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Fatty acids, essential amino acids, and chlorogenic acids profiles, *in vitro* protein digestibility and antioxidant activity of food products containing green coffee extract

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Abstract

The aim of this study was to determine the effect of addition of green coffee extract (GCE) in concentrations from 0.25 to 1 g 100 g^{-1} on the physical, sensory and chemical properties, and antioxidant activity of some food products. Two kinds of *gnocchi* (from fresh and dried potatoes), sponge cake, bread, mayonnaise, marshmallows, caramel candies and jellies were prepared. Among supplemented products food with the maximal GCE addition best sensory properties showed mayonnaise, followed by marshmallows and jellies. Addition of GCE caused changes in color of products, where the smallest changes were observed in mayonnaise and *gnocchi* from dried potatoes (ΔE 1.20 and 2.93 respectively) and the highest in jellies and sponge cake (ΔE 20.38 and 12.80 respectively). GCE added to food products allowed to maintain higher amount of polyunsaturated fatty acids (PUFA), especially prepared with the use of high temperature, such as marshmallows. In this product with 1 g 100 g^{-1} of GCE the PUFA content was 5.82 g 100 g^{-1} while in control it amounted 4.55 g 100 g^{-1}. The losses of polyphenol introduced to the food products with GCE were the smallest in mayonnaise and jellies prepared under low temperature. Addition of GCE contributed to the increase of antioxidant activity of enriched products. The highest increase of antioxidant activity was observed in sponge cake, followed by candies, marshmallows and mayonnaise. The losses of amino acids caused by the addition of GCE were greatest in products treated at a moderately high temperature and subject to further gelation, i.e. jellies and marshmallows. Despite higher losses of amino acids the effect of the addition of GCE on protein pepsin digestibility the did not exceed 3%.

Introduction

Polyphenols counteract oxidative changes in food as well as show an effect of reducing the oxidative stress in human organism. A rich source of polyphenols, especially phenolic acids in human diet is coffee (Daglia *et al.*, 2007). Among antioxidants, green coffee contains mainly isomers of caffeoylquinic acid (which are referred to as chlorogenic acid) and caffeic acid (Hennig *et al*., 2004). Brewed coffee is consumed for many centuries, due to its stimulating properties, and other health beneficial activities (Seeram *et al*., 2006). It exhibits anti-inflammatory and anti-mutagenic effects, which prevents from chronic disorders, such as tumors, cardiovascular and rheumatologic diseases (Cheng *et al.*, 2007). Consumption of coffee is also helpful in the fight against obesity and limits the effects of type II diabetes (Bassoli *et al*., 2008).

Due to lack of consumers' trust towards synthetic antioxidants in food, the use of natural extracts to enhance the oxidative stability of products is intensively studied, including green coffee extract (Schwarz *et al*., 2001). Also, coffee contains caffeine in the amount of even few percent, which gives it additional pro-health benefits compared to extracts from fruits, vegetables, cereals, herbs, spices or legumes (Harland, 2000). The levels of supplemented antioxidants in food products rarely exceed 0.1%, which is sufficient to effectively prolong the stability of lipids in foods (Marinova *et al*., 2009). Current food production trends include not only the protection of food components but also giving products pro-health properties through the introduction of antioxidants (Maat *et al*., 2005). However, to meet these aims the concentration of antioxidants in food products has to be significantly higher than the amounts used for protection of food lipids from oxidation, on a level of even 1 g of polyphenols in a typical daily portion of a product (Williamson and Holst, 2008). In the light of previous studies, which showed that in some food materials the concentration of polyphenols, including coffee phenolic acids, even at a level below 0.1% gave a pro-oxidant effect on lipids (Luzia *et al.*,

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1997), the issue of testing oxidative stability of food products with a relatively high level of enrichment with plant extracts must be verified (Decker, 1998). The impact of such a large addition of antioxidants on the composition also raises some doubts due to the fact that polyphenols and their oxidation products are highly reactive with proteins, limiting their bioavailability (Viljanen *et al*., 2004; Renouf *et al.*, 2010).

The objectives of the study was to determine the effect of addition of green coffee extract on the physical, chemical and sensory properties, and. antioxidant activity of some food products (Lindley, 1998).

Materials and Methods

Source of materials

Caffeic acid (CA), 5-O-caffeoylquinic acid (5-CQA), 5-O-feruloylquinic acid (5-FQA), benzoic acid (BA), caffeine, 37 component mix (fatty acid methyl esters), linoleic acid methyl ester cis/trans mix, cis-10-pentadecenoic acid, phenol, hexane and heptane were obtained from Sigma Aldrich (St. Louis, MO, USA). Ethanol and methanol were obtained from J.T Baker (Phillipsburg, NJ, USA) and acetonitrile from Scharlab (Barcelona, Spain). NaCl, HCl, petroleum ether, NaOH, formic acid and citric acid were obtained from Chempur (Piekary Śląskie, Poland). Standards of amino acids, and 2,2-di(4-tert-octylphenyl)-1-picrylhydrazyl ($DPPH^{\cdot}$) were supplied by Fluka (Mumbai, India), triethylamine by Fluka (Buchs, Switzerland), phenyl isothiocyanate (PITC) and L-norleucine by Sigma-Aldrich (Steinheim, Germany), sodium acetate by Bioultra Sigma (Taufkirchen, Germany), acetic acid by Fisher Scientific (Loughborough, UK). All chemical reagents used in this study were of analytical grade. Ultrapure water (resistivity, 18.2 MΩ cm^{-1}) was obtained from a Millipore Milli-Q Plus purification system (Bedford, MA, USA).

Green Robusta coffee (*Coffea canephora*) beans originating from Brasil, dehulled by dry method, purchased from Bero Polska (Gdynia, Poland) were used. Agar, wheat starch syrup and citric acid were purchased from Plus (Lodz, Poland). Another raw materials used for obtaining food products, such as eggs, wheat and rye flour, fresh and dried potatoes, potato starch, sugar, slim powdered milk, sunflower oil, butter, gelatin, orange concentrate, mustard, yeast, and NaCl were purchased from a local market.

Preparation of green coffee extract (GCE)

An aqueous extract of green coffee was prepared. Beans were ground and sieved to a particle size ranging from 480 to 680 μm, because the material obtained after grinding was not homogeneous. The isolated fraction comprised approximately 90% of the material obtained after grinding, where very fine particles limiting the effectiveness of filtration, and relatively high particles with low efficiency of polyphenols extraction were eliminated. The extract was obtained using a 1: 5.75 ratio of ground coffee to the solvent, by boiling in pressure vessel (PS-5682 Vienna, Austria) at 110°C for 10 min, cooled in a water bath to a temperature of 40°C for 20 min and filtered under vacuum using a vacuum pump KNF 18 035.3 N (Neuberger NJ, USA). The extract was then freeze-dried in a DELTA 1-24LSC Christ freeze drier (Osterode am Harz, Germany). The resulting lyophilisate contained: 37.6 g 100 g^{-1} of chlorogenic acids, 14.5 g 100 g^{-1} of protein, 0.7 g 100 g^{-1} of fat, 11.9 g 100 g^{-1} of ash, 6.9 g 100 g^{-1} of caffeine, 6.8 g 100 g^{-1} of soluble fiber, 19.3 g 100 g^{-1} carbohydrates and 2.3 g 100 g^{-1} of water. The extract was characterized by higher antioxidant activity than extracts obtained by brewing with boiling water or boiling in water for 10 min (Budryn *et al*., 2009).

