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Effect of coffee roasting on in vitro α-glucosidase activity: Inhibition and mechanism of action

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ABSTRACT

In vitro α-glucosidase inhibitory activity of unroasted, and medium, dark and very dark roasted robusta coffee was studied. Coffee extracts significantly inhibited the enzyme activity in a dose-dependent way. The inhibitory activity was well correlated with the degree of roast. Coffee components were separated by gel permeation chromatography into low (1 < MW < 6 kDa), intermediate (15 < MW < 60 kDa) and high (MW > 100 kDa) molecular weight fractions, which were analyzed for the α-glucosidase inhibitory capacity. Only fractions obtained from dark and very dark roasted coffee exhibited inhibitory effect. When the same fraction was obtained from coffee presenting different roasting degree, changes in α-glucosidase inhibition extent were observed. This was attributed to compositional changes within each fraction as induced by roasting. Coffee extracts and their fractions exerted a mixed-type to competitive inhibition against α-glucosidase and these mechanisms are consistent with the complexity of coffee composition.

1. Introduction

Coffee has been consumed over countless centuries and currently belongs to the most marketed food items (Esquivel & Jiménez, 2012). The brew obtained from coffee beans contains a huge number of compounds, which are mainly produced during roasting, upon chemical modification of green coffee composition (Hečimović, Belščak-Cvitanović, Horžić, & Komes, 2011; Moreira, Nunes, Domingues, & Coimbra, 2012). Roasted coffee composition depends on roasting time and temperature, whose choice allows obtaining coffees with different degree of roast. The latter are appreciated for their flavour as well as physiological and psychoactive effects (Dórea & Da Costa, 2005; Hečimović et al., 2011; Yanagimoto, Ochi, Lee, & Shibamoto, 2004). Roasting leads to the formation of many bioactive compounds, thus affecting not only coffee sensory properties but also its beneficial health effects. The latter allow defining coffee as a functional food (Budryn et al., 2017; Dórea & Da Costa, 2005; Hečimović et al., 2011; Ludwig, Clifford, Lean, Ashiharad, & Crozier, 2014; Wang, Qian, & Yao, 2011). For instance, the melanoidins developed upon Maillard reaction demonstrated antioxidant, antimicrobial and anti-inflammatory properties (Morales, Somoza, & Fogliano, 2012). Coffee consumption was also associated to a reduced incidence of Parkinson, Alzheimer, cardiovascular diseases and cancer (Baspinar, Eskici, & Ozcelik, 2017; Hu, Bidel, Jousilahti, Antikainen, & Tuomilehto, 2007; Kleemola, Jousilahti, Pietinen, Vartiainen, & Tuomilehto, 2000; Lindsay et al., 2002). Several epidemiological studies highlighted that a moderate and prolonged coffee consumption also contributed to the reduction of type 2 diabetes risk (Lecoultre et al., 2014; Van Dam & Hu, 2005; Van Dijk et al., 2009). This effect was associated with the ability of some compounds contained in coffee to reduce the blood glucose level, thus promoting a hypoglycemic effect (Johnston, Clifford, & Morgan, 2003; Krebs, Parry-Strong, Weatherall, Carroll, & Downie, 2012; Salazar-Martinez et al., 2004; Shearer et al., 2003). Despite some information about the action site of these compounds is available, their mechanism of action has not been clarified yet. In particular, chlorogenic acids (Iwai et al., 2012; Johnston et al., 2003; Lecoultre et al., 2014), caffeic acid and quercetin (Murase et al., 2012), trigonelline (Hamden, Bengara, Amri, & Elfeki, 2013; Van Dijk et al., 2009), and Amadori compounds (Ha et al., 2011), can cause the inhibition of α-glucosidase. This enzyme is a hydrolase located on the intestinal cell membrane of the ciliate epithelium and is required to obtain glucose from oligo- and disaccharides (Chiba, 1997). α-Glucosidase plays thus a key role in the final step of carbohydrates digestion and its inhibition currently represents a common therapeutic approach to reduce postprandial hyperglycemia (Goto et al., 2012; Hu, Wang, & Kong, 2013; Kwon, Apostolidis, & Shetty, 2008). The inhibitory effect is obtained by the so-called glycomimetic drugs (i.e.

