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Spent coffee grounds, an innovative source of colonic fermentable compounds, inhibit inflammatory mediators *in vitro*

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ABSTRACT

Spent coffee grounds (SCG), rich in dietary fiber can be fermented by colon microbiota producing short-chain fatty acids (SCFAs) with the ability to prevent inflammation. We investigated SCG anti-inflammatory effects by evaluating its composition, phenolic compounds, and fermentability by the human gut flora, SCFAs production, nitric oxide and cytokine expression of the human gut fermented-unabsorbed-SCG (hgf-NDSCG) fraction in LPS-stimulated RAW 264.7 macrophages. SCG had higher total fiber content compared with coffee beans. Roasting level/intensity reduced total phenolic contents of SCG that influenced its colonic fermentation. Medium roasted hgf-NDSCG produced elevated SCFAs (61:22:17, acetate, propionate and butyrate) after prolonged (24 h) fermentation, suppressed NO production (55%) in macrophages primarily by modulating IL-10, CCL-17, CXCL9, IL-1β, and IL-5 cytokines. SCG exerts anti-inflammatory activity, mediated by SCFAs production from its dietary fiber, by reducing the release of inflammatory mediators, providing the basis for SCG use in the control/regulation of inflammatory disorders. The results support the use of SGC in the food industry as dietary fiber source with health benefits.

1. Introduction

In 2012–13, 146 million bags (60 kg each) of coffee provided an estimated 4 billion of coffee cups consumed daily in the world (International Coffee Organization (ICO), 2014), generating large quantity of by-products during processing. Spent coffee grounds (SCG), the residue obtained after brewing, are rich in polysaccharides, proteins, phenolic compounds, melanoidins and dietary fiber (DF) (Campos-Vega, Loarca-Piña, Vergara-Castañeda, & Oomah, 2015). Spent coffee-dietary fiber (SCDF) with 84% insoluble and 16% soluble dietary fiber relative to total dietary fiber, exhibit antioxidant properties and can be categorized as antioxidant dietary fiber (Campos-Vega, Loarca-Piña, et al., 2015; Campos-Vega, Vázquez-Sánchez, et al., 2015). These dietary fibers strongly

Abbreviations: ABTS, 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid); AC, Antioxidant capacity; CB, Coffee beans; CBDR, Coffee bean dark roasted; CBMR, Coffee bean medium roasted; DF, Dietary fiber; DPPH, 2,2-diphenyl-1-picrylhydrazyl; DR, Dark roasted; FBS, Fetal bovine serum; GI, Gastrointestinal; GO, Gene ontology; hgf-NDSCG, Human gut fermented-unabsorbed-SCG; HPLC-DAD, High-performance liquid chromatography-diode array detection; ICO, International Coffee Organization; IDF, Insoluble dietary fractions; IL, Interleukin; KEGG, Kyoto encyclopedia of genes and genomes; LPS, Lipopolysaccharides; MR, Medium roasted; MTT, Methylthiazolyltetrazolium; NDSCG, Unabsorbed spent coffee grounds; NO, Nitric oxide; SCDF, Spent coffee-dietary fiber; SCFAs, Short-chain fatty acids; SCG, Spent coffee grounds; SCGDR, Spent coffee grounds dark roasted; SCGMR, Spent coffee grounds medium roasted; SDF, Soluble dietary fractions; TDF, Total dietary fiber; TNF-α, Tumor necrosis factor-α.

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associated/bound to tannins are metabolized primarily during colonic fermentation and subsequently absorbed and distributed to tissues with potential health benefits (Campos-Vega, Vázquez-Sánchez, et al., 2015). Fermentation of dietary fiber from SCDF with human gut flora can potentially release short chain fatty acids (SCFAs), similar to those of nondigestible bean fraction that modulates genes and proteins involved in the anti-inflammatory process/pathway (Campos-Vega et al., 2012). SCFAs affect different cells involved in the inflammatory and immune responses. They modulate the function of leukocytes (e.g., production of inflammatory mediators and ability of leukocytes to migrate) and induce apoptosis in lymphocytes, macrophages and neutrophils (Vinolo, Rodrigues, Nachbar, & Curi, 2011).

The physiological potential and health benefits of SCG have not been adequately investigated although it is a rich antioxidant and DF (~60%) source, and has been proposed as a healthy bakery ingredient for the general population and for people with special nutritional requirements (Campos-Vega, Vázquez-Sánchez, et al., 2015; Galanakis, Martinez-Saez, del Castillo, Barba, & Mitropoulou, 2015). This study aimed at evaluating the nutraceutical and anti-inflammatory activity of bioactive compounds from unabsorbed spent coffee grounds (NDSCG) fraction. *In vitro* gastrointestinal assay and colonic fermentation were used to determine the fate of SCFAs and the anti-inflammatory activity was evaluated on lipopolysaccharide (LPS)-induced mouse RAW 264.7 macrophages.

2. Materials and methods

2.1. Materials

Roasted coffee arabica beans (medium- and dark-roasted), purchased directly from the manufacturer were grown and harvested in the state of Chiapas, México. Butyrate was purchased from Fluka (Sigma-Aldrich Canada Ltd., Oakville, ON, Canada). Vanillin, D-(+)-raffinose, and (+)-catechin were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Acetate, propionate and formic acid, and other chemicals were purchased from Sigma Chemical Co and J. T. Baker (México City, México).