Table1. Ingredients proportion for preparation of food products containing green coffee extract (GCE)

Ingredient	Product							
	GF	GD	SC	B	MY	MR	C	J
	Content of main ingredients (g 100 g^{-1})							
Cooked potatoes	70	-	-	-	-	-	-	-
Dried potatoes	-	18	-	-	-	-	-	-
Wheat flour	25	25	18	47	-	-	-	-
Rye flour	-	-	-	11	-	-	-	-
Potato starch	-	-	9	-	-	-	-	-
Sugar	-	-	27	-	-	37	40	8
Eggs	4	4	46	-	-	-	-	-
Egg yolk	-	-	-	-	4	-	-	-
Egg white	-	-	-	-	-	10	-	-
Slim powdered milk	-	-	-	-	-	3	-	-
Sunflower oil	-	-	-	1	69	-	-	-
Butter	-	-	-	-	-	16	-	-
Gelatin	-	-	-	-	-	-	-	4
Agar	-	-	-	-	-	2	-	-
Wheat starch syrup	-	-	-	-	-	19	30	-
Orange concentrate	-	-	-	-	-	-	-	13
Mustard	-	-	-	-	6	-	-	-
Yeast	-	-	-	2	-	-	-	-
NaCl	1	1	-	1	1	-	-	-
Citric acid	-	-	-	-	-	-	-	1
Water	-	52	-	38	20	13	30	72

GF – gnocchi from fresh potatoes; GD - gnocchi from dried potatoes; SC – sponge cake; B- bread; MY – mayonnaise; MR – marshmallows; C – candies
"-" – ingredient not used in a formula

Preparation of products enriched with GCE

All control products were obtained from 400 g of ingredients according to recipe compositions presented in Table 1. Then enriched products were obtain using the same compositions and additionally

different concentrations of GCE, i.e. 0.25, 0.5 and 1.0 g 100 g^{-1}. The food products prepared include:

Gnocchi (a traditional Italian dish of cooked potatoes): Potatoes were peeled, cooked and mashed. All the ingredients (Table 1) used for gnocci from fresh potatoes (GF), i.e. besides potatoes also wheat flour, eggs and NaCl were mixed in a blender together with GCE. *Gnocchi* were formed and boiled in water for 2 minutes. A similar procedure was used for preparation of *gnocchi* from dried potatoes (GD), substituting fresh potatoes with a mixture of industrially dried potato puree and water.

Sponge cake (SC): Cold method was used to prepare the sponge cake. Egg whites (Table 1) were whipped in a blender with sugar and then yolks, wheat flour and potato starch were added along with GCE and gently mixed. The cake was baked at 170 °C for 40 minutes.

Bread (B): Bread dough was prepared using two-phase method. In a first phase a leaven was prepared using 60 ml of water, 80 g of wheat flour and whole amount of yeast (Table 1) mixed together gently in a blender. After 10 minutes in ambient conditions the leaven was mixed with the remaining ingredients, i.e. the remainder of water and wheat flour, rye flour, sunflower oil and NaCl. The dough was put into a fermenter at 31°C and relative humidity of 60% for 1 hour. The different concentrations of GCE were added, the dough was mixed and baked in an electric oven at 200°C for 25 minutes.

Mayonnaise (MY): A middle fat mayonnaise emulsion was prepared. Egg yolks and water (Table 1) were mixed thoroughly in a blender. Sunflower oil and seasonings were added in small portions until a homogenous emulsion was obtained. This was followed by addition of different concentrations of GCE.

Marshmallows (MR): Marshmallows as confectionery foams were obtained based on the structure of whipped and denatured egg whites settled with agar as a gelling agent. First agar was quenched with water, allowed to swell, heated to dissolve to about 100°C and mixed manually with sugar. The mixture was poured into starch syrup and heated to reach 110°C. The mass was cooled to 100°C and added to earlier whipped foam from egg whites and aerated by whipping for 5 min. Butter was liquefied and milk powder dissolved in a small amount of water was added in portions to butter and then the foam from egg whites and different concentrations of GCE were added to it at about 90°C. Then the mixture was poured into molds.

Caramel candies (C): Caramel candies were obtained from amorphous sugar by dissolving of sugar in hot water, and then evaporating the solvent after addition of wheat starch syrup as an anti-crystallization agent. To achieve this sugar and water (Table 1) were heated to 90°C. The solution was poured into starch syrup. The mixture was concentrated by heating in an open beaker for about 30 min to achieve 141°C, and then cooled to a temperature of 120°C. At this temperature, different concentrations of GCE were added. The resulting caramel mass was poured into molds.

Jellies (J): Jellies were obtained as a confectionery gel with gelatin used as a gelling agent. 32 g of sugar and 4 g of citric acid were dissolved in 144 g of water (Table 1). 16 g of gelatin was allowed to swell for 15 min in the rest 144 g of water, and then heated to dissolve at a temperature of 75°C. The prepared solution of sugar and citric acid was mixed with 52 g of orange concentrate and poured into dissolved gelatin and the appropriate addition of GCE was added. Then the mixture was poured into molds.

Water activity and pH

Water activity and pH were determined as described by Budryn *et al.* (2011).

Sensory analysis

A trained eight-member panel consisting of students was selected for the sensory evaluation. Four samples of one food product with different concentrations of GCE coded with three–digit number were presented to the panelists for evaluation. Panelists evaluated two different kinds of products daily with an interval of half an hour between evaluation of products. The first session included the evaluation of *gnocchi* from fresh and then from dried potatoes, second sponge cake and bread, third mayonnaise and foams, and fourth, candies and jellies. Each sensory attribute, i.e. color and flavor was rated on a 5-point hedonic scale (1 = extremely poor quality while 5 = typical, characteristic, highly acceptable attributes).

Texture profile analysis

Texture analysis of the prepared food products containing different concentrations of GCE was determined using a TA.XTplus texture analyzer, Stable Macro System (Godalming, UK). For the caramel candies, a needle insertion test was performed at a certain time under constant force, in which a measure of the textural properties is the cavity depth, and greater depth means a more soft structure. For mayonnaise a test of measuring force needed to push a sample between a cylindrical vessel and a round cylinder moving in the vessel, evaluating cohesion of a sample, was applied. For other products such

as gnocchi, sponge cake, bread and marshmallows the compression test was adopted. Analyses were performed according to the manufacturer's application manual.

Color measurement

Color measurement in CIE $L^*a^*b^*$ system was made using an automatic colorimeter Konica Minolta CR-400 with Spectra Magic NX 1.3 software (Osaka, Japan). Color parameters, i.e., the brightness L^* (from 0 – black to 100 – white), a^* (from (-50) – green to 50 – red), b^* (from (-50) – blue to 50 – yellow), and dE value, equal to square root of $[(dL^*)^2 + (da^*)^2 + (db^*)^2]$, characterizing the total change of color were measured (Charurin *et al*., 2002).

