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Antioxidant activity and bioactive compounds of lettuce improved by espresso coffee residues

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ABSTRACT

The antioxidant activity and individual bioactive compounds of lettuce, cultivated with 2.5–30% (v/v) of fresh or composted espresso spent coffee grounds, were assessed.

A progressive enhancement of lettuce's antioxidant capacity, evaluated by radical scavenging effect and reducing power, was exhibited with the increment of fresh spent coffee amounts, while this pattern was not so clear with composted treatments. Total reducing capacity also improved, particularly for low spent coffee concentrations. Additionally, very significant positive correlations were observed for all carotenoids in plants from fresh spent coffee treatments, particularly for violaxanthin, evaluated by HPLC. Furthermore, chlorophyll *a* was a good discriminating factor between control group and all spent coffee treated samples, while vitamin E was not significantly affected.

Espresso spent coffee grounds are a recognised and valuable source of bioactive compounds, proving herein, for the first time, to potentiate the antioxidant pool and quality of the vegetables produced.

1. Introduction

Coffee is one of the most important agricultural commodities in world's trade but its processing produces significant amounts of undervalued by-products. These residues are highly rich in organic and inorganic compounds, with high environmental pollution if released into the ecosystem without adequate pre-treatment (Mussatto, Machado, Martins, & Teixeira, 2011). Coffee wastes reuse has been therefore a major priority of producing and consuming countries, both for ecological, as well as economic and social reasons.

Following these concerns, numerous attempts have been considered to recycle coffee residues through compost preparation, biogas production, animal feed and mushrooms production, or more recently by extraction of value-added fractions, as lipids for biodiesel production (Mussatto et al., 2011; Oliveira, Franca, Camargos, & Ferraz, 2008). In the particular case of spent coffee grounds (SCG), recent reports emphasised their richness in coffee bioactive compounds (Bravo et al., 2012; Cruz, Cardoso, et al., 2012), as caffeine or chlorogenic acids, derived from the incomplete extraction process of beverage preparation. Indeed, these are the same compounds receiving health claims on coffee consumption, being among the main contributors for its antioxidant capacity and bioactivity (Bravo et al., 2012). Thus, their presence in SCG makes it senseless to depose them without further use.

Supporting an increased application of this residue in domestic agriculture, apparently with benefits on plant protection and appearance, recent studies highlighted the possibility of vegetable enrichment in bioactive compounds, particularly carotenoids, when cultivated in the presence of reduced amounts of fresh-SCG (Cruz, Baptista, Cunha, Pereira, & Casal, 2012). However, many other antioxidant substances may receive benefits from this pre-harvest treatment, requiring detailed evaluation. Furthermore, the influence of other coffee-based amendments, such as the composted ones which are traditionally applied at domestic levels, has not been studied so far. In order to support an effective and sustained reuse of this coffee waste, this study aimed to clarify the effect of espresso SCG as soil amendment for agricultural purposes, as is or after composting, on the nutritional quality of green leafy vegetables. A particular focus was devoted to their antioxidant features as these are amongst the most important bioactive attributes resulting from vegetable consumption.

The combined assessment of lipophilic and hydrophilic compounds, together with an estimation of the plant total antioxidant activity by *in vitro* assays will allow a first and better elucidation about SCG's influence on green leafy vegetables nutritional characteristics, thus exploring and supporting the reuse of these coffee residues for agriculture purposes.

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2. Materials and methods

2.1. Standards and reagents

Authentic standard *trans*-β-carotene, and the internal standard (IS) *trans*-β-apo-8′-carotenal were purchased from Fluka Chemie (Germany), while gallic acid, lutein, and chlorophyll *a* were obtained from Sigma–Aldrich (Germany), and pheophytin *a* was obtained from an acidified chlorophyll *a* solution (Sievers & Hynninem, 1977). Tocopherols (α-, and γ-) were acquired from Supelco (USA) and tocol, used as IS for tocopherol quantification, was from Matreya Inc. (USA). The concentration of the individual carotenoids (lutein and *trans*-β-carotene), chlorophyll *a* (≈0.1 mg mL^{-1}), and tocopherols (≈5 mg mL^{-1}) were evaluated by UV/VIS spectrophotometry (UV-1800, Shimadzu, Japan), using published absorption coefficients (Britton, 1995; Nesaretnam, Yew, & Wahid, 2007). A stock solution of *trans*-β-apo-8′-carotenal (0.65 mg mL^{-1}) was prepared in ethyl acetate while tocol was prepared in *n*-hexane (1.0 mg mL^{-1}).