2.2. Spent coffee grounds preparation

Arabica spent coffee grounds were recovered from the filter of a coffeemaker (MOULINEX, Heliora comfort, México) after 6 min brewing (7:100, w/v) at 90 °C. The SCG were freeze-dried, defatted (Soxhlet extraction, petroleum ether; 6 h) and then stored in the dark until use.

2.3. Proximate composition

AOAC procedures were used to determine moisture (method 925.10), lipid (method 920.39), ash (method 923.03), and nitrogen (method 920.87) contents of the ground bean samples (coffee beans and SCG) (Association of Official Analytical Chemists, 2002). Moisture was assessed based on weight loss after oven drying at 105 °C until constant weight was reached. Nitrogen content was determined using the micro-Kjeldahl method with sodium sulfate as catalyst. Protein content was calculated as nitrogen × 6.25. Lipid content was obtained from Soxhlet extraction (6 h) with petroleum ether. Ash content was calculated from the weight of the sample after incineration in a muffle furnace at 550 °C for 2 h. Carbohydrate values were obtained by difference.

2.4. Total dietary fiber (TDF) and resistant starch

Dietary fiber fractions, containing soluble dietary fractions (SDF), and insoluble dietary fractions (IDF) were determined following the enzymatic-gravimetric method of Shiga, Lajolo, and Filisetti (2003). Resistant starch was quantified following the gravimetric method of Saura-Calixto, Goñi, Bravo, and Mañas (1993) described briefly in our earlier study (Campos-Vega et al., 2009).

2.5. Polyphenols extraction

Microwave extraction was performed by the modified method described previously (Campos-Vega, Vázquez-Sánchez, et al., 2015). Spent coffee grounds and 20% ethanol solution (1:9 solid/liquid ratio) were heated in 100 mL Erlenmeyer flasks at 80 W for 20 s in a microwave oven (LG MS-1145KYL); cooled, then re-extracted under the same conditions. Extracts were centrifuged (5000 rpm, 10 min, 4 °C; Hermle Z 323 K, Hermle Labortechnik GmbH), and the recovered supernatant was filtered (45 μm) for HPLC.

2.6. Analysis of polyphenols compounds and caffeine by HPLC-DAD

Polyphenols and caffeine were analyzed by HPLC following the method described by Ramírez-Jiménez, Reynoso-Camacho, Mendoza-Díaz, and Loarca-Piña (2014) with an Agilent 1100 Series HPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a binary pump and an automated sample injector. An Agilent reversed-phase Zorbax Eclipse XDB C-18 (5 μm particle size, 250 × 4.6 mm) column was used at 35 °C. The chromatographic separation was performed using a gradient of Milli-Q water acidulated with acetic acid (pH 3.0) (solvent A) and acetonitrile, (solvent B) at 1 mL/min constant flow rate. Detection was accomplished with a diode array detector (DAD) and chromatograms were recorded at 280 nm. Quantification was performed using the external standard method with commercial standards of (+)-catechin, quercetin, rutin, caffeine, and caffeic, chlorogenic, *p*-coumaric, ellagic, ferulic and gallic acids.

2.7. In vitro gastrointestinal digestion

The adapted method of Campos-Vega, Vázquez-Sánchez, et al. (2015) was followed to mimic physiologic conditions. Briefly, four healthy volunteers, who had consumed their last meal at least 90 min prior to the test, were recruited. All participants provided written informed consent prior to participating in the study. The subjects chewed the test products under standardized conditions after brushing their teeth without toothpaste. Spent coffee grounds (1 g) were chewed 15 times for ~15 s. After chewing, the product was expectorated into a beaker containing 5 mL of distilled water. The subjects rinsed their mouths with another 5 mL of distilled water for 60 s. Subsequently, the suspensions of each sample were mixed in a single vessel and an aliquot (10 mL) was adjusted to pH 2 using HCl solution (150 mM, 2·81 ml). Pepsin (0.055 g, Sigma) dissolved in 0.94 ml of 20 mM HCl was added to each sample and incubated for 2 h at 37 °C. An intestinal extract was prepared 30 min before use by dissolving 3 mg of gall Ox (bile salts) and 2.6 mg pancreatin (enzymatic components including trypsin, amylase and lipase, ribonuclease, and protease) in 5 ml Krebs-Ringer buffer [118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 11 mM glucose and 2.5 mM $CaCl_2$, pH 6.8]. This solution (5 ml) was added to each sample; the suspension (15 ml) was transferred to a vessel containing an everted gut sac, prepared from male Wistar rats (body wt. 250–300 g, n = 6). Prior to the surgical procedure, the rats were fasted overnight (16–20 h) with water *ad libitum*. The rats were anesthetized with pentobarbital sodium (60 mg/kg, i.p.). The intestine of the rats was exposed by a midline abdominal incision and a 20–25 cm segment of the proximal rat jejunum was excised and placed in the gasified (CO_2) buffer solution Krebs-Ringer at 37 °C. The intestine was