Wijngaard, Hossain, Rai, & Brunton, 2012). New and promising studies with the aim to chemically characterise fruit and vegetable by-products and waste biomass have been performed, highlighting their potential use as valuable source of bioactive components such as polyphenols, dietary fibre (Lozano-Sánchez et al., 2011; O'Shea, Arendt, & Gallagher, 2012) and other molecules (Pfaltzgraff, De Bruyn, Cooper, Budarin, & Clark, 2013).

Among food manufacturing sectors, the coffee industry produces high quantities of solid wastes and by-products, which recent studies describe to be rich in phytochemicals and bioactive molecules that have potential in the formulation of functional foods (Esquivel & Jiménez, 2012; Franca & Oliveira, 2009; Mussatto, Machado, Martins, & Teixeira, 2011). Spent coffee grounds (SCGs) obtained in large quantities from coffee brewing are one of the most interesting organic post-consumer coffee residues. A total of 50% of SCG come from the industrial preparation of instant soluble coffee (Esquivel & Jiménez, 2012) and the remaining 50% come from the worldwide production of different coffee brews in cafeterias, restaurants and homes. Currently, disposable espresso capsules are among the most popular ways to consume coffee brew and are an interesting and widely method adopted in homes or at offices (Parenti et al., 2014). Spent coffee grounds have recently been characterised showing high quantities of water-soluble organic bioactive antioxidant compounds, such as caffeine, chlorogenic acids and melanoidins (Bravo, Monente, Juániz, Paz De Peña, & Cid, 2013; Bravo et al., 2012; Panusa, Zuorro, Lavecchia, Marrosu, & Petrucci, 2013; Ramalakshmi, Rao, Takano-Ishikawa, & Goto, 2009; Zuorro & Lavecchia, 2012) and inorganic components, such as minerals (Cruz et al., 2012). Nevertheless, the studies mentioned above have been carried out on fresh spent coffee grounds analysed after stages of preparation such as drying, defatting and/or freeze-drying to preserve the original organic sample. While SCG produced from soluble coffee production can be immediately treated at the industrial level (Bravo et al., 2012), SCG from capsules can be stored in the place where capsules are consumed. Therefore, because one half of the SCG production originates from the consumption of espresso capsules, it would be interesting to know how storage can

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affect the composition of the bioactive of polyphenolic compounds present in this solid residue and its potential for industrial reuse.

Therefore the aim of this study was to define the stability of antioxidant activity, polyphenolic compounds, bacteria and fungi present in Arabica spent coffee capsules during storage for up to one month in air at room temperature to reflect real storage conditions at home or in the workplace before the industrial reuse of these residues.

2. Materials and methods

2.1. Chemicals and reagents

n-Hexane, acetone, ethanol, methanol, formic acid, *trans*-5-*O*-caffeoylquinic acid (*trans*-5-CQA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis-(3-ethylbenzothiazolin-6-sulfonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) and 3′,6′-dihydroxyspiro[isobenzofuran-1[3H],9′[9H]-xanthen]-3-one (fluorescein), Folin–Ciocalteu's phenol reagent, sodium nitrite, aluminium chloride, potassium persulphate, hydrochloric acid and sodium hydroxide were purchased from Sigma-Aldrich (Milan, Italy). All chemicals were of reagent- or HPLC grade level. Ultra-pure water was produced with a Milli-Q System (Millipore, Milan, Italy). Plate Count Agar (PCA), Malt Extract Agar (MTA) and Ringer's solution were supplied by Oxoid (Milan, Italy).

2.2. SCG sample preparation and storage

Commercial aluminium coffee capsules (Lavazza Blue Tierra 2 Intenso, 100% Arabica) from the same batch (one hundred capsules) were provided by Lavazza S.p.A. (Turin, Italy) and used to produce a typical Italian espresso coffee brew with an automatic espresso machine (Lavazza Blue LB 1000, Lavazza, Italy). In particular, one espresso coffee was obtained from each capsule (8 g of ground coffee), using oligomineral water (electrical conductivity (20 °C) 69.5 μS/cm) by stopping the espresso machine to obtain an espresso with a final volume of 20 mL. The extraction parameters for espresso production are as follows: water temperature of approximately 90 °C, water pressure of 9 bar, percolation time of 10 s for 20 mL of espresso. All used capsules were collected and stored in air at room temperature (22 °C ± 3) in a polyethylene terephthalate container. Spent capsules had twenty-two small holes (each of 2 mm diameter) in the aluminium cover. After 0, 7, 15 and 28 days of storage, ten capsules were randomly opened, the SCGs were collected, mixed and immediately used to perform all chemical and microbiological analyses. Unused ground coffees from capsules were analysed as a reference for chemical analyses. Three replicates were carried out for each analysis and each step of storage.

