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Nutritional potential and inhibitory activity of bread fortified with green coffee beans against enzymes involved in metabolic syndrome pathogenesis

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ABSTRACT

The nutritional potential and selected enzyme inhibitory properties of wheat bread fortified with coffee beans (1, 2, 3, 4, and 5 g/100 g, B1-B5, respectively) were investigated. Replacement of wheat flour with powdered green coffee beans (GCB) significantly decreased the total protein content only in the case of B4. The characteristic starch parameters such as the total starch content, resistant starch, and starch digestibility did not differ significantly between the control bread (C) and the enriched bread samples. Only potentially bioavailable starch was significantly lower in B4 and B5 (311.84 and 325.34 mg/g d.w., respectively), compared with C (392.67 mg/g d.w.). The bread with GCB was characterized by a higher hydrolysis index and expected glycemic index than C but these changes had no significant effect. Moreover, the EC_{50} value of ACE inhibition noted for bread B2-B5 was significantly lower (in the range of 1.6–7.77 mg/mL) than in C (17.96 mg/mL). The fortification with GCB had an influence on lipase inhibitory activity, whereas this property was not detected for C. The lowest EC_{50} value was noted for bread B3 and B5 (3.00 and 2.02 mg/mL, respectively). The addition of GCB to bread had no significant effect on α-amylase and α-glucosidase inhibitory activity.

1. Introduction

Bakery products, especially bread, are the most common foods worldwide (Rosell, 2015, pp. 173–192). They are characterized by high dietary fiber and mineral content and low salt, added sugar, and fat content. Due to the variety of ingredients, different types, and high nutritional value, bread is one of the most commercially available products and often a primary product for consumers, who are nowadays more aware of the influence of diet on improvement of the quality of their lives and health (Sandvik, Nydahl, Kihlberg, & Marklinder, 2018).

Metabolic syndrome (MS) is one of the most common diseases in the population of western countries. It constitutes a risk factor for the development of cardiovascular diseases, diabetes, and arteriosclerosis. It has become a very common global disorder (Pahwa, Adams-Huet, & Jialal, 2017) prevalent in adults and children. MS is characterized by glucose intolerance, imbalance in the content of glucose in blood, hypertension, dyslipidemia, and/or obesity (Cornier et al., 2008). One of the methods of reducing glucose in blood is to inhibit enzymes involved in the release of glucose from complex sugars (Fernandez-Gomez et al., 2016). α-Glucosidase is a membrane-bound enzyme located in the epithelium of the small intestine and is involved in the catalysis of glucose release from disaccharides and oligosaccharides into the blood stream. Therefore, inhibition of this enzyme can decrease the glucose content and, subsequently, glucose uptake. Another enzyme involved in glucose content regulation is α-amylase, which catalyzes the hydrolysis of polysaccharides and releases reduced sugar from starch or glucagon. The clinical use of the inhibitory activity of this enzyme is advisable because controlled reduction of starch digestion can influence carbohydrate uptake in obesity or diabetes mellitus. Specific inhibitors of animal α-amylases have long been known; they were obtained from plants, particularly wheat and beans (Layer, Carlson, & Dimagno, 1985) and used as weight control remedies.

Additionally, two enzymes have an influence on the pathogenesis of MS: the angiotensin converting enzyme (ACE) and pancreatic lipase. The former is a part of the hormone renin-aldosterone-angiotensin system (RAAS), which plays an important role in the regulation of fluid balance, hypertension, and blood volume. ACE catalyzes inactive angiotensin I (decapeptide) to active angiotensin II (octapeptide), which is a potent vasoconstrictor, promotes aldosterone release and the main active product of the RAAS, and has other potentially harmful effects on the cardiovascular system. The inhibition of this enzyme has a beneficial influence on protection against heart and renal diseases, stroke, and heart attack. Drugs with mechanisms based on ACE activity inhibition are used in hypertension treatment; however, they may cause

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side effects such as rush, cough, edema, or sleepiness. For these reasons, ACE inhibitors that can be characterized and identified as food ingredients are sought.

