LWT - Food Science and Technology 61 (2015) 12–18

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Revalorization of coffee by-products. Prebiotic, antimicrobial and antioxidant properties

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ARTICLE INFO

Article history:
Received 11 August 2014
Received in revised form
1 November 2014
Accepted 16 November 2014
Available online 26 November 2014

Keywords:
Coffee by-products
Coffee melanoidins
Prebiotic activity
Antimicrobial activity
Antioxidant capacity

ABSTRACT

Coffee industries are a key sector in the global economy due to income reporting and job creation. Coffee companies produce annually more than 2 billion tons of by-products such as coffee spent grounds (CSG) and coffee silverskin (CS). And most of which are thrown away and are not recycled for other purposes. Here we show the prebiotic, antimicrobial and antioxidant capacity of CSG and CS, as well as those melanoidins (a coffee component generated during the roasting process) obtained from the former. The prebiotic activity was important in both CSG and CS, although the presence of coffee melanoidins (CM) interfered with such beneficial properties. On the contrary, CM exerted an intense antimicrobial activity that could be used to avoid the growth of pathogenic bacteria in food products. In addition, CSG, CS and CM were highly antioxidant, even their indigestible fraction, which were the most relevant. Finally, we found that the addition of sugar during coffee roasting, namely torrefacto, increased the antioxidant and antimicrobial activity due to a larger generation of CM, although prebiotic activity was not affected. Therefore, CSG, CS and CM should be recycled in order to be used as a source of new food ingredients.

1. Introduction

Coffee is one of the most valuable primary products in world trade due to the high consumption of coffee beverage (Mussatto, Machado, Martins, & Teixeira, 2011). Due to the great production of this food, large amounts of residues are generated in the coffee industry. The main generated by-products are coffee spent grounds (CSG) and coffee silverskin (CS), which represent serious environmental problems due to their toxic nature against plants and microorganisms living in the soil (Nabais et al., 2008). CSG are obtained both during instant coffee production and as a result of coffee beverage consumption in restaurants, coffee shops, etc. (Mussatto, Carneiro, Silva, Roberto, & Teixeira, 2011). In addition, CS is produced as a result of the beans roasting.

CSG is a by-product with fine particle size, high organic load and humidity while CS is a residue with high concentration of soluble dietary fiber. Their chemical composition is based on cellulose, hemicelluloses, proteins, fat, polyphenols, minerals and different products formed by the Maillard reaction during the roasting process, such as melanoidins (Borrelli, Esposito, Napolitano, Ritieni, & Foglianao, 2004). The presence of organic material makes CSG and CS highly pollutant due to a great demand of oxygen to be degraded (Silva, Nebra, Machado-Silva, & Sanchez, 1998). Even more, caffeine, polyphenols or tannins confer a toxic nature to these by-products, especially for the soil ecosystem, representing a pollution hazard when discharged into landfields (Cruz et al., 2012; Mussatto, Machado, et al., 2011; Ricardo, Páscoa, & Magalhaes, 2013). Contrary, recent studies report considerable evidence of health benefits for healthy adults as a result of moderate coffee consumption, partially attributed to the antioxidant activity of polyphenols (Butt & Sultan, 2011; Vignoli, Bassoli, & Benassi, 2011).

CSG can be used for pellets production due to its high calorific power (around 5000 kcal/kg) as stated by Silva et al. (1998). Other valuable alternatives are biodiesel production, H_2 and ethanol (Kondamudi, Mohapatra, & Misra, 2008), as well as the use of CSG as a substrate for fermentation technology (Ramalakshmi, Rao, Takano-Ishikawa, & Goto, 2009). Regarding CS, it is also a good source of nutrients during fructooligosaccharides and

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http://dx.doi.org/10.1016/j.lwt.2014.11.031
0023-6438/

fructofuranosidase production by fermentation (Mussatto & Teixeira, 2010). In addition, CS has been proposed as a new potential functional ingredient due to the prebiotic and antioxidant capacity (Borrelli et al., 2004). Finally, coffee melanoidins (CM), high-molecular weight compounds formed during coffee roasting, have been also demonstrated as potential food ingredients due to their antioxidant (Rufián-Henares & Morales, 2007a), antimicrobial (Rufián-Henares & de la Cueva, 2009) or antihypertensive activities (Rufián-Henares & Morales, 2007a,b).

Nevertheless, there is a lack of information regarding the potential effect of unprocessed CSG, CS and CM as food ingredients, both in a joint manner or separately. Considering the large amount yearly produced, we evaluated the *in vitro* antimicrobial, prebiotic and antioxidant activities of the combination of CSG, CS and CM. These products behave as non-digestible ingredients that selectively stimulated the growth of limited number of bacteria in a separate manner, while the joint prebiotic activity was limited due to strong antimicrobial activity of CM. We finally also evaluated the nutritional composition of three different commercial coffees by-products.

2. Material and methods

2.1. Reagents

6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox®), potassium persulphate, ethanol, methanol, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,3,5-triphenyltetrazolio chloride (TPTZ), hydrochloric acid, sodium acetate, anhydrous ferric chloride, alpha amylase (A1031-5KU), pepsin, pancreatin and bile salts were from Sigma–Aldrich (Germany). Other reactives for antimicrobial activity were also from Sigma–Aldrich (Germany). Authentic reference standards of 5-caffeoylquinic acid (5-CQA), 3-CQA, 4-CQA, 3,4-, 3,5-, and 4,5-dicaffeoylquinic acids were purchased from Sigma–Aldrich.