2.3. Moisture and pH determination

The moisture content was determined using an electronic moisture balance (Eurotherm, Gibertini Elettronica, Milan, Italy) with 5 g of sample. For the determination of pH, samples of coffee or SCG were mixed with ultrapure water 1:25 (w/v) with continuous shaking (Asal srl, Stirrer 711, Cernusco sul Naviglio, Milan, Italy) for 15 min at room temperature. The pH of the decanted liquid phase was measured for 5 min using a pH-metre (Micro pH 2002, Crison, Italy).

2.4. Extraction of phenolic compounds

Extraction of phenolic compounds was carried as reported by Pinelo, Tress, Pedersen, Arnous, and Meyer (2007), in agreement with the recent literature (Panusa et al., 2013). Briefly, 2 g of coffee or SCG was added to 20 mL of a mixture of ethanol/water 60:40 (v/v), shaken on an orbital shaker for 30 min at a constant oscillation (100 oscillations per min) in the dark and at room temperature and then centrifuged (15 min, 4 °C, 16800 *g*) (Heraeus Megafuge 11R, Thermo Electron, LED GmbH, Germany). The supernatant was filtered (0.45 μm), diluted to 25 mL with ethanol/water 60:40 (v/v) and immediately analysed. The extractions were performed in triplicate for each sample. Extracts were used for the determination of the total phenolic content, antioxidant capacity, HPLC–photodiode array detector (PDA) and MS/MS analyses.

2.5. Total phenolic content (TPC) assay

TPC was spectrophotometrically assayed by means of the modified Folin–Ciocalteu's method (Singleton, Orthofer & Lamuela-Raventós, 1999; Singleton & Rossi, 1965). Briefly, 0.5 mL of phenolic extract was appropriately diluted and mixed with 2.5 mL of Folin–Ciocalteu's reagent that had been diluted with water 1:10 (v/v). The mixture was incubated at room temperature for 3 min, and 2 mL of 7.5% (w/v) aqueous sodium carbonate solution was added. The mixture was incubated at 45 °C for 15 min and finally cooled in a water–ice bath to stop the reaction. The specific absorbance at 765 nm was immediately measured at room temperature with a UV–visible spectrophotometer (UV-1700 PharmaSpec, Shimadzu, Milan, Italy). A mixture of solvent and reagents was used as blank. The total phenolic content was expressed as mg gallic acid equivalents (GAE) per gram of sample on a dry basis, through a calibration curve of gallic acid. The linearity range of the calibration curve was 0–250 mg/L ($r^2 = 0.998$).

2.6. In vitro antioxidant capacity (AC) assays

2.6.1. Trolox equivalent antioxidant capacity (TEAC) assay

The TEAC values of phenolic extracts were estimated according to the original analytical procedure described by Re et al. (1999), with slight modifications. ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulphate (final concentration). The mixture was allowed to stand in the dark at room temperature for 12–16 h before use. Immediately before the analysis, the $ABTS^{\bullet+}$ stock solution was diluted with ethanol to reach an absorbance of 0.70 (±0.02) at 734 nm, and equilibrated at 30 °C. Sample solutions (or standard) (30 μL) were mixed with $ABTS^{\bullet+}$ solution (3 mL). Absorbance readings were taken at 30 °C exactly 6 min after the initial mixing. An appropriate solvent blank was obtained by mixing 60% ethanol (30 μL) with $ABTS^{\bullet+}$ solution (3 mL), while absolute ethanol was used as a control. The $ABTS^{\bullet+}$ scavenging effect (% Inhibition) was calculated using the equation:

$$\%\ \text{Inhibition} = \left[\left(A_{734\text{blank}} - A_{734\text{sample}}\right)/A_{734\text{blank}}\right] \times 100$$

where $A_{734\text{blank}}$ and $A_{734\text{sample}}$ are the absorbances of $ABTS^{\bullet+}$ solution at 734 nm before and after sample addition. Results are expressed as μmol Trolox equivalent (TE) per gram of sample on a dry basis, by means of a dose–response curve for Trolox (0–350 μM).

2.6.2. DPPH radical scavenging capacity (DPPH RSC) assay

The DPPH RSCs of the phenolic extracts were measured based on the discolouration of the purple coloured methanol solution of the 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$). The spectrophotometric assay was conducted according to the method reported by von Gadow, Joubert, and Hansmann (1997). Briefly, 75 μL of sample extract was added to 3 mL of 6.1×10^{-5} M $DPPH^{\bullet}$ solution in methanol. The decrease in absorbance at 515 nm was recorded at room temperature condition until stable values (1 h) using methanol as control and methanol solution of $DPPH^{\bullet}$ as blank. All operations were performed in the dark or dim light (Sharma & Bhat, 2009). The inhibition percentage (IP) of the $DPPH^{\bullet}$ by phenolic extracts was calculated according to the formula

$$\text{IP} = [(A_{0\ \text{min}} - A_{60\ \text{min}})/A_{0\ \text{min}}] \times 100$$