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Coffee with ginger – Interactions of biologically active phytochemicals in the model system

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ABSTRACT

This study evaluated the interactions between antiradical and anti-inflammatory compounds from coffee and ginger. Results obtained for whole plant material extracts were compared with those for chlorogenic and caffeic acids (the main hydroxycinnamic acids of plant material). All the tested samples showed the ability to scavenge free radicals and to inhibit lipoxygenase (LOX) activity. Both of these activities increased after simulated gastrointestinal digestion. Aromatic additives, such as ginger, are able to change the antioxidant properties of coffee extract and antioxidant interactions may be identified using two methods. Antiradical phytochemicals from coffee and ginger acted synergistically – isoboles adopted a concave form, while after digestion *in vitro* an additive reaction was observed; in turn, chemical standards acted antagonistically. Water extractable LOX inhibitors acted antagonistically; however, after digestion *in vitro* synergism was observed. The same kind of interaction was determined for standard compounds. These results were confirmed by IF (interaction factor) analysis.

1. Introduction

The oxidation of polyunsaturated fatty acids in biological membranes leads to serious damage such as coronary atherosclerosis, emphysemas, cancer and cirrhosis. Safeguarding fats against oxidation is normally effected by restricting the access of oxygen or adding antioxidants. The most commonly applied antioxidants are synthetic phenols, such as butylated hydroxytoluene and butylated hydroxyanisole (BHA). Their safety, however, has been questioned (Imadia et al., 1983). Therefore, natural antioxidants are readily acceptable by consumers, as they are considered safe. Unlike their synthetic counterparts which are faced with tight governmental legislation (Hancock & Viola, 2005), antioxidants from natural sources are identical to the food that people consume regularly or use as condiments and they play important roles in the human diet. Antioxidants act as free radical-scavengers and inhibit lipid peroxidation and other free radical-mediated processes; thereby helping to protect the human body from several diseases attributed to the reactions of radicals (Atoui, Mansouri, Boskou, & Kefalas, 2005). Reactive oxygen species generation during inflammation is bound, inter alia, with lipoxygenase (LOX) activity. Phenolic plant compounds have been suggested as inhibitors of inflammation via deactivation of a range of pro-oxidative enzymes, including inhibition of LOX-mediated arachidonic acid metabolism (Gawlik-Dziki et al., 2012).

Plants such as herbs have long been used in traditional/folk medicine in various cultures throughout the world. *Zingiber officinale* is one such traditional folk medicinal plant that has been used for over 2000 years (Tepe, Sokmen, Akpulat, & Sokmen, 2006). The rhizome of ginger is known for its contribution to food and has antioxidant (Ghasemzadeh, Jaafar, & Rahmat, 2010) and antimicrobial potentials (Martins et al., 2001). In addition to its aromatic contribution to foods, ginger has been reported to improve blood circulation, reduce blood glucose levels in diabetic patients, aid digestion, and is used in the treatment of nausea (Ernst & Pittler, 2000; Riddell & Perkins, 2009). Ginger is an indispensable component of many food additives. With regards to antioxidant properties, it can be successfully used as a component in curry powder, sauces, gingerbread and ginger-flavoured carbonated drinks and also in the preparation of dietaries for its aroma and flavour. Many authors discuss the chemical composition of ginger (Bartley & Jacobs, 2000; Nishimura, 1995), but no toxic effect has been found.

In recent years, due to increased interest in finding physiologically functional foodstuffs, the relationship between coffee and health has been extensively studied (Higdon & Frei, 2006). Antioxidant activity in foods and beverages is one of the properties that has generated much interest within the scientific community

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(Yukawa et al., 2004). In coffee, antioxidant activity is generally associated with the levels of indigenous phenolic compounds contained therein, as well as Maillard reaction products, the latter being generated during roasting (Del Castillo, Ames, & Gordon, 2002). Among the different phenolic compounds in coffee, the most abundant are hydroxycinnamic acids which exist mainly in esterified form. The best example is chlorogenic acid (5-caffeoylquinic acid) (CGA) with an average level of 100 mg per cup of coffee (Clifford, 1999). Few free phenolic acids are present in coffee, although small quantities of caffeic, ferulic, and vanillic acids have been detected (Dórea & da Costa, 2005).

In contrast to synthetic pharmaceuticals based upon single chemicals, many phytomedicines exert their beneficial effects through the additive or synergistic action of several chemical compounds acting at single or multiple target sites associated with a physiological process (Williamson, 2001). The method used for the identification of interactions between active compounds is isobolographic analysis. This method is independent of any activity mechanism; however, it should be emphasised that this analysis is quite complicated and labour-intensive. A definitely less complicated method for the determination of interactions between mixture components – interaction factor (IF) – was proposed by Gawlik-Dziki (2012). This way of studying interactions, as with isobolographic analysis, is independent of the mechanism of an activity and requires a linear relationship between an activity and sample concentration. Moreover, the "strength" of an interaction may be approximately estimated based on the IF value.

There have been reports on the antioxidant activities of coffee compounds (Gomez-Ruiz, Leake, & Ames, 2007) and ginger (Ghasemzadeh et al., 2010). However, there is limited information on the antioxidant property of coffee with ginger as an aromatic supplement, and its potential for the management of oxidative stress-related metabolic disorders. Therefore, this work reports on a preliminary investigation into the antioxidant and anti-inflammatory properties of hot water extracts of coffee and ginger mixtures and their potential as functional food.