2.2. Samples

Three different types of coffee consumed regularly in Spain were provided by a Spanish coffee roaster: regular coffee (C), torrefacto coffee 20% (T_{20}) and torrefacto coffee 100% (T_{100}). T_{20} was composed by 80% of regular coffee +20% of T_{100}. Regular coffee was an Arabica medium roasted coffee, while torrefacto coffee is a special type of coffee highly consumed in Spain obtained after roasting regular coffee in the presence of sugar. From these different types of coffees, three coffee by-products were obtained as follows:

- CS: coffee silverskin is produced by roasting coffee beans (*Coffea arabica*) and obtained from Cafés Cumbal (Granada, Spain). CS easily peels off roasted coffee beans in the roasting process of green coffee beans. CS separated from roasted coffee beans in the iron pot of the roasting machine was collected by aspiration of air to another container. The yield of CS obtained was 1.1% of the weight of roasted coffee beans.
- CSG: coffee spent grounds were obtained as the main residue after coffee brewing. Briefly, 50 g of ground roasted coffee (C, T_{20} or T_{100}) was extracted with 1000 ml of water using a filter coffeemaker (Cucina, Philips, Netherlands) for 5 min at 92 °C. CSG were lyophilized to obtain a dry residue (LyoQuest −80, Telstar, Spain). CSG+CM were the spent grounds obtained after coffee brew obtaining. In the case of CSG-CM, coffee melanoidins were removed by ultrafiltration. The mean yield of CSG+CM obtained was 82.4% from ground roasted coffee (regular, T_{20} and T_{100}) and 60.8% for CSG-CM.
- CM: coffee melanoidins were obtained by ultrafiltration and subsequent lyophilization as stated by Pastoriza and Rufián-Henares (2014). Ultrafiltration was performed by means of a peristaltic pump (Masterflex LS), connected with PVC tubes to a 5 kDa polyetilensulfone membrane (Vivaflow 200, Sartorius, Germany) working at a pressure of 3 bars and a recirculation stream of 300 ml/min. Fifty grams of CSG were extracted with 5 l of deionized water at 50 °C. The retentate, enriched with melanoidins, was washed three times with distilled water and lyophilized. The solid CSG-CM fraction was also lyophilized. The mean yield of CM obtained was 21.6% of the weight of roasted coffee beans (regular, T_{20} and T_{100}).

2.3. In vitro digestion

CSG, CS and CM were submitted to *in vitro* gastrointestinal digestion following the technique described by Pastoriza, Delgado-Andrade, Haro, and Rufián-Henares (2011) partially modified in order to include a fermentation step following the procedure of Borrelli et al. (2004). Briefly, samples were digested consecutively with a-amylase, pepsin, pancreatin and bile salts. The insoluble fraction plus a 15% of the soluble fraction were submitted to a 24-h fermentation step with the faecal slurry of three healthy human donors under strictly anaerobic conditions. Once finished, the bioaccesible (soluble) and the non-bioaccesible (or insoluble) fractions of the samples were separated. The digestion protocol was applied in triplicate to each sample.

2.4. Prebiotic activity

The ability of bacteria to utilize CSG, CS or CM as carbon source was performed as described by Borrelli et al. (2004). After the digestion-fermentation step, the growth of different bacterial strains was assessed by qRTi-PCR by the method of Muros et al. (2014). For DNA extraction, the QIAamp DNA Stool Mini Kit (Qiagen, Germany) was used according to the manufacturer's instructions after diluting the stool contents 1:10 (w/v) in PBS. DNA was eluted in buffer AE (provided in the kit), and then purified DNA extracts were stored at −20 °C. A series of genus-specific primer pairs were used (Muros et al., 2014). PCR amplification and detection was performed in an Eco Illumina thermocycler (Eco Illumina, USA) as follows. Each reaction mixture (10 μL) was composed of 5 μL of KAPA SYBR Fast Master Mix (Kapa Biosystems, USA), 0.25 μL of each specific primer (at a concentration of 10 μM) and 2 μL of template DNA. Standard curves were created using serial 10-fold dilutions of bacterial DNA extracted from pure cultures with a bacterial population ranging from 2 to 9 log10 CFUs, as determined by plate counts. One strain belonging to each of the bacterial genera or groups targeted in this study was used to construct the standard curve. More specifically, the strains from which the DNA was extracted were the following: *Bifidobacterium longum* CECT 4551, *Clostridium coccoides* DSMZ 935, *Bacteroides fragilis* DSMZ 2151, *Lactobacillus salivarius* CECT 2197. All of them were obtained from the Spanish Collection of Type Cultures (CECT) or the German Collection of Microorganisms and Cell Cultures (DSMZ).

2.5. Antimicrobial activity

The antimicrobial activity of CSG, CS and CM was assessed by the method of Rufián-Henares and Morales (2008a). Briefly, overnight suspensions of *Escherichia coli* American Type Culture Collection (ATCC) 11775 and *Staphylococcus aureus* subsp. *aureus* (ATCC 6538P) were growth at 37 °C in BHI until a concentration of 10^9 colony forming units (cfu)/ml was reached. Culture of bacteria were