Ethyl acetate, *n*-hexane and methanol, all HPLC grade, were from Sigma–Aldrich. All the remaining reagents were analytical grade from several suppliers and included: butylated hydroxytoluene (BHT), chloroform, 1,4-dioxane, sodium chloride, triethylamine, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical and iron (III) chloride, hexahydrate, Folin and Ciocalteu's reagent, sodium carbonate and trichloroacetic acid. Phosphate buffer (pH 6.6) was prepared from sodium dihydrogen phosphate and disodium hydrogen phosphate.

2.2. Experimental design

2.2.1. Soil and spent coffee material

Two different assays were developed simultaneously, one using fresh-SCG and another using composted-SCG. Each SCG batch was assembled with coffee wastes collected from several coffee shops, in Porto metropolitan area (NW Portugal), serving espresso coffee.

Regarding the study using fresh-SCG, five mixtures with plain vegetable soil (Siro® Germe, Portugal) were prepared: 2.5%, 5%, 10%, 15% and 20%, all on a volume basis, and distributed by 1 L plastic pots. For the second assay, SCG were previously composted for 6 months at a specialised municipal solid waste treatment facility (Lipor, Porto, NW Portugal). Fresh-SCG were collected and composted on the following day. Equivalent volumes of fresh grass, straw and fresh-SCG were piled in a 100 L composter, at ambient temperature, from July to January. Temperature and moisture were controlled weekly with a digital thermo-hygrometer, being revolved and watered when compost's moisture dropped below 60%. After a six-month period, the compost was completely stabilized, being transported to the greenhouse where five final mixtures were prepared with plain soil: 5%, 10%, 15%, 20% and 30%, also on a volume basis. Plain vegetable soil was used as control (0%) for both experiments.

2.2.2. Plant material and growth conditions

The experimental setup for this study was a randomized greenhouse pot design performed at the School of Agriculture, Polytechnic Institute of Bragança (NE Portugal), under controlled conditions (day/night thermal regime of 23/18 ± 2 °C, 70 ± 10% relative humidity, natural light).

Butterhead lettuce seeds (*Lactuca sativa* L. var. *capitata* cv. "Four Seasons") were germinated with organic substrate (Siro® Germe, Portugal), during spring season in the Northern hemisphere. After 4 weeks, the healthy plantlets were transferred to the plastic pots containing the mixtures prepared. For each SCG percentage (5 levels) and assay (fresh and composted), plus control, thirty pots were prepared, giving a total of 330 plants. All pots were watered after transplanting, and distributed randomly through the greenhouse longitudinal extension (Fig. 1). Every two days all plants were irrigated (50 mL) and, at the 7th and 21st days, irrigation water was supplemented with 0.2% (v/v) of Complesal 12-4-6 (N/P/K) nutritive solution (Bayer, Portugal).

Lettuce plants were harvested after 5 weeks, carefully washed with deionized water, and the edible part was separated and weight. Due to the reduced individual plants weight for all the assays, the 30 plants from each group were divided into 10 subgroups of 3 plants, named hereafter as samples.

For the antioxidant capacity assays, five samples from each treatment were dried in a ventilated oven at 60 °C (Memmert GmbH & Co. KG, Germany) until constant weight, whose procedure was already been verified not to have any effect on the antioxidant capacity results (Gomes et al., 2013). For quantification of lipophilic compounds, the remaining samples were frozen at −18 °C in polyethylene bags. Before analysis, plants were carefully homogenised in food processors (Silvercrest, Germany), and immediately sampled for the chemical analyses. Moisture was evaluated by oven drying at 103 ± 2 °C (WTC Binder, Germany).

2.3. Chemical analysis

2.3.1. Antioxidant activity

2.3.1.1. Extraction procedure. Triplicate amounts of dried lettuce samples (1 g) were macerated (1 h; at 60 °C), under stirring (25 mL; 30% methanol, v/v), as previously described by Gomes and coworkers (2013). The extracts were filtered through Whatman No. 42 paper and stored at −18 °C.

2.3.1.2. Radical scavenging activity. Lettuce samples were analysed for their capacity to scavenge the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, according to the method described by Hatano, Kagawa, Yasuhara, and Okuda (1988). Briefly, extract solution (0.3 mL) was mixed with DPPH radical methanolic solution (2.7 mL; 6×10^{-5} M), kept in the dark for 60 min and the absorbance measured at 517 nm (Genesys 10S UV–Vis Thermo Scientific, USA). DPPH radical scavenging effect was evaluated by the following equation: DPPH Radical Scavenging Effect (%) = $[(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, where A_S was the absorbance of the lettuce solution and A_{DPPH} the absorbance of DPPH control solution. Different concentrations of lettuce extract were prepared, allowing the determination of half maximal effective concentration (EC_{50}) values, which corresponded to the extract concentration that originates a DPPH scavenging effect equal to 50%.

Fig. 1. Distribution of lettuce samples in the greenhouse during the experiment.