Lipase, which is mainly produced in the pancreas, catalyzes the hydrolysis of lipids to monoaclyglycerol and fatty acids that can be absorbed in the blood system. An excessive concentration of free fatty acids in the blood may cause increased insulin resistance, formation of free radicals, or formation of fat tissue, which results in obesity. It has been suggested that inhibition of pancreatic lipase may result in increased fecal lipid content and final body weight (Grove, Sae-Tan, Kennett, & Lambert, 2012).

Since the diet has an impact on every component of the metabolic syndrome, it is considered to be a major contributor to the syndrome (Cornier et al., 2008). In this work, wheat bread was fortified with coffee beans and its potential to be used in diet therapy of metabolic syndrome was determined. As the most common food product worldwide and a food matrix for food enrichment with bioactive compounds, bread has been widely investigated. Coffee beans are characterized as a controversial food product that may have a beneficial effect on health. Coffee is the most common beverage in Europe and America and its aroma and flavor are used as an additive to food, as this is desired by consumers. Investigation results suggest that polyphenol compounds from coffee may inhibit lipoxygenase (LOX), which is regarded as an inflammatory factor (Gawlik-Dziki, Durak, Jamioł, & Świeca, 2016), or may exert an antioxidant effect (Liang, Xue, Kennepohl, & Kitts, 2016). Therefore, the aim of our study was to investigate the nutritional potential and inhibitory activity against enzymes involved in MS of bread fortified with coffee beans.

2. Material and methods

2.1. Material

Green coffee beans were obtained from company Cofeina Romuald Zalewski, Marki, Poland. Wholemeal wheat flour type 2000 (protein content 14 mg/100 g, ash content 1.89 mg/100 g, moisture content 13.6 mg/100 g; WF) was purchased in the local supermarket in Lublin, Poland.

Chemical compounds: HHL (Hippuryl-L-Histidyl-L-Leucine, PubChem CID: 94418), pepstatin A, PMSF (phenylmethanesulfonyl fluoride, PubChem CID: 4784), α-amylase from hog pancreas (50 U/mg), pepsin from porcine gastric mucosa (250 U/mg), pancreatin from porcine pancreas, bile extract, PubChem CID: 11045), DNS (3,5-dinitrosalicylic acid, PubChem CID: 11873), 2-Mercaptoethanol PubChem CID: 1567, DTT (Dithiothreitol PubChem CID: 446094), SDS (sodium dodecyl sulfate PubChem CID: 3423265), pNPA (*p*-nitrophenyl acetate PubChem CID: 13243), DMSO (dimethyl sulfoxide, PubChem CID: 679), starch solution from Sigma-Aldrich Company, USA.

2.2. Bread preparation

The flour used in the formula of control bread (C) was wheat bread flour (600 g), type 2000 (average 1.9 g/100 g ash content, humidity 14%). The wheat flour was replaced with green coffee bean (GCB) at 1, 2, 3, 4 and 5 g/100 g (B1-B5, respectively). The percentage of green coffee flour addition was chosen on the basis of a previous test on antioxidant activity (data not published). Besides this 1 g/100 g of instant yeast (equivalent to 3 g/100 g compressed yeast) and 2 g/100 g of salt were used for dough preparation. The quantity of water necessary for the preparation of the dough was established through the marking of water absorption properties in flour of a consistency of 350 Brabender units. The batches of dough were mixed in a spiral mixer for 6 min. Dough was fermented at 30 °C and 80% relative humidity (RH) for 60 min (with 1 min transfixion after 30 min). Pieces of dough (300 g) were molded by hand, panned, and proofed at 30 °C and 75% RH over the period required for optimal dough development. After fermentation, the pieces of dough (300 g) were put into an oven heated up to a temperature of 230 °C. The baking time was 30 min. After baking, the bread was left to stand for 24 h at room temperature. Subsequently, the samples were sliced (slices about 1.5 cm thick). The crust was removed aseptically and crumb was kept frozen (at −20 °C) until analysis. After thawing, the slices were freeze dried and then manually crumbed, ground in a mill and screened through a 0.5 mm sieve to obtain bread powder (Swieca, Gawlik-Dziki, Dziki, Baraniak, & Czyż, 2013).

