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Antiglycative and antioxidative properties of coffee fractions

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ABSTRACT

In this work the inhibitory activity of coffee low molecular weight compound (LMWC) and high molecular weight compound (HMWC) fractions against *in vitro* advanced glycation end-products (AGEs) formation was investigated. The HMWC fraction was characterised for its content in total phenolic groups, proteins and carbohydrates. The chlorogenic acids of LMWC fraction were identified by liquid chromatography coupled with tandem mass spectrometry. HMWC inhibited bovine serum albumin glycation by acting as radical scavenger and Fe-chelator in the post-Amadori phase of the reaction and by inhibiting dicarbonyl reactive compounds production during glucose autoxidation. LMWC fraction was able to inhibit protein glycation and dicarbonyl reactive compounds formation more than HMWC fraction. Chlorogenic acids are the main compounds responsible for the antiglycative activity of LMWC fraction.

This study clearly shows that coffee contains molecules with *in vitro* antiglycative activity, in particular chlorogenic acids, are of particular interest for their known bioavailability *in vivo*.

1. Introduction

In the past, a large number of scientific reports have suggested an important role of antioxidants in the prevention of chronic diseases (Perez et al., 2002). Amongst these, phenolic compounds have received considerable attention for their biological effects, such as antiglycative (Wu & Yen, 2005), antiatherogenic and cardioprotective (Dell'Agli, Buscialà, & Bosisio, 2004) activities. Another class of antioxidants receiving attention in recent years is that of melanoidins, polymeric brown compounds formed in the last stage of the Maillard reaction and involved in the colour and flavour of thermally-treated food. They are present in food and beverages such as coffee, beer, traditional balsamic vinegar, cocoa and bread (Borrelli & Fogliano, 2005; Rufián-Henares & Morales, 2007; Tagliazucchi, Verzelloni, & Conte, 2008).

Oxidative stress in humans has been linked to various diseases, such as cancer (Halliwell, 2007), atherosclerosis (Madamanchi, Hakim, & Runge, 2005) and diabetes (Thorpe & Baynes, 1996). Advanced glycation end-products (AGEs) are the result of a non-enzymatic glycation reaction between amino groups of proteins and aldehydic groups of reducing physiological sugars (Wautier & Guillausseasu, 2001).

Carboxymethyllysine (CML) is one of the most important products of the fragmentation of Amadori compounds, together with pentosidine (Peyroux & Sternberg, 2006). CML is present in plasma, renal tissue, retinas, and collagen of diabetic patients and its concentration in human tissue is proportional to age (Peyroux & Sternberg, 2006). Its presence has also been reported in intracellular neurofibrillary deposits, in patients affected with Alzheimer's disease (Cervantes-Lauren et al., 2006).

Protein glycation is a spontaneous reaction depending *in vivo* on the degree and duration of hyperglycaemia. AGEs build up slowly and can permanently alter proteins' structure and function; they accumulate mainly in proteins with long half-life, such as extracellular collagens and lens crystallin, altering their structural and biochemical properties. AGEs also contribute to reducing artery, heart and lung tissue elasticity and they seem to have a significant role in the progression of diabetic and atherosclerosis complications, Alzheimer's disease, neuropathy, nephropathy, and joint stiffness (Baynes & Thorpe, 2000; Cervantes-Lauren et al., 2006; Thorpe & Baynes, 1996). Another way of AGE formation is glucose autoxidation, through the generation of dicarbonyl compounds, such as glyoxal, which evolve to ketoimines and finally to AGEs (Wolff & Dean, 1987). During the first phase of the autoxidation and the degradation of Amadori to AGEs, metal ions, superoxide anion, hydrogen peroxide and hydroxyl radical intervene.

Coffee is a beverage consumed daily worldwide. Its consumption has been associated with reduction of chronic diseases risk and, in particular, type 2 diabetes (Van Dam & Hu, 2005). These positive properties may be due to naturally-occurring compounds present in the green beans (chlorogenic acids, trigonelline or caffeine) and/or to molecules formed during the roasting process (Maillard reaction products, melanoidins). The aim of this work was to study the inhibitory activity of coffee fractions against AGE formation.

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2. Materials and methods

2.1. Materials

2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), catechin, 5-caffeoylquinic acid, and bovine serum albumin (BSA) were supplied by Sigma (Milan, Italy). All other chemical reagents and solvents were supplied by Carlo Erba (Milan, Italy). Rabbit polyclonal antibody to carboxymethyllysine was supplied by Abcam (Cambridge Science Park, Cambridge, UK). Biotinylated anti-rabbit IgG, streptavidin–horseradish peroxidase conjugate and 4-chloro-1-naphthol were supplied by Calbiochem (Darmstadt, Germany). All other western blotting and electrophoresis reagents were from Biorad (Hercules, CA). Absorbance was read using a Jasco V-550 UV/Vis spectrophotometer (Orlando, FL). Fluorescence was read using a FLUOstar Optima microplate reader (BMG Labtech, Offenburg, Germany).