Thus, the aim of this study was to test two hypotheses: (1) that interactions between bioactive phytochemicals play a crucial role in the creation of the nutraceutical potential of coffee fortified with ginger, (2) that interaction with the food matrix and/or changes during simulated gastrointestinal digestion cause significant differences in the relationship between the main hydroxycinnamic acids contained in extracts compared to pure chemicals (standard compounds).

2. Materials and methods

2.1. Chemicals

ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), α-amylase from hog pancreas (50 U/mg), pepsin from porcine gastric mucosa (250 U/mg), pancreatin from porcine pancreas, bile extract, lipoxygenase, linoleic acid, PBS (phosphate buffered saline) were purchased from Sigma–Aldrich company. Acetonitrile and methanol gradient HPLC grade and formic acid LC–MS grade for LC–UV–MS separations were purchased from J.T. Baker (Phillipsburg, New Jersey, USA). Water was purified in-house with a Milli-Q water purification system Simplicity-185 (EMD Millipore Corporation, Billerica, Massachusetts, USA). All other chemicals were of analytical grade.

2.2. Materials

The experimental material consisted of ground coffee available on the Polish market, typical average quality coffee. Ginger was bought from a local market (Lublin, Poland; Manufacturer: Tchibo GmbH, Hamburg, Germany) in the form of ground spice.

The coffee brew and the ginger brew – the aromatic supplement, were analysed separately and in appropriate combinations (4:1; 3:2; 1:1; 2:3; 1:4 v/v) coffee/ginger.

Model compound solutions were prepared in water and the final concentration was 10 μg/mL. The chlorogenic acid solution and the solution of caffeic acid were analysed separately and in appropriate combinations (4:1; 3:2; 1:1; 2:3; 1:4 v/v) chlorogenic acid/ caffeic acid.

2.3. Extraction procedures

2.3.1. Raw extracts preparation

For extraction of water-soluble antioxidants and phenolics 0.5 g of coffee and ginger were poured into 8 mL of boiling water, the samples were shaken for 30 min at 37 °C. After centrifugation (15 min, 20 °C, 8000*g*), the supernatants were combined, and the final volume was brought to 10 ml with distiled water. The final extract concentration was 50 mg dry weight (DW)/ml.

2.3.2. In vitro digestion

In vitro digestion was carried out according to the method described by Gawlik-Dziki (2012) with slight modification. For simulated gastrointestinal digestion 15 ml of each extract were mixed with 5 ml of simulated salivary fluid (2.38 g Na_2HPO_4, 0.19 g KH_2PO_4 and 8 g NaCl), 200 U α-amylase (E.C. 3.2.1.1) in 1 L H_2O, pH 6.75 and shaken for 10 min at 37 °C in the absence of light. Next, the samples were adjusted to pH 1.2 with HCl (5 mM), suspended in 15 mL of simulated gastric fluid (300 U/ml of pepsin A, EC 3.4.23.1 in 0.03 M HCl, pH 1.2) and shaken for 120 min at 37 °C in the dark. After simulated gastric digestion, samples were adjusted to pH 6 with 0.1 M $NaHCO_3$ and suspended in simulated intestinal juice (0.05 g of pancreatin (activity equivalent 4×USP) and 0.3 g of bile extract in 35 mL 0.1 M $NaHCO_3$), adjusted to pH 7 with 1 M NaOH and finally 5 mL of 120 mM NaCl and 5 mM KCl were added to the sample. The prepared samples underwent *in vitro* intestinal digestion for 120 min in the dark. The final concentration of the resulting gastrointestinally digested extract was 20 mg DM/ml.

2.4. Analytical procedures

Ultra-Performance Liquid Chromatography was used to analyse compounds of interest using a Waters ACQUITY UPLC™ system (Waters Corp., Milford, MA, USA), consisting of a binary pump system, sample manager, column manager and PDA detector (also from Waters Corp.). Waters MassLynx software v.4.1 was used for acquisition and data processing. The samples were separated on a BEH C18 column (100 mm × 2.1 mm i.d., 1.7 μm, Waters Corp., Milford, MA, USA), which was maintained at 40 °C. The flow rate was adjusted to 0.40 ml/min. The following solvent system: mobile phase A (0.1% formic acid in Milli-Q water, v/v) and mobile phase B (0.1% formic acid in MeCN, v/v) was applied. The gradient program was as follows: 0–1.0 min, 5% B; 1.0–24.0 min, 5–50% B; 24.0–25.0 min, 50–95% B; 25.0–27.0 min, 95% B; 27.0–27.1 min, 95–5% B; 27.1–30.0 min, 5% B. Samples were kept at 8 °C in the sample manager. The injection volume of the sample was 2.0 μL (full loop mode) and samples were analysed in triplicate. Strong needle wash solution (95:5, methanol–water, v/v) and weak needle wash solution (5:95, acetonitrile–water, v/v) were used. The detection wavelength was set at 250 nm at a 5 point/s rate, at 3.6 nm resolution. The separation was completed in 30 min. Peaks were assigned on the basis of their UV spectra, mass to charge ratio (*m*/*z*) and ESI–MS/MS fragmentation patterns. Chlorogenic acid