Abbreviations: 3-CQA, 3-caffeoylquinic acid; 5-CQA, 5-caffeoylquinic acid; CGA, chlorogenic acids; HMW, high molecular weight; I, inhibitor; IMW, intermediate molecular weight; LMW, low molecular weight; S, substrate; TP, total phenols

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acarbose, voglibose, miglitol) that are able to interact with the enzymatic active site (Akkarachiyasit, Charoenlertkul, Yibchok-Anun, & Adisakwattana, 2010; Dubois, 2014). Nonetheless, these drugs carry undesired side effects, such as weight increase, hypoglycemia and gastrointestinal diseases (Cheng & Fantus, 2005). To avoid these undesired effects, researchers are looking for natural α-glucosidase inhibitors, which could represent a valuable option to artificial drugs (Cheng & Fantus, 2005; Kwon et al., 2008; Ríos, Francini, & Schinella, 2015). According to Ludwig, Clifford, et al. (2014), coffee consumption can reduce by 60% diabetes risk and this value is in the same range as observed with pharmacological approaches. Kim (2015) demonstrated that a β-carboline alkaloid norharman isolated from coffee inhibits α-glucosidase with an uncompetitive mechanism. However, little is known about the inhibitory effect carried out by the whole coffee brew (Singh et al., 2014). The latter consists of a complex mix of compounds, whose interaction may modify the overall effect towards α-glucosidase (Farah, De Paulis, Trugo, & Martin, 2005). Further, different roasting conditions substantially modify coffee composition and this may potentially affect the α-glucosidase inhibitory effect.

Therefore, the aim of this study was to investigate the effect of different roasting degrees on the in vitro inhibitory capacity against α-glucosidase, exhibited by coffee extracts and their fractions. Furthermore, the mechanism of inhibition carried out by the extracts and fractions was studied.

2. Materials and methods

2.1. Sample preparation

Coffee samples were prepared by using green coffee beans from the species *Coffea canephora* var. *robusta* Pierre ex Froehn *(Rubiaceae)*, from Vietnam. Coffee was roasted in an air circulation oven (TC 40 Thermocenter, Salvis, Reussbühl, Switzerland) at 200 °C during 15, 45 and 60 min. After the treatments, samples were immediately removed from the oven, cooled to room temperature and left to stand at room temperature for 12 h. The roasted samples were ground in a mill (Moulinex mod. 505, Paris, France) and sieved through a 35 Mesh sieve. Afterwards, coffee powders were transferred into plastic vessels with pressure lid and stored at −18 °C until analyses were performed. Coffee brews were prepared by solid-liquid extraction with milli-Q deionized water of the ground green and roasted beans. The ratio between coffee powder and water was 1:8 (*w*/w). The coffee-water mixture was boiled for 5 min under stirring at 300 rpm (Yellow line magnetic IKA, Staufen, Germany), cooled, filtered through filter paper and freeze-dried (Laboratory and Pilot Freeze Dryer Mini-fast, mod. 1700, Edwards Alto Vuoto, Milano, Italy). Freeze-dried material was added with a mixture of methanol and water (1,9 *v*/v) to obtain 0.4 g/mL coffee extracts which were used for further analyses.

2.2. Coffee fractions separation

The method of Borrelli, Visconti, Mennella, Anese, and Fogliano (2002) was followed. A HPLC system (LC-10AT VP, Shimadzu Corporation, Kyoto, Japan) equipped with a UV/VIS detector (SPD-10 AT VP, Shimadzu Corporation, Kyoto, Japan) was used. The coffee extract was filtered (Whatman 0.45 μm) and loaded onto a Sephadex G-25 gel filtration chromatography column (60 × 1.1 cm i.d.; Pharmacia, Uppsala, Sweden). The injection volume was 20 μL and the mobile phase, delivered at a flow rate of 1.2 mL/min, was milli-Q water. The detection wavelength was 280 nm. Peak integration was performed by Polyview 2000 software (Ver. 5.3, Varian, Texas, USA). In according with Borrelli et al. (2002) four fractions were collected: fraction I, containing high-molecular-weight (HMW) material, indicated as coffee melanoidins; fractions II and III, containing intermediate-molecular-weight (IMW) compounds; and fractions IV, containing small compounds such as low-molecular-weight (LMW) phenols. Since fractions II and III both contained IMW compounds, in the present experiment they were mixed together. Thus, three fractions, i.e. high, intermediate and low molecular weight, were obtained from each coffee sample. The coffee fractions were freeze-dried and stored in a desiccator, until analyses were performed. Prior to analyses, freeze dried fractions were added with a mixture of methanol and water (1:9 *v*/v) to obtain coffee fractions with a concentration of 0.1 g/mL.