Fatty acids analysis

Fat was extracted from the prepared food products containing different concentrations of GCE in a Soxtec Avanti 2000 extractor (Foss, Hoeganaes, Sweden) using petroleum ether (boiling point of 40-60°C) as an extraction solvent. Fatty acids composition was determined by gas chromatography (Varian 450 gas chromatograph, Varian Inc., Palo Alto, California, USA) with flame ionization detection (GC-FID) using a standard method (AOAC 963.22, 2000).

Fatty acids separation was performed on a FactorFour VF-23ms (60 m x 0.25 mm x 0.25 μm) column (Varian Inc., Palo Alto, California, USA). For separation, the temperature range 180 - 220°C for 25 min was used with 2°C min^{-1} rise. The temperature of injector was 220°C, and detector temperature was 250°C. Helium was used as a carrier gas with a flow rate of 1 mL min^{-1} and 1:100 split. For identification and quantitative analysis the 37 component mix and *cis/trans* isomer of linoleic acid methyl ester standards were used. As an internal standard 20 μL of *cis*-10-pentadecenoic acid methanol solution (200 mg mL^{-1}) was added to the sample.

Amino acids analysis

A 5 g sample of a product was defatted three times with 30 mL of hexane for 5 min with constant shaking and subsequently centrifuged for 10 min at 6000 rpm. Then, the hexane solution was discarded and the residual organic solvent eliminated under a gentle stream of nitrogen. Amino acids quantitative analysis included pre-column derivatization after acid hydrolysis according to the method of Kwanyuen and Joseph (2010). 10 mg of a defatted sample was placed in a vial, 400 μL 6 M HCl containing 1% of phenol (antioxidant agent) was added and connected to a stream of nitrogen and a vacuum pump for 5 min. Hydrolysis was performed at 110°C for 24 h. After this vacuum drying under nitrogen stream at room temperature until completely dry was done. After evaporation 150 μL of methanol:water:triethylamine 2:2:1 (v/v/v) buffer was introduced into vials. Samples were shaken vigorously, and next, added buffer was evaporated as above, this step was performed twice. Then the vials were supplemented with 150 μl methan ol:water:triethylamine:PITC 7:1:1:1 (v/v/v/v) buffer, mixed and allowed to stand for 20 minutes. After the reaction, the buffer was evaporated as above. Next twice 150 μL of methanol was added to the vials and samples were vacuum dried under nitrogen. Resulting residue was dissolved in 1 mL of 10 mM sodium acetate buffer in 6% acetic acid and centrifuged. The supernatant was filtered through a nylon syringe filter with a pore size of 0.20 μm. Then the aliquot of 20 μL of the filtrate was injected to Dionex Ultimate 3000 UHPLC$^+$ (CA, USA) using a BioBasic C18, 5 μm, 150 x 4.6 mm column. The used eluents were: A - 10 mM sodium acetate, pH 6.4, B – acetonitrile:water 6:4 (v/v). Elution was performed with the gradient starting at 95% A and 5% B, to reach 80% A and 20% B at 5.5 min, 60% A and 40% B at 10 min, 100% B at 10.5 and becoming isocrating for 5 min, and 100% A at 18 min with a flow rate of 1 mL min^{-1}. Detection was performed with the use of UV-DAD MWD-3000 RS detector at a wavelength of 254 nm. Amino acids quantities were identified by comparing retention times of standard amino acids mixture with sample peaks and calculated on the basis of the equation of the calibration curve and taking into account the loses of internal standard – L-norleucine.

In vitro pepsin digestibility

The method of Zhang *et al.* (2009) was adopted for the determination of in vitro pepsin digestibility of food products. A 10 mg of a product sample was dissolved in 800 μL of 0.1 M HCl solution, then 200 μL of pepsin (1 mg mL^{-1}, enzyme:substrate 1:50, m/m) was added and incubated at 35°C for 2 h. The reaction was terminated by adding 100 μL of 20% trichloroacetic acid. Then 10 μL of the solution was injected to Dionex Ultimate 3000 UHPLC$^+$ (CA, USA) using a C18 BioBasic, 5 μm, 150 x 4.6 mm column. The used eluents were: A - 0.1% aqueous solution of trifluoroacetic acid, B - 0.1% solution of trifluoroacetic acid in acetonitile. Elution was performed using the following program: 0 min - 10 min - 100% A, 10 - 43 min from 0 to 80% B with a linear gradient and a flow rate of 1 mL min^{-1}. Detection was carried out using UV DAD MWD-3000 RS detector at a wavelength of 215 nm. Peak areas from HPLC analysis of protein from products without pepsin digestion but after dissolving in water adjusted to pH

9 by 0.1 M NaOH allowed calculating the quantity of initial protein. Digestibility of protein was then established as a percent of protein hydrolyzed into peptides, namely the decrease of protein peaks area after the hydrolysis.

Caffeine and phenolic acids analysis

A 5.0 g sample of a food product was defatted three times with 30 ml of hexane for 5 min with constant shaking and subsequently centrifuged for 10 min at 6000 rpm. Then, the hexane solution was discarded and the residual organic solvent eliminated under a gentle stream of nitrogen. The defatted sample was mixed with 100 µl of internal standard solution (benzoic acid, 10 mg•ml^{-1}). Phenolic compounds and caffeine were extracted three times from defatted products with 100 mL of Milli-Q Plus water, for 30 min in an orbital shaker. After each extraction, the mixture was centrifuged as above and the supernatant was decanted. The supernatants from the centrifuged tubes were combined and filtered through a 0.20 µm nylon syringe filters.

Chromatographic analysis UHPLC/DAD was carried out using a UHPLC$^+$ Ultimate 3000 system with an auto sampler and a diode array detector (DAD) from Dionex (CA, USA). The analytical column Accucore™ C18 (100 mm x 3.0 mm x 2.6 µm) from Thermo Scientific (PA, USA) was used. The mobile phase consisted of 1% formic acid (solvent A) and acetonitrile:1% formic acid (80:20, v/v) (solvent B) with flow rate of 0.5 mL min^{-1}. The elution was performed with a gradient starting at 5% B to reach 35% B at 23 min and becoming isocratic for 5 min. 2 µL injection volume and 25°C column temperature were used. Detection was performed with two wavelengths, 280 nm for caffeine and 320 nm for phenolic acids. Phenolic compounds and caffeine were identified by comparing their retention times and UV spectra with those of reference standards when available or with literature data (Budryn *et al*., 2009). The quantification was performed using the internal standard method. Standard calibration curves were constructed in the concentration range of 1 to 10 mg L^{-1} of 5-O-CQA. All caffeic acid ester derivatives were quantified as 5-CQA equivalents.

Antioxidant activity of products

Radical scavenging ability of the samples was determined as described by Scherer and Godoy (2009). DPPH$^·$ was used as a standard radical. The test was carried out using aqueous suspension of products at concentrations of 0.2, 0.5, 1 and 2 g•100 g^{-1}. 0.1 mL of a suspension was reacted with 3.9 mL of methanolic radical solution (DPPH$^·$:methanol, 1:20000 (m/m). Methanol was used as a blank sample and 0.1 mL of water with 3.9 mL of methanolic DPPH$^·$ solution as a control. Based on the measurement of absorbance of a filtered sample after 30 min reaction in the darkness at 517 nm (UV/VIS spectrophotometer U-2800 A, Hitachi, Tokyo, Japan) a calibration curve of concentration of a product versus chain-breaking activity was obtained. Chain-breaking activity % = $[(A_{control} - A_{test})/A_{control}]$ x 100%. The concentration of a product at which the concentration of radical form was reduced by 50% (IC_{50}) was calculated.

