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Coffee and spent coffee extracts protect against cell mutagens and inhibit growth of food-borne pathogen microorganisms

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ABSTRACT

Coffee consumption decreases the risk of oxidative stress-related diseases. The by-product obtained after brewing process (spent coffee) also has antioxidant capacity. Spent coffee and coffee brews (filter and espresso) extracts were obtained from Arabica and Robusta coffees, respectively. Spent coffee showed slightly high amounts in chlorogenic acids, but caffeine content was similar to their respective coffee brew. All samples exhibited strong protection activity against indirect acting mutagen 2-AF (≤92%), whereas the protection against NPD (direct mutagen) was 12–35% (Ames Test). The growth inhibition of common food-borne pathogen and food spoilage microorganisms by coffee extracts was also studied. Spent coffee showed antimicrobial activity, mainly against Gram-positive bacteria (*Staphylococcus aureus*, *Listeria monocytogenes*) and yeast (*Candida albicans*). The role of phenolic acids, caffeine and melanoidins in the antimutagenic and antimicrobial activities is discussed. Thus, spent coffee extracts could be a potential source of bioactive compounds, thereby becoming a promising new functional food ingredient.

1. Introduction

Coffee is a worldwide food product with a total production of 8,700,000 kg in 2013 (International Coffee Organization ICO, 2013). Several studies have positively linked coffee consumption with a decreased risk of oxidative stress-related diseases, such as cancer, cardiovascular ailments, and diabetes, among others; thus, coffee has been proposed as a potential functional food due to the presence of caffeine and phenolic compounds (Dorea & Da Costa, 2005). Because different brewing processes extract different amounts of bioactive compounds, the by-product generated after brewing processes, referred to as spent coffee, could partially retain some of the bioactive

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Abbreviations: CGA, chlorogenic acid; CQA, caffeoylquinic acid; DiCQA, dicaffeoylquinic acid; NPD, 4-nitro-*O*-phenylenediamine; 2-AF, 2-aminofluorene; MIC, minimum inhibitory concentration; CFU, colony forming unit

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compounds and consequently their health-related properties, and it could possibly be considered as a novel and sustainable functional ingredient. In a recent study, spent coffee was tested for its antioxidant capacity measured by using chemical-based assays and in *in vitro* cell cultures showing good capability for protecting against oxidation and DNA damage in human cells (Bravo, Arbillaga, De Peña, & Cid, 2013a; Bravo et al., 2012).

Nowadays, consumers are requesting safe food products with beneficial health effects. Therefore, the food industry is searching for new functional ingredients. The addition of bioactive compounds capable of preventing pathological conditions caused by DNA damages, such as cancer, might be a good strategy. Cancer is a leading cause of death worldwide (8.2 million deaths in 2012) (WHO, International Agency for Research on Cancer, 2012). Reactive oxygen species (ROS) from endogenous and exogenous sources induce DNA changes, which can lead to cell mutation (Klaunig & Kamendulis, 2004). Initial studies on coffee found potential mutagenicity of coffee, although excessively heated brewed coffee samples (Kato, Hiramoto, & Kikugawa, 1994) or extremely high coffee concentrations (Duarte et al., 1999) were used for these analyses. In addition, non-physiological doses of coffee compounds such as melanoidins or caffeine have been associated with a prooxidant effect (Azam, Hadi, Khan, & Hadi, 2003; Caemmerer et al., 2012). On the contrary, a small amount of coffee has a strong protective effect against oxidants (Stadler, Turesky, Müller, Markovic, & Leong-Morgenthaler, 1994). Moreover, fruits, vegetables or herbs with antioxidant properties and genoprotective effects have shown antimutagenic effects (Edenharder, Sager, Glatt, Muckel, & Platt, 2002).

Another consideration that is essential in the development and production of foods, including functional foods, is food safety. The European Food Safety Authority (EFSA) reported high rates of outbreaks per population (1.2 per 100,000) in the EU in 2011 (EFSA, (European Food Safety Authority), ECDC (European Centre for Disease Prevention and Control), 2013), commonly caused by *Escherichia coli*, *Salmonella*, *Bacillus*, *Shigella* and *Staphylococcus aureus*, among others. *Listeria monocytogenes* is a major risk concern; in 2011, approximately 90% of the cases resulted in hospitalization and the fatality rate was 10% (EFSA, (European Food Safety Authority), ECDC (European Centre for Disease Prevention and Control), 2013). In addition to health consequences, microorganisms may cause food spoilage that can result in considerable economic loss to producers and consumers. The food industry commonly uses preservatives, preferably naturally occurring, to prevent microbial growth. Coffee has shown antimicrobial activity against a broad range of microorganisms, including foodborne pathogens (Almeida et al., 2012; Daglia, Cuzzoni, & Dacarro, 1994; Martínez-Tomé et al., 2011), but to the best of our knowledge, the antimicrobial activity of spent coffee has not yet been evaluated.

