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Interactions of green coffee bean phenolics with wheat bread matrix in a model of simulated *in vitro* digestion

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ABSTRACT

Interactions of phenolics from green coffee bean flour (GCS) with the matrix of wheat bread have been studied employing direct (electrophoretic and chromatographic techniques) and indirect tests (nutrient digestibility). According to the chromatograms of digests, the antiradical activity of enriched bread was exhibited by free phenolics. An increase the area of chromatograms and some additional peaks observed for enriched bread may confirm some interactions of proteins with phenolics. The electrophoretic profile of these extracts showed that the band corresponding to a protein with molecular mass of 38 kDA had much higher intensity in enriched bread. Electrophoretic analysis of pellets remaining after digestion revealed GCS dose-dependent differences in bands corresponding to proteins with molecular masses of 52 kDa and 23 kDa. The relative digestibility of both starch and proteins was slightly decreased by addition of GCS; however, these changes did not exceed 10%, which justifies the use of this functional material.

1. Introduction

In recent years, there has been growing interest in application of green coffee beans as a natural food additive to produce functional foods (Babova, Occhipinti, & Maffei, 2016). They are a rich source of bioactive compounds, especially phenolic antioxidants, wherein chlorogenic acids (CGAs) are the most abundant molecules (accounting for up to 10% of the weight of green coffee) (Babova et al., 2016; Budryn, Zaczyńska, & Oracz, 2016; Clifford, 2000; Gómez-Ruiz, Ames, & Leake, 2008). CGAs represent a group of phenolic compounds that are esters of hydroxycinnamic acids, e.g. caffeic acid, ferulic acid, and *p*-coumaric acid with (−)-quinic acid (Babova et al., 2016; Clifford, 2000). 5-O-caffeoylquinic acid commonly referred to as CGA is the most abundant phenolic compound in green coffee (Bicho, Lidon, Ramalho, & Leitão, 2013). CGA represents about 77% of total CGAs in green coffee (Fortunato et al., 2010).

Given its promising composition, green coffee and its bioactive compounds have been used so far as functional ingredients improving the pro-health properties of a few food products such as bread (Budryn, Zaczyńska, & Rachwał-Rosiak, 2016; Dziki, Gawlik-Dziki, & Krzykowski, 2015; Glei, Kirmse, Habermann, Persin, & Pool-Zobel, 2006; Świeca, Gawlik-Dziki, Dziki, & Baraniak, 2017), cookies (Budryn & Nebesny, 2013; Budryn et al., 2016), chocolate (Budryn & Nebesny, 2013), caramel, nut filling, mushroom and meat stuffing (Budryn et al., 2016), and soy-bean milk (Seczyk, Swieca, & Gawlik-Dziki, 2017).

Bread is one of the most popular foodstuff in Europe and European-derived cultures such as both Americas, the Middle East, and North Africa (Goh et al., 2015). It provides more than 50% of the total energy intake. Due to its relatively low cost, availability, acceptability, and widespread consumption, bread is considered to be one the best vehicle for food fortification, supplementation and/or enrichment (Dziki, Różyło, Gawlik-Dziki, & Świeca, 2014; Mervat, Mahmoud, Bareh, & Albadawy, 2015; Rosell Cristina, Joanna, & El, 2015).

Enrichment of certain food with phenolic compounds influences its pro-health quality; however, it may also affect the nutritional value, including bioaccessibility/digestibility (Jakobek, 2015). Such changes are usually caused by interactions with the food matrix as well as an inhibitory effect of phenolics on digestive enzymes (Świeca et al., 2017). Therefore, studies concerning a potential undesirable/limiting effect of enrichment are a very important step in evaluation of the quality of functional food obtained through enrichment with phenolics. The methodology usually involves a direct study conducted with electrophoretic or/and chromatographic techniques for visualization of the relationships as well as indirect tests e.g. assays of nutrient digestibility and the level of bioactivity masked by the interactions (Świeca et al., 2017). The effect of phenolics from green coffee beans (GCPs) on the quality of bread has already been confirmed (Świeca et al., 2017) but, in the current study, we have determined whether and to what extent

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the interactions determine the quality of enriched bread.

2. Materials and methods

2.1. Chemicals

ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)), α-amylase, pancreatin, pepsin, bile extract, were purchased from Sigma-Aldrich company (Poznan, Poland). All others chemicals were of analytical grade.

2.2. Green coffee flour (GCS) preparation

Green coffee beans of *Coffea Arabica* L. from Kenya were prepared to grinding by adding water to adjust moisture content to 10% (w.b.) and storing for 48 h at room temperature. The beans were ground using a laboratory hammer mill (POLYMIX-Micro-Hammermill MFC, Kinematica. AG, Littau/Lucerne, Switzerland) equipped with round holes 3.0 mm (Dziki, Gawlik-Dziki, Pecio, et al., 2015). GCS contains 14.3% of protein, 78.2% of starch and 0.2% of phenolics.

2.3. Bread preparation

The flour used in the formula of control bread (C) was commercial wheat bread flour (600 g), type 2000 (humidity 14%, average ash content 1.95%). The flour was replaced with GCS at 1%, 2%, 3%, 4%, and 5% levels (B1-B5, respectively) to obtain enriched breads. The percentage of the supplement was chosen on the basis of the previous studies concerning consumer acceptance (Dziki, Gawlik-Dziki, Pecio, et al., 2015). Additionally, 6 g of instant yeast and 12 g of salt were used. The quantity of water for the dough preparation was established based on water absorption properties of flour determined with a Farinograph. Dough was mixed with distilled water to optimum consistency of 350 Brabender units. The batches of dough were mixed in a spiral mixer for 6 min. The dough was fermented at 30 °C and 75–80% relative humidity (RH) for 60 min (with 1-min transfixion after 30 min of resting). After fermentation, pieces of dough (300 g) were put into an oven heated up to a temperature of 230 °C and baked for 30 min. After baking, the bread was left to stand for 24 h at room temperature, sliced (approx. 1.5 cm thick slices), lyophilized, and grounded in a laboratory mill. The ground bread samples were stored in darkness at −20 °C until analysis.