Statistical analysis

Each batch of products was prepared twice. Analyses were carried out in triplicate and their results were subjected to statistical analysis. It comprised determination of average values of six measurements and their standard deviation as well as one-way ANOVA (analysis of variation) at the significance level $p < 0.05$.

Results and Discussion

Physical and organoleptic properties

Potato *gnocchi* obtained from fresh and dried potatoes exhibited good sensory properties apart from the sample with 1 g 100 g^{-1} GCE, which strongly deviated from the standard in terms of color and flavor attributes (Table 2). A gray color of the product could be caused by activity of oxidative enzymes present in the added wheat flour, which oxidized phenolic acids of GCE. In sponge cake and bread, the addition of GCE caused a moderate influence on the sensory characteristics. It resulted in a drop of color and flavor values, but the new features were well tolerated by evaluating panelists based on their comments on the questionnaire, where new features were described as nutty, caramel and almond. Polyphenols are known as precursors of many substances responsible for desired flavor of heated foods, including the formation of the toast, caramel and roasted flavors (Jiang and Peterson, 2010).

Significant difference in water activity (a_w) between the control and the GCE enriched product was observed for both types of *gnocchi* (Table 2). Other products showed a similar level of a_w whatever the GCE addition caused a slight tendency ($p < 0.05$) for a_w growth together with increasing supplementation with GCE.

Regardless of the type of product a drop of pH associated with the enrichment in GCE was observed ($p < 0.05$) (Table 2). This was generally caused by the introduction of the extract, which contains caffeic acid and its esters with quinic acid, partially dissociated in

Table 2. Physical and sensory properties of products containing green coffee extract (GCE)

Type of product and concentration of GCE (g 100 g^{-1})	Water activity	pH	Sensory evaluation (1-5)		Texture	Color coordinates			
			color	flavor		L*	a*	b*	ΔE
GF control	0.651^{d,e}	5.37^{e,f}	5.0^{a}	5.0^{a}	1.59*^{f}	75.11^{a}	-1.57^{i,j}	16.46^{d}	-
GF 0.25	0.719^{f}	5.29^{d,e}	5.0 ^{a}	5.0 ^{a}	1.97*^{e}	70.88^{b,c}	-1.60^{j}	13.21^{g,h}	1.18
GF 0.5	0.721^{f}	5.22^{d,e}	3.5 ^{c,d}	4.0 ^{b,c}	2.07*^{d,e}	70.18^{b,c}	-1.77^{j}	12.63^{h}	5.78
GF 1.0	0.753^{f,g}	4.86^{c}	1.0^{e}	1.0^{e}	2.71*	64.49^{f}	-1.05^{h}	12.02^{i}	6.49
GD control	0.617^{c}	5.74^{f}	5.0 ^{a}	5.0 ^{a}	1.33*^{g}	92^{a,b}	-1.36^{i}	13.66^{g}	-
GD 0.25	0.730^{f}	5.72^{f}	4.5 ^{a,b}	5.0 ^{a}	1.45*^{f,g}	72.09^{a,b}	-1.72^{j}	13.09^{g,h}	1.59
GD 0.5	0.724^{f}	5.48^{e,f}	3.5^{c,d}	4.0 ^{b,c}	1.59*^{f}	71.38^{b}	-2.52	12.04^{i}	1.78
GD 1.0	0.736^{f}	5.10^{d}	1.0^{e}	1.0^{3}	2.38*^{c}	70.61^{b,c}	-0.72^{g,h}	11.60^{j}	2.93
SC control	0.776^{g}	7.20^{i}	5.0 ^{a}	5.0 ^{a}	1.37*^{g}	78.39	0.58^{d}	14.65^{f}	-
SC 0.25	0.781^{g,h}	6.89^{h,i}	5.0 ^{a}	5.0 ^{a}	1.62*^{f}	67.47^{d,e}	3.76	16.42^{d,e}	11.51
SC 0.5	0.804^{h}	6.64^{h}	4.5 ^{a,b}	4.5 ^{a}	1.66*^{f}	66.77^{e}	4.80^{a}	18.33^{b,c}	12.67
SC 1.0	0.798^{h}	6.50^{g,h}	3.5 ^{c,d}	4.0 ^{b,c}	1.78*^{e,f}	66.46^{e,f}	5.07 ^{a}	18.75^{b}	12.80
B control	0.796^{h}	5.79^{f}	5.0 ^{a}	5.0 ^{a}	1.45*^{f,g}	72.50^{b,c}	0.69	16.23^{e}	-
B 0.25	0.791^{h}	5.58^{e,f}	4.5 ^{a,b}	5.0 ^{a}	2.03*^{d,e}	69.20^{c}	0.18^{e}	14.08^{f}	4.38
B 0.5	0.815^{h,i}	5.56^{e,f}	4.0^{b,c}	4.5 ^{a}	2.13*^{d}	68.46^{c}	0.09^{e}	13.42^{g}	4.63
B 1.0	0.820^{i}	5.53^{e,f}	3.0^{c}	3.5 ^{c,d}	2.50*^{b}	68.17^{c,d}	-0.33^{f}	12.74^{h}	5.58
MY control	0.674^{e}	6.81^{h}	5.0 ^{a}	5.0 ^{a}	0.12$^{\#}$	60.33^{g}	-0.61^{g}	11.94^{i,j}	-
MY 0.25	0.679^{e}	6.56^{g,h}	5.0 ^{a}	5.0 ^{a}	0.17$^{\#a}$	59.16^{g}	-0.67^{g}	11.73^{j}	0.51
MY 0.5	0.712^{f}	6.55^{g,h}	5.0 ^{a}	5.0 ^{a}	0.18$^{\#a}$	58.82^{g}	-0.87^{h}	11.44^{k}	0.53
MY 1.0	0.698^{e,f}	6.50^{g,h}	5.0 ^{a}	5.0 ^{a}	0.18$^{\#a}$	58.76^{g}	-0.87^{h}	11.40^{k}	1.20
MR control	0.613^{c}	6.56^{g,h}	5.0 ^{a}	5.0 ^{a}	2.28*^{c}	85.36	-0.30^{f}	16.84^{d}	-
MR 0.25	0.611^{c}	6.53^{g,h}	5.0 ^{a}	5.0 ^{a}	2.33*^{c}	81.87^{a}	-0.19	20.19^{a}	0.65
MR 0.5	0.631^{d}	6.50^{g,h}	5.0 ^{a}	5.0 ^{a}	2.36*^{c}	81.33^{a}	0.66^{d}	21.05^{a}	1.35
MR 1.0	0.634^{d}	6.35^{g}	5.0 ^{a}	4.0 ^{b,c}	2.55*^{b}	81.26^{a}	0.99^{c}	21.19^{a}	5.55
C control	0.373^{a}	5.33^{d,e}	5.0 ^{a}	5.0 ^{a}	0.36°^{a}	48.18^{h}	-0.32^{f}	17.01^{c,d}	-
C 0.25	0.363^{a}	5.18^{d}	5.0 ^{a}	5.0 ^{a}	0.38°^{a}	49.06^{h}	1.18^{c}	16.68^{d}	1.07
C 0.5	0.392^{b}	5.03^{c,d}	5.0 ^{a}	5.0 ^{a}	0.56°	49.04^{h}	1.74^{b}	13.92^{f,g}	3.55
C 1.0	0.403^{b}	4.80^{c}	4.0^{b,c}	4.0 ^{b,c}	0.83°	45.62	1.60^{b}	12.48^{h,i}	5.22
J control	0.681^{e,f}	3.49	5.0 ^{a}	5.0	5.32*	60.78^{g}	5.02^{a}	28.81	-
J 0.25	0.720^{f}	3.20^{b}	5.0 ^{a}	5.0	5.70*^{a}	59.90^{g}	4.36	25.05	9.87
J 0.5	0.722^{f}	3.13^{3,b}	5.0 ^{a}	5.0	5.74*^{a}	58.84^{g}	3.12	13.01^{h}	16.63
J 1.0	0.738^{f}	3.03^{a}	5.0 ^{a}	4.0 ^{b,c}	5.80*^{a}	49.36^{h}	2.85	12.03^{i}	20.38