The main aim of this study was to assess the potential antimutagenic and antimicrobial activity of spent coffee extracts due to the presence of high amounts of bioactive compounds in order to suggest their use as natural functional food ingredients. Therefore, the protection of spent coffees and their respective coffee brews against acting mutagens (Ames Test), as well as the capability to act as a food preservative inhibiting the growth of a broad range of foodborne pathogens and food spoilage microorganisms, has been evaluated.

2. Material and methods

2.1. Preparation of coffee brews and spent coffee extracts

Roasted coffee from Guatemala (*Coffea arabica*, referred to as Arabica, 3.03% water content, $L^* = 25.40 \pm 0.69$, roasted at 219 °C for 905 s) and Vietnam (*Coffea canephora* var. robusta, referred to as Robusta, 1.59% water content, $L^* = 24.92 \pm 0.01$, roasted at 228 °C for 859 s) was provided by a local factory. Coffee beans were ground for 20 s using a grinder (Moulinex super junior "s", Paris, France). The L^* value was analyzed by means of a tristimulus colorimeter (Chromameter-2 CR-200, Minolta, Osaka, Japan), using the D65 illuminant and CIE 1931 standard observer.

Filter coffee brew was prepared with an Ufesa Avantis coffeemaker (24 g coffee/400 mL water, 6 min at 90 °C). Espresso coffee brew was prepared with a Saeco Aroma coffeemaker (7 g coffee/40 mL, 24 s at 90 °C). Coffee residues, called spent coffee, were dried for 2 h at 102 ± 3 °C in a JP Selecta oven (Barcelona, Spain) and defatted with petroleum ether (1:11, w/v) for 3 h at 60 °C in a Soxhlet extraction system (Büchi, Flawil, Switzerland). Next, 24 g of spent coffee was extracted with 400 mL of water using a filter coffeemaker (6 min at 90 °C). Both coffee brews and spent coffee extracts were lyophilized using a Cryodos Telstar (Terrassa, Spain).

2.2. Chlorogenic acids (CGA) and caffeine HPLC analysis

Extract preparation and cleanup were carried out on a C_{18} Sep-Pak cartridge (Millipore Waters, Milford, MA, USA) according to Bicchi, Binello, Pellegrino, and Vanni (1995). Briefly, an aliquot of the sample (6 mL) was loaded onto the cartridge, previously conditioned with MeOH (5 mL) and Milli-Q water (3 mL). The cartridge was then eluted with 20 mL of MeOH/Milli-Q water (40/60). The compounds were analyzed by HPLC following the method described by Farah, De Paulis, Trugo, and Martin (2005), with some modifications (Bravo et al., 2012). Briefly, 100 μL of sample was injected into an analytical HPLC unit model 1100 (Agilent Technologies, Palo Alto, CA, USA) equipped with a reversed-phase Poroshell 120 C-18 (2.7 μm particle size, 250 × 4.6 mm) column at 25 °C. The chromatographic separation was performed using a gradient of methanol (Panreac, Barcelona, Spain) (solvent A) and Milli-Q water acidulated with phosphoric acid (pH 3.0, solvent B) at a constant flow of 0.8 mL/min as described by Bravo et al. (2012). Chromatograms were recorded at 325 nm for chlorogenic acids (CGA) and 276 nm for caffeine. Individualized identification of chlorogenic acid (3-, 4- and 5-caffeoylquinic acids and 3,4-, 3,5-, and 4,5-dicaffeoylquinic acids) and caffeine was carried out by comparing the retention time and the photodiode array spectra with those of their reference standard compounds. Pure reference standards of 5-caffeoylquinic acid (5-CQA) and caffeine were obtained from Sigma-Aldrich (St. Louis, MO, USA), and pure reference standards of 3,4-, 3,5-, and 4,5-dicaffeoylquinic acids were purchased from Phytolab (Vestenbergsgreuth,