2.4. Preparation of extracts

2.4.1. Buffer extracts (BE)

The samples (1 g of dry weight (DW)) were extracted for 1 h with 20 mL of PBS buffer (phosphate buffered saline, 137 mmol/L NaCl, 2.7 mmol/L KCl, 10 mmol/L Na_2HPO_4, 2 mmol/L KH_2PO_4, pH 7.4). The extracts were separated by decantation and the residues were extracted again with 20 mL of PBS buffer. The extracts were combined and stored in darkness at −20 °C.

2.4.2. In vitro digestion

The *in vitro* digestion was performed as described previously by Świeca, Baraniak, and Gawlik-Dziki (2013). A simulated saliva solution was prepared by dissolving 2.38 g Na_2HPO_4, 0.19 g KH_2PO_4, and 8 g NaCl, and 100 mg of mucin in 1 L of distilled water. The solution was adjusted to pH 6.75 and α-amylase (E.C. 3.2.1.1.) was added to obtain 200 U per mL of enzyme activity. For gastric digestion, 300 U/mL of pepsin (from porcine stomach mucosa, pepsin A, EC 3.4.23.1) was prepared in 0.03 mol/L HCl, pH 1.2. Further, simulated intestinal juice was prepared by dissolving 0.05 g of pancreatin (activity equivalent 4 × USP) and 0.3 g of bile extract in 35 mL 0.1 mol/L $NaHCO_3$. The samples were subjected to simulated gastrointestinal digestion as follows: 1 g of a powdered sample was homogenized in a Stomacher laboratory blender for 1 min to simulate mastication in the presence of 15 mL of simulated salivary fluid; subsequently, the samples were shaken for 10 min at 37 °C. The samples were adjusted to pH 1.2 using 5 mol/L HCl; next, 15 mL of simulated gastric fluid was added. The samples were shaken for 120 min at 37 °C. After digestion with the gastric fluid, the samples were adjusted to pH 6 with 0.1 mol/L of $NaHCO_3$ and then 15 mL of a mixture of the bile extract and pancreatin were added. The extracts were adjusted to pH 7 with 1 mol/L NaOH and finally 5 mL of 120 mmol/L NaCl and 5 mL of mmol/L KCl were added to each sample. Once prepared, the samples were submitted to *in vitro* digestion for 120 min at 37 °C and in darkness. Thereafter, the samples were centrifuged and the supernatants were used for further analysis.

After the *in vitro* digestion (AD), the samples were centrifuged for 30 min with 6800 × *g* at 4 °C, and the supernatants were discharged and stored at −20 °C in dark containers until analysis. Pellets were also stored at −20 °C and used for electrophoretic studies.

2.5. Relative digestibility of nutrients

2.5.1. Relative digestibility of proteins

The relative digestibility of proteins was expressed as the differences in the amounts of free amino groups determined for the control and for the enriched bread after the *in vitro* digestion (Sęczyk, Świeca, & Gawlik-Dziki, 2016).

The content of free amino groups was determined using the trinitrobenzenesulfonic acid (TNBS) method (Adler-Nissen, 1979). Briefly, samples (20 μL) were mixed with 0.980 mL of 0.2 M sodium phosphate buffer, pH 8.0, and 0.5 mL of 0.1% TNBS. After cooling (30 min), the absorbance of the reaction mixture was measured at 340 nm and the content of free amino groups was expressed as L-leucine equivalents (μg/mL).

The following equation was used to calculate the relative digestibility of proteins:

$$RDP = \frac{Aa_F - Ab_F}{Aa_C - Ab_C} \times 100\%$$

where: RDP– relative digestibility of proteins (%), Aa– content of free amino groups after *in vitro* digestion, Ab– content of free amino groups before *in vitro* digestion, F– enriched sample,

C– control sample.

2.5.2. Relative digestibility of starch

The relative digestibility of starch was expressed as the difference in the amounts of reducing sugars determined for the control and the enriched bread after the *in vitro* digestion (Sęczyk et al., 2016).

The content of reducing sugars was determined using the 3,5-dinitrosalicylic acid (DNSA) method (Miller, 1959). Samples (0.2 mL) were mixed with 0.3 mL distilled water and 0.5 mL DNSA reagent. Then, the mixture was incubated in a water bath at 100 °C for 10 min. After cooling to room temperature, the absorbance of the reaction mixture was measured at 540 nm. The level of reducing sugars was expressed as maltose equivalents (μg/mL).

The following equation was used to calculate the relative digestibility of starch:

$$RDS = \frac{RSa_F - RSb_F}{RSa_C - RSb_C} \times 100\%$$

where:

RDS– relative digestibility of starch (%), RSa– content of reducing sugars after *in vitro* digestion, RSb– content of reducing sugars before *in vitro* digestion, F– enriched sample, C– control sample.

2.6. Size exclusion chromatography (SEC)

Size exclusion chromatography of the bread samples was carried out using Sephadex G-50 as a gel filtration medium. 0.5 mL of the sample

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