* - maximum force of compressing (kg); # - maximum force during squeezing through a gap (kg); ◊ - maximum depression (mm). Different letters or lack of them in a column indicate statistically significant differences ($p > 0.05$); ($n = 6$). Labeling of products as described in Table 1.

the environment of the obtained products (Beltrán *et al.*, 2003)

The obtained products were analyzed using different tests characterizing their texture (Table 2). Caramel candies with the addition of GCE were softer ($p < 0.05$) than the control product as GCE limited crystallization of saccharose. It also caused the growth of hygroscopicity, as evidenced by the rise of aw. For other products, addition of coffee extract caused an increase in their hardness ($p < 0.05$). To the highest degree it concerned both types of *gnocchi* and bread. In these products we can expect a covalent interaction of polyphenols and proteins, resulting in increased cross-linking of proteins (Selinheimo *et al.*, 2007). In jellies, marshmallows and sponge cake the increase of hardness ($p < 0.05$) due to the addition of GCE was relatively smaller than in bread, which may be caused by the presence of saccharose limiting the interactions between polyphenols and proteins (Gonçcalves *et al.*, 2011). The influence of GCE on the texture of mayonnaise was also observed, which was statistically independent of the concentration of the additive, and caused an increase ($p < 0.05$) of cohesiveness and lowering of the spill effect.

GCE addition for each of the products (beside mayonnaise) caused its darkening ($p < 0.05$) (decrease of the L^* parameter) (Table 2). The maximum darkening concerned the sponge cake, and also fresh potato *gnocchi* and jellies. The lowest changes of L^* were found for mayonnaise. In this case, the control product was relatively dark, mainly due to the content of traditional components, which showed a masking effect for GCE. In the analyzed products the contents of the green and red dyes were rather low. GCE addition caused an increase of the parameter a^* in sponge cake, caramel candies and marshmallows ($p < 0.05$). These products were obtained at high temperature and at a relatively low humidity, where the addition of GCE contributed to the more intense formation of pigments as a result of non-enzymatic browning. In the case of gnocchi, in the process of boiling, a high degree of hydration of the product counteracts the non-enzymatic darkening. Other products have been obtained at lower temperatures. The b^* parameter in products with the addition of GCE increased only in the case of sponge cake and marshmallows ($p < 0.05$). These products were intensely aerated during preparation, so the oxidation of the components of GCE could affect the formation of yellow pigments in these two products (Namiki *et al.*, 2001). In other products the addition of the components of green coffee has caused the decrease of the b^* parameter ($p < 0.05$). Additionally the ΔE indicator was calculated, which determines the total changes of color. The biggest ΔE was found for jellies, followed by sponge cake. In these products darkening and changes in the content of red and yellow pigments were clearly observed,

Table 3. Concentration of major fatty acids in mayonnaise and marshmallows enriched with green coffee extract (GCE)

Type of product	Fatty acid	Concentration of GCE (g 100 g^{-1}) Control	0.25	0.5	1.0
		fatty acid concentration (g 100 g^{-1} of total fatty acids)			
MY	Palmitic	5.80^{a}	5.65^{b}	5.70^{a,b}	5.69^{a,b}
	Stearic	3.65^{a}	3.65^{a}	3.55^{b}	3.52^{b}
	Oleic	26.30^{a}	26.16^{a,b}	26.05^{b}	26.08^{b}
	Linoleic	61.32	61.61	61.80^{a}	61.85^{a}
	SFA	10.87	10.73	10.60	10.55
	MUFA	26.87	26.71	26.59	26.62
	PUFA	62.26	62.58	62.81	62.83
MR	Lauric	1.32	1.09^{a}	0.93^{a}	0.70
	Myristic	5.72	4.46	3.28	2.67
	Palmitic	39.83	41.95^{a}	42.65^{a}	44.83
	Stearic	12.05^{a}	12.32^{a}	11.34^{b}	10.61^{b}
	Oleic	29.16^{c}	30.04^{b,c}	31.18^{a,b}	32.07^{a}
	Linoleic	3.54^{b}	4.01^{b}	4.81^{a}	4.95^{a}
	ΣSFA	62.02	62.08	60.27	60.35
	ΣMUFA	33.74	33.11	34.04	34.16
	ΣPUFA	4.55	5.16	5.96	5.82

SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids. Different letters or lack of them in a row indicate statistically significant differences ($p > 0.05$); (n = 6). Labeling of products as described in Table 1.

i.e. the increase in sponge, and in the case of jellies a significant decrease of both a^* and b^* coordinates of the color. The lowest ΔE was found for mayonnaise. This product with GCE showed ΔE below the level of detection of the human eye (<3).

Fatty acids composition

The determination of fatty acids composition was performed only for those products, in which fat content exceeded 5%. These products were mayonnaise and marshmallows. In mayonnaise fat was composed mainly from sunflower oil and egg yolk lipids. In marshmallows it was mainly milk fat. In mayonnaise linoleic acid was present in largest amounts (Table 3). In fat from marshmallows, among unsaturated fatty acids, a significant content of oleic acid and linoleic acid was found. In both of these fatty acid cases the addition of GCE had a positive influence on their content in the product ($p < 0.05$). Simultaneously the stearic acid content was lowered. The reduction potential of polyphenols is lower than that of polyunsaturated fatty acids. Polyphenols therefore more willingly react with lipid hydroperoxide radicals than polyunsaturated fatty acids and terminate chain reaction, what explains the beneficial effects of the GCE addition on the fatty acid composition. However, for high used concentrations of antioxidants from coffee beans in the fatty food products this effect has not been earlier confirmed (Leonardis *et al*., 2008; Valencia *et al*., 2008). The presence of chlorogenic acid may be particularly advantageous in products containing vitamin E, such as mayonnaise, because of the synergistic effect of chlorogenic with α-tocopherol confirmed earlier by Sim *et al*. (2010). Moreover, in more complex food products polyphenols protect protein against radicals produced by the oxidation of lipids (Salminen and Heinonen, 2008).