2.2. Sample preparation

Coffee was prepared using a Bialetti Italian moka (coffee maker). Coffee was 100% Arabica and was a ready-made product bought in a local supermarket. Coffee was used for the analysis immediately after opening the package. Seventeen grams of coffee powder were put into the Italian moka with 188 ml of water; the coffee obtained was then diluted 1:5 with double distilled water and filtered with Whatman no. 4 filter paper (Maidstone, UK), to avoid clogging of the ultrafiltration units.

2.3. Coffee fractionation

Filtered sample (4 ml) was subjected to ultrafiltration with Amicon Ultra-4 nominal cutoff 10 kDa (Millipore, Milan, Italy), at 7500*g* for 50 min at 4 °C (Tagliazucchi, Verzelloni, & Conte, 2010). At the end of the separation two fractions were obtained: the retentate containing high molecular weight compounds (HMWC fraction) and the filtrate containing low molecular weight compounds (LMWC fraction). The two fractions were made up to 4 ml with distilled water for analysis. To quantify filtrate and retentate dry matter content, they were freeze-dried and the residues were weighed.

2.4. Measurements of browning

The browning index of the coffee fractions was determined by measuring the colour as absorbance at 420. The absorption at 420 nm is used to determine the concentration of melanoidins in the coffee fractions. The absorption at 280 and 325 nm was also determined. The results were expressed as l/g/cm.

2.5. Analysis of HMWC fraction

The protein content of the HMWC fraction was determined by Kjeldahl automated apparatus (Borrelli, Visconti, Mennella, Anese, & Fogliano, 2002). Total phenolic group content was determined with Folin–Ciocalteu reagent (Bekedam, Schols, Van Boekel, & Smit, 2006) and 5-caffeoylquinic acid was used as standard. Carbohydrate content was assayed by phenol–sulphuric acid method (Tagliazucchi et al., 2010). Mannose was used as standard.

2.6. HPLC–ESI-MS/MS analysis of LMWC fraction

The LMWC fraction was analysed by a modification of the method of Del Rio and colleagues (2004), using a Waters 2695 Alliance separation module equipped with a Micromass Quattro Micro API mass spectrometer fitted with an electrospray interface (ESI). The chlorogenic acids were all quantified as 5-caffeoylquinic acid equivalents, monitoring the loss of caffeoyl moiety with resultant ionisation of quinic acid.

2.7. Bovine serum albumin glycation

BSA (50 mg/ml) was incubated at 37 °C for 7 days with glucose (0.8 M) in 1.5 M phosphate buffer (pH 7.4; sodium azide 0.012%) in the presence or not of a variable amount of HMWC or LMWC fraction (Wu & Yen, 2005). Since the main part of coffee polyphenols is chlorogenic acids, the antiglycative activity of 5-caffeoylquinic acid was also tested. Catechin was tested as positive control.

2.8. Fluorescence measurement of AGE products

Formation of AGEs can be measured with fluorescence at the excitation and emission maxima of 355 and 405 nm, respectively, *versus* an incubated blank containing BSA and inhibitor. The percent inhibition by different concentrations of inhibitor was calculated and data are expressed in terms of IC_{50} (concentration in μg of inhibitor/ml required to inhibit glycation by 50%) calculated from the log dose inhibition curve.

2.9. Detection of N^{ε}-carboxymethyllysine (CML)

Samples were subjected to electrophoresis by using 7.5% polyacrylamide separating gel (Laemmli, 1970) and then blotted onto a PVDF membrane. The membrane was blocked with 0.5% non-fat dry milk proteins in TBST (10 mM Tris pH 7.5, 100 mM NaCl and 0.1% Tween 20) and incubated with rabbit polyclonal antibody to carboxymethyllysine (1:10000 dilution in TBS). After washing in TBST, a biotinylated goat anti-rabbit IgG (1:1000 dilution in TBS) was added. After washing, a streptavidin–horseradish peroxidase conjugate (1:1000 dilution in TBS) was added. Proteins recognised by the antibody were revealed by colorimetric reaction with 4-chloro-1-naphthol.

2.10. Quantitative measurements of Amadori products

The concentration of Amadori compounds was determined in samples glycated with glucose in the presence or not of coffee HMWC or LMWC fractions, using a fructosamine kit (FAR; Verona, Italy; Voziyan et al., 2003). The possible interference of coffee compounds was considered by subtracting the contribution of an incubated blank containing BSA and coffee fractions. Results are expressed as mM glycosylated albumin.

2.11. Glucose autoxidation

Glucose (0.8 M in phosphate buffer 1.5 M, pH 7.4, 0.012% sodium azide) was incubated at 37 °C for 7 days. The degree of autoxidation of glucose was tested by measuring the formation of glyoxal with a specific spectrophotometric test that applies Girard-T reagent (Wolff & Dean, 1987). Results are expressed as μM glyoxal.

2.12. Fe^{2+}-chelation ability and radical-scavenging activity of coffee fractions

The method used to measure Fe^{2+}-chelation ability of HMWC fraction was developed according to Tagliazucchi et al. (2010). The Fe^{2+}-chelation ability of LMWC fraction was evaluated by the ferrozine assay (Karama & Pegg, 2009).

Radical-scavenging activity was measured with the ABTS assay (Verzelloni, Tagliazucchi, & Conte, 2007) and the results were