2.3. Weight loss and total solid content determinations

Sample weight loss upon roasting was calculated as the percentage weight difference between the initial and final weights of the coffee beans. Total solid content was determined by gravimetric method (AOAC, 1995).

2.4. Color analysis

Color analysis was carried out on ground coffee using a tristimulus colorimeter (Chromameter-2 Reflectance, Minolta, Osaka, Japan) equipped with a CR-300 measuring head. The instrument was standardized against a white tile before measurement. Color was expressed in CIE units as L* (lightness/darkness), a* (redness/greenness) and b* (yellowness/blueness). The parameters a* and b* were used to compute the hue angle ($\tan^{-1}$ b*/a*) (Clydesdale, 1978).

2.5. Phenolic compounds quantification

Chromatographic quantification of chlorogenic acids was performed on coffee extracts and fractions following the method proposed by Hečimović et al. (2011) modified according to Llorach, Tomás-Barberán, and Ferreres (2004). A HPLC pump (LC-10AT VP, Shimadzu Corporation, Kyoto, Japan) equipped with a diode array detector (SPD-10 AT VP, Shimadzu Corporation, Kyoto, Japan) and an inverse phase apolar C18 column (5 μm, 250 × 4.6 mm, Alltima, Lokeren, Belgium) were used. The injection valve (Rheodyne, Sigma-Aldrich, Milano, Italy) was equipped with a 20 μL plastic loop and samples were injected using a syringe (SGE LC, 100 μL, FN). The elution was in gradient mode using a mixture of 5% formic acid (Sigma-Aldrich, Milano, Italy) in water (solvent A) and methanol (Sigma-Aldrich, Milano, Italy) (solvent B) as mobile phase at a flow rate of 1 mL/min. Gradient was set as follows: solvent A was held at 90% for the first 25 min, decreased to 80% and held at this level for 15 min; then decreased further to 50% and held for 5 min; finally, 90% solvent B was reached and held for 15 min. The detection was conducted at 280, 335 and 350 nm. Quantification was carried out using external standards. Calibration curves were linear ($R^2 > 0.995$) in the 2.0 to 200.0 mg/L concentration interval. Peaks integration was performed by using Polyview 2000 software (Ver. 5.3, Varian, Texas, USA). Total phenolics (TP; i.e., the overall number of phenolic compounds) was computed by summing up the concentration of phenolic compounds detected at 280, 335 and 350 nm. TP, chlorogenic acids (CGA), 3-caffeoylquinic acid (3-CQA) and 5-caffeoylquinic acid (5-CQA) concentrations were expressed as mg/g_{dm} of the coffee extract.

2.6. α-Glucosidase inhibition assay

The inhibitory activity of coffee extracts and their fractions against α-glucosidase was assessed spectrophotometrically (UV-2501PC, UV-VIS Recording Spectrophotometer, Shimadzu Corporation, Kyoto, Japan), following the method of Singh et al. (2014) with some modifications. Freeze-dried coffee extracts and fractions were diluted to proper concentrations with a mixture of methanol and deionized water (1:9 *v*/v). Different aliquots of coffee extracts (0.4 g/mL) and fractions (0.1 g/mL), or 3.33 mg/mL 3-CQA and 5-CQA (Sigma-Aldrich, Milano, Italy) and 0.004 mg/mL acarbose (Sigma-Aldrich, Milano, Italy) aqueous solutions were introduced in 1 mL capacity cuvettes in the