Amino acids composition and in vitro pepsin digestibility

Analysis of amino acids and protein *in vitro* pepsin digestibility was not performed in caramel candies and mayonnaise due to the low protein content of these products. Addition of GCE contributed to the greater degradation of amino acids in food products after their preparation as compared to controls (Table 4). These losses associated with GCE addition were summarized from 7% in bread to 16% in jellies, but the last of these product contained gelatin, which generally contains proteins of low nutritional value. During the preparation of bread, the loss of amino acids was statistically lower than in case of sponge cake both baked with GCE, even though these products were obtained under similar conditions. The reason may be lower pH of bread that limits the interactions of proteins and polyphenols. The relatively large losses of amino acids in jellies were somewhat surprising in view of the fact that the product was of a relatively low pH, which limits the interaction of proteins with polyphenols, moreover, the product was not aerated or exposed to temperatures above 100°C. Thus, in this case, also another mechanism has to work that predestined gelatin for greater derivatization, such as cross-linking in the presence of GCE. Similar observation was also confirmed in the studies of Gramza-Michalowska and Regula (2007) by impact of green tea polyphenols on jellies consistency and Kosaraju *et al.* (2010) on gelatin cross-linked by phenolic acids.

The greatest loss of amino acids in the tested products was generally related to such amino acids as tryptophan and isoleucine, although in individual products the high level of degradation could also concern other amino acids, such as lysine in fresh potato *gnocchi* and sponge cake, valine in jellies, leucine and phenylalanine in marshmallows. For most of these amino acids strong interactions with chlorogenic acid or its oxidized form were previously shown (Prigent *et al*., 2008). However, derivatization of proteins in the presence of polyphenols does not necessarily mean lowering their susceptibility to digestion (Petzke *et al*., 2005). Despite the significant loss of amino acids, severe changes in pepsin digestibility in the resulting products were not observed, the lowering of protein digestibility did not exceed 3%. Non-covalently linked adducts of

Table 4. Essential amino acid profile and *in vitro* protein digestibility of food products containing green coffee extract (GCE)

Type of product and concentration of GCE (g 100 g^{-1})		Amino acid (g 100 g^{-1})									Pepsin digestibility (%)
		HIS	THR	VAL	MET	ILE	LEU	PHE	LYS	TRY	
GF	Control	0.017^{a,b}	0.073^{a}	0.175^{a}	0.207	0.164^{a}	0.207^{a}	0.156	0.123	nd	80.77^{d,e}
	0.25	0.018^{a}	0.068^{a,b}	0.169^{a,b}	0.194^{a}	0.155^{a,b}	0.206^{a}	0.143^{a}	0.113^{a}	nd	81.90^{d}
	0.5	0.015^{b}	0.066^{a,b}	0.157^{b}	0.191^{a}	0.148^{b}	0.199^{a}	0.141^{a}	0.107^{a,b}	nd	81.23^{d}
	1.0	0.013^{b}	0.062^{b}	0.154^{b}	0.191^{a}	0.146^{b}	0.197^{a}	0.136^{a}	0.103^{b}	nd	78.20^{e}
GD	Control	0.005^{a}	0.093	0.228	0.342^{a}	0.154^{a}	0.220^{a}	0.190^{a}	0.125^{a}	0.067	76.85^{f}
	0.25	0.005^{a}	0.083^{a}	0.216^{a}	0.337^{a}	0.147^{a,b}	0.219^{a}	0.181^{a,b}	0.122^{a,b}	0.051^{a}	76.28^{f,g}
	0.5	0.001^{b}	0.084^{a}	0.215^{a}	0.326^{b}	0.143^{b}	0.215^{a,b}	0.171^{b,c}	0.117^{a,b}	0.051^{a}	75.91^{g}
	1.0	0.002^{b}	0.083^{a}	0.213^{a}	0.316^{b}	0.138^{b}	0.207^{b}	0.163^{c}	0.110^{b}	0.049^{a}	75.23^{g}
SC	Control	0.198^{a}	0.266^{a}	0.633^{a}	0.485^{a}	0.320^{a}	0.694^{a}	0.399^{a}	0.508^{a}	0.070^{a}	87.45^{c}
	0.25	0.191^{a,b}	0.261^{a}	0.620^{a}	0.475^{a,b}	0.311^{a,b}	0.693^{a}	0.383^{a,b}	0.497^{a,b}	0.065^{a,b}	89.19
	0.5	0.188^{b,c}	0.246^{b}	0.591^{b}	0.473^{a,b}	0.312^{a,b}	0.678	0.378^{b}	0.487^{a,b}	0.056^{b,c}	87.66^{c}
	1.0	0.175^{c}	0.235^{b}	0.582^{b}	0.467^{b}	0.294^{b}	0.649	0.354	0.443	0.044^{c}	85.59^{c}
B	Control	0.234^{a}	0.242^{a}	0.411^{a}	0.149	0.252^{a}	0.644^{a}	0.489^{a}	0.160^{a}	0.127	64.46^{h}
	0.25	0.229^{a,b}	0.238^{a}	0.400^{a,b}	0.133^{a}	0.245^{a}	0.631^{a,b}	0.478^{a,b}	0.155^{a,b}	0.101^{a}	63.16^{i}
	0.5	0.227^{a,b}	0.230^{a}	0.396^{b}	0.131^{a}	0.235^{b}	0.627^{a,b}	0.465^{b}	0.147^{b}	0.101^{a}	63.37^{i}
	1.0	0.217^{b}	0.229^{a}	0.395^{b}	0.126^{a}	0.233^{b}	0.618^{b}	0.459^{b}	0.146^{b}	0.094	63.92^{h}
MR	Control	0.043^{a}	0.144^{a,b}	0.188	0.072^{a}	0.041^{a}	0.189	0.161^{a}	0.554	0.021^{a}	36.43
	0.25	0.041^{a,b}	0.134^{b}	0.172^{a}	0.067^{a,b}	0.040^{a}	0.172^{a}	0.159^{a}	0.532^{a}	0.019^{a}	38.70
	0.5	0.041^{a,b}	0.133^{b}	0.166^{a,b}	0.063^{b}	0.033^{b}	0.163^{a,b}	0.152^{a,b}	0.523^{b}	0.019^{a}	34.52^{j}
	1.0	0.038^{b}	0.128^{b}	0.155^{b}	0.062^{b}	0.030^{b}	0.154^{b}	0.146^{b}	0.525^{a,b}	0.011	33.80^{j}
J	Control	0.064^{a}	0.104	0.114^{a}	0.053^{a}	0.048^{a}	0.135^{a}	0.113	0.180^{a}	0.046^{a}	97.84^{a}
	0.25	0.058^{a,b}	0.090^{a}	0.109^{a}	0.053^{a}	0.048^{a}	0.132^{a,b}	0.100^{a}	0.179^{a}	0.045^{a}	97.57^{a}
	0.5	0.058$_{a,b}$	0.085^{a}	0.090^{b}	0.053^{a}	0.045^{a}	0.126^{b}	0.095^{a}	0.177^{a}	0.031^{b}	96.81^{b}
	1.0	0.055^{b}	0.085^{a}	0.087^{b}	0.045	0.038	0.118	0.089^{a}	0.176^{a}	0.030^{b}	96.92^{a,b}

HIS – histidine; THR – threonine; VAL – valine; MET methionine; ILE isoleucine; LEU – leucine; PHE – phenylalanine; LYS – lysine; TRP – tryptophan; nd - not detected Different letters or lack of them in a column indicate statistically significant differences ($p > 0.05$); (n = 6). Labeling of products as described in Table 1.

polyphenols with proteins can be hydrolyzed and do not affect the absorption of proteins and polyphenols, and released polyphenols again show antioxidant activity *in vivo* (Rawel *et al*., 2001; Rufián-Henares and Morales, 2007). It should be pointed out that some limits of protein digestibility are a compromise in relation to the beneficial effect of products fortified with polyphenols, which help to lower triglycerides and LDL cholesterol and increase HDL cholesterol in a cholesterol rich diet or reduce absorption of glucose in obese and people suffering from type II diabetes (Zduńczyk *et al.*, 2002).