2.3. In vitro digestion

In vitro digestion was performed as described previously (Minekus et al., 2014). For simulated mastication and gastrointestinal digestion, 250 mg of freeze-dried bread samples were homogenized in 0.5 mL PBS buffer and 1 mL of simulated salivary fluid [15.1 mmol/L KCl, 3.7 mmol/L KH_2PO_4, 13.6 mmol/L $NaHCO_3$, 0.15 mmol/L $MgCl_2(H_2O)_6$, 0.06 mmol/L $(NH_4)_2CO_3$, 1.5 mmol/L $CaCl_2$, α-amylase (75 U/mL)] and shaken for 10 min at 37 °C. Next, the samples were adjusted to pH 3 with 6 mol/L HCl, suspended in 2 mL of simulated gastric fluid [6.9 mmol/L KCl, 0.9 mmol/L KH_2PO_4, 25 mmol/L $NaHCO_3$, 47.2 mmol/L NaCl, 0.1 mmol/L $MgCl_2(H_2O)_6$, 0.5 mol/L $(NH_4)_2CO_3$ 0.15 mmol/L $CaCl_2$, pepsin (2000 U/mL)] and shaken for 120 min at 37 °C. After simulated gastric digestion, samples were adjusted to pH 7 with 1 mol/L NaOH and suspended in 4 mL simulated intestinal fluid [6.8 mmol/L KCl, 0.8 mmol/L KH_2PO_4, 85 mmol/L $NaHCO_3$, 38.4 mmol/L NaCl, 0.33 mmol/L $MgCl_2(H_2O)_6$, 0.15 mmol/L $CaCl_2$, 10 mmol/L bile extract, pancreatin (2000 U/mL)]. The prepared samples underwent *in vitro* intestinal digestion for 120 min.

2.4. Nutritional potential of fortified bread

2.4.1. Protein content assay

Total proteins (TP) were measured according to procedure described by Hurkman and Tanaka (2007). For this method, 50 mg of flour was suspended in 200 µl of urea buffer (2 mol/L urea, 10% glycerol, 65 mmol/L dithiothreitol (DTT), and 20 mmol/L Tris, pH 8.0), the suspension was incubated at room temperature for 1 min, and insoluble material was removed by centrifugation (16 000 × g for 10 min). The proteins were then precipitated by addition of 4 vol (vol.) of cold acetone and recovered by centrifugation. The pellet (total proteins) was dried by lyofilization and solubilized in 1% SDS (w/v), 0.1 mol/L 2-mercaptoethanol in 60 mmol/L Tris- HCl pH 6.8.

The protein content in extracts was determined with the Bradford method using bovine serum albumin as the standard protein (Brand-Williams, Cuvelier, & Berset, 1995). Fifty microliters of samples were mixed with 950 mL of Bradford reagent and incubated for 15 min at room temperature. After this time, absorbance at 595 nm was measured. Protein content was calculated in mg/g of dry weight (d.w.).

2.4.1.1. Proteins digestibility in vitro. The *in vitro* digestibility of protein (PD) was evaluated on the basis of protein content before (TP) and after digestion *in vitro* (RP) (Świeca & Baraniak, 2014).

$$PD[\%] = 100\% - \left(\frac{RP}{TP} \times 100\%\right)$$

For determination of the content of proteins after digestion procedure proteins were extracted from pellets according to procedure described by Hurkman and Tanaka (2007). Albumins and globulins content was described as the proteins present in supernatants obtained after digestion *in vitro* (correction for components of digestive system was performed). Proteins content after digestion *in vitro* (RP) were determined as a sum of proteins from pellets and proteins presented in fluids from digestion.