Caffeine and phenolic acids contents

In GCE and prepared food products, caffeine, and caffeic acid derivatives contents were analyzed (Table 5). The listed compounds were subject to little changes in mayonnaise, which is obtained at room temperature. Comparing with the content of caffeine and polyphenols introduced into mayonnaise with GCE it can be concluded that the amount of caffeine was statistically the same ($p > 0.05$), the total polyphenol content also did not differ ($p > 0.05$). A partial isomerization and hydrolysis of the phenolic acids took place, which resulted in the drop of concentration of acids such as 3-*O*-caffeoylquinic acid and diesters, namely dicaffeoylquinic acids in favor of 5-*O*-caffeoylquinic acid. A relatively small decrease of polyphenols content was determined in jellies ($p < 0.05$). Given the increased degradation of amino acids in GCE-enriched jellies it should be assumed that the loss of polyphenols (about 10%) resulted probably from their interaction with proteins. In *gnocchi* small amount of polyphenols was observed in the control product, due to the content of chlorogenic acid in potatoes. In both types of enriched in GCE *gnocchi* the polyphenol losses, taking into account the increased hydration of the product after cooking, did not exceed 15%. Losses in this case were mainly due to dissolution in water, as indicated by the loss of caffeine not occurred in any other product ($p > 0.05$). Also partial isomerization and hydrolysis of chlorogenic acids took place in gnocchi. Similar polyphenol contents were found in marshmallows and caramel candies, in which the losses amounted to less than 20%, taking into account partial evaporation of water during processing. In the other two products - bread and sponge cake the greatest losses of polyphenols were observed, and taking into account water evaporation caused by baking process, the actual losses reached 40%.

From data on degradation of amino acids and polyphenols it can be concluded that significant deterioration in both groups of components can be associated with interactions between them. However, the ratio of amino acids to polyphenols has to be taken into account. And so in jellies there was relatively the least amount of amino acids, so perhaps their degradation was so far advanced. Instead, in sponge cake there was much more of essential amino

Table 5. Caffeine and phenolic acids contents of green coffee extract (GCE) *(mg g^{-1}) and food products containing green coffee extract (GCE) (mg 100 g^{-1})

Type of product and concentration of GCE (g 100 g^{-3})	Caf.	3-*O*-CQA	4-*O*-CQA	5-*O*-CQA	4-*O*-FQA	5-*O*-FQA	3,4-*O*-diCQA	3,5-*O*-diCQA	4,5-*O*-diCQA	Total polyphenols
GCE*	$62.57^{f,g}$	80.78	83.36^{b}	100.09^{f}	7.62^{e}	$12.08^{f,g}$	25.84	17.23	$14.26^{a,b}$	341.25^{a}
GF control	-	0.31	2.72	2.65	-	-	-	-	-	5.68
0.25	15.37^{g}	18.66	16.21^{f}	31.48^{e}	1.46^{b}	3.82^{d}	0.39^{h}	0.33^{g}	0.20^{i}	72.55^{c}
0.5	26.28^{i}	$34.12^{b,c}$	35.15^{d}	$66.66^{d,e}$	$2.85^{b,c}$	7.61^{d}	$0.65^{h,i}$	$0.57^{g,h}$	0.55^{i}	148.16^{c}
1.0	56.04	53.56^{g}	$69.13^{d,e}$	129.45^{e}	5.33^{c}	14.01^{e}	1.05^{i}	0.93^{h}	0.92^{i}	274.38^{d}
GD 0.0	-	0.17	1.81	1.82	-	-	-	-	-	3.80
GD 0.25	14.94^{h}	14.32^{f}	17.90^{d}	$37.74^{b,c}$	0.44	2.70^{g}	1.12^{g}	0.95^{e}	$0.91^{f,g}$	$6.08^{b,c}$
GD 0.5	26.24^{i}	$25.99^{h,i}$	32.36^{f}	68.75^{d}	1.93^{e}	$6.99^{e,f}$	2.25^{g}	1.92^{e}	1.98^{f}	142.17^{d}
GD 1.0	52.89^{j}	$51.28^{i,j}$	68.73^{e}	122.73^{e}	$4.48^{d,e}$	13.17^{f}	2.48	2.02	2.16^{h}	267.05^{d}
SC 0.25	18.57^{a}	16.29^{e}	17.75^{d}	$24.08^{f,g}$	$1.24^{c,d}$	2.50^{g}	1.65^{f}	$1.54^{c,d}$	$1.49^{d,e}$	66.54^{e}
SC 0.5	33.11^{d}	24.35^{h}	25.22	45.49^{g}	1.56^{e}	$4.09^{h,j}$	4.28^{d}	3.92^{b}	3.83^{c}	112.72^{f}
SC 1.0	63.11^{f}	54.25^{g}	56.96^{h}	106.92^{f}	3.73^{e}	10.22^{g}	8.84^{d}	7.82^{b}	7.48^{c}	256.22^{e}
B 0.25	18.05^{a}	17.82^{a}	14.76^{g}	18.97	1.00^{d}	2.22^{h}	$1.94^{d,e}$	1.69^{c}	1.60^{d}	59.99
B 0.5	$33.64^{c,d}$	35.46^{a}	29.91^{g}	35.85^{h}	$1.74^{e,f}$	2.07^{j}	3.36^{f}	2.96^{d}	2.82^{e}	114.18^{f}
B 1.0	62.17^{f}	71.66^{a}	55.85^{h}	70.48^{h}	$4.49^{d,e}$	7.17^{i}	6.73^{f}	6.44^{c}	6.37^{d}	229.20^{f}
MY 0.25	15.42^{g}	16.60^{e}	22.84^{a}	36.45^{c}	1.65^{a}	4.60^{a}	1.25^{g}	0.96^{e}	1.28^{e}	82.14^{b}
MY 0.5	$30.64^{g,h}$	30.46^{d}	45.08^{a}	79.69^{b}	3.01^{b}	8.63^{a}	$3.99^{d,e}$	3.93^{b}	4.05^{c}	178.84^{a}
MY 1.0	$62.56^{f,g}$	$58.92^{e,f}$	80.43^{c}	144.86^{c}	$6.10^{a,b}$	17.42^{a}	13.44^{c}	10.77	10.25	352.19^{a}
MR 0.25	15.40^{g}	14.26^{f}	17.75^{d}	26.10^{f}	0.78^{e}	3.16^{f}	$.87^{e,f}$	1.67^{c}	1.63^{d}	67.22^{e}
MR 0.5	$31.96^{e,f}$	$26.82^{g,h}$	27.52^{h}	63.59^{e}	0.95^{f}	$4.01^{h,j}$	$7.50^{b,c}$	7.00^{a}	6.88^{b}	$144.27^{c,d}$
MR 1.0	$63.91^{e,f}$	52.35^{h}	60.89^{g}	120.95^{e}	1.84^{f}	7.40^{i}	$16.33^{a,b}$	14.95^{a}	$14.49^{a,b}$	289.22^{c}
C 0.25	17.35^{b}	15.29^{d}	22.25^{a}	38.93^{b}	0.07	0.50^{k}	0.17	0.08^{f}	0.06	67.35^{e}
C 0.5	$33.81^{c,d}$	$32.34^{c,d}$	40.25^{c}	$71.96^{c,d}$	0.52^{g}	2.25^{j}	$3.91^{d,e}$	0.23^{f}	1.52^{g}	$152.98^{b,c}$
C 1.0	$63.94^{e,f}$	$60.02^{d,e}$	$81.70^{b,c}$	131.92	1.22^{g}	2.65^{k}	7.05^{e}	0.13	1.92^{h}	$286.61^{c,d}$
J 0.25	15.53^{g}	12.93^{i}	12.47^{i}	48.57^{a}	$1.11^{d,e}$	$4.07^{b,c}$	4.42^{a}	3.91^{a}	3.57^{a}	91.05^{a}
J 0.5	$31.25^{f,g}$	25.12^{j}	23.94^{i}	95.80^{a}	$2.18^{d,e}$	$8.57^{a,b}$	9.60	11.40	8.10	187.71
J 1.0	62.88^{f}	48.47^{h}	56.86^{h}	149.11	$4.18^{d,e}$	15.43^{c}	15.18^{b}	14.08^{a}	13.86^{b}	317.18^{b}

Statistical analysis of the losses of particular compounds was calculated taking into account the differences resulting from the added amounts of GCE (for products with the addition of 0.25 g 100 g^{-1} content was multiplied times 4, for 0.5 g 100 g^{-1} times 2 and then compared with the content in products with addition of 1 g 100 g^{-1} and with content in initial GCE).
Caf – Caffeine, 3-CQA - 3-*O*-Caffeoylquinic acid, 4-CQA - 4-*O*-Caffeolylquinic acid, 5-CQA - 5-*O*-Caffeoylquinic acid, 4-FQA - 4-*O*-Feruloylquinic acid, 5-FQA - 5-*O*-Feruloylquinic acid, 3,4-diCQA - 3,4-*O*-Dicaffeoylquinic acid, 3,5-diCQA - 3,5-*O*-Dicaffeoylquinic acid 4,5-diCQA - 4,5-*O*-Dicaffeoylquinic acid. Different letters or lack of them in a column indicate statistically significant differences ($p > 0.05$); (n = 6). Labeling of products as described in Table 1.

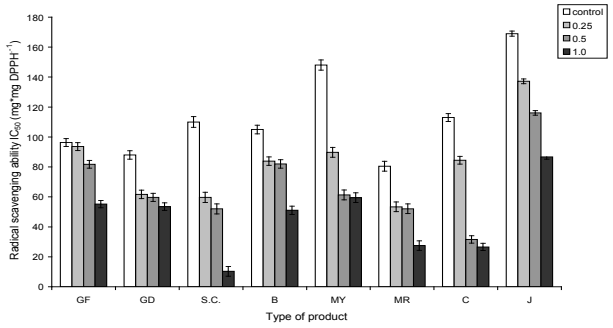

Figure 1. Radical scavenging ability of food products containing green coffee extract (GCE) in concentrations of 0.25, 0.5 and 1 g 100 g^{-1}. Labeling of products as described in Table 1. Bars represent standard error of the mean; (n = 6)

acids, which may decide on their lesser degradation despite the substantial loss of polyphenols. In regard to caffeine, the decrease was observed only in the boiled products, due to its partial dissolution in water. In some products a relative increase in caffeine content caused by a partial evaporation of moisture during the preparation was observed.

Antioxidant activity

Radical scavenging ability of the received food products was determined in the reaction with the DPPH$^{\cdot}$ radical. All the obtained control products without the addition of GCE showed little ability to scavenge the radical in the range from 88 to 170 mg mg^{-1} of DPPH$^{\cdot}$ (Figure 1). For example, Trolox has a capacity of 0.28 mg mg^{-1} of DPPH$^{\cdot}$, and 5-*O*-CQA, which is a major component of GCE, 1.63 mg mg^{-1} of DPPH$^{\cdot}$ (Budryn and Nebesny, 2008). The best properties in this regard were found for marshmallows. This could be caused by natural antioxidants introduced with butter (possibly fortified with antioxidants). Preferable properties have also been found for gnocchi, particularly the ones obtained from dried potatoes concentrate. The potatoes contain a certain amount of chlorogenic acid, and vitamin C, and in the case of *gnocchi* made from dried potatoes, this ability could be increased by the presence of the antioxidants added during the drying processing. Further positions were given to bread, sponge cake and caramel candies. The weakest ability to scavenge DPPH$^{\cdot}$ radical was shown by mayonnaise and jellies.

Addition of coffee extract resulted in case of each of the products in an increase of radical scavenging ability. The largest increase in activity occurred in the case of sponge cake. IC_{50} for sponge cake reached

even a value of 10 mg mg^{-1} of $DPPH^{\cdot}$. Sponge cake with GCE was characterized by a significant increase in the content of pigments, especially red, which, as indicated by many studies, favors the increase of antioxidant properties. Large increase of scavenging capacity was also found for caramel candies, processed at relatively high temperature. Since in case of these products the losses of polyphenols were significant, it is believed that the high antioxidant activity may come from compounds resulting from thermal degradation of polyphenols (Manzocco *et al.*, 2001). A similar antioxidant activity was found for enriched marshmallows but in this case the increase in antioxidant activity due to the GCE addition was not as significant as for first two products. A significant increase ($p < 0.05$) in antioxidant activity was however showed by supplemented mayonnaise, *gnocchi* made from fresh and dried potatoes and bread. In case of jellies the highest IC_{50} was observed, which was also due to low radical scavenging ability of the control product. Perhaps, in this case, an increase in the prooxidative activity of polyphenols undergoing short heating was observed (Andueza *et al*., 2009).

The highest increase of antioxidant activity in sponge cake and caramel candies after GCE supplementation indicates the formation of compounds with high antioxidant potential due to exposure to high temperatures at a relatively low humidity of the product. The increase of scavenging capacity does not require the interaction of polyphenols with proteins at high temperature, as evidenced by the low value of the IC_{50} in GCE-enriched caramel candies, where the protein content is practically negligible. However, product formulation can affect the antioxidant activity changes in heated products, as evidenced by the differences in radical scavenging ability of sponge cake and bread, obtained under similar conditions of temperature and humidity of the product. In this case, probably the presence of saccharose favored the increase of antioxidant activity in sponge (Charurin *et al.*, 2002).

Conclusions

Green coffee extract was added to food products to increase their antioxidant activity and to introduce additional amount of polyphenols along with typical diet. Polyphenols were the most stable in mayonnaise and jellies, the products obtained at low temperature, while the antioxidant activity increased by the addition of the GCE mostly in sponge cake, caramel candies, marshmallows, and also mayonnaise. Thus, products obtained at high temperatures, in which a significant degradation of polyphenols was observed, were characterized by a large increase in antioxidant activity. This indicates that the products of thermal degradation of polyphenols next to the same polyphenols cause an increase of antioxidant activity of food products.

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