Catechin stability, antioxidant properties and sensory profiles of rye breads fortified with green tea extracts

JOANNA BAJERSKA – SYLWIA MILDNER-SZKUDLARZ – JAN JESZKA – ARTUR SZWENGIEL

Summary

The effect of green tea extracts (GTE) on catechin stability, antioxidant properties and sensory profiles were analysed in rye breads (RB). The following samples of RB were prepared: control bread (C), breads with 0.5, 0.8 and 1.1% of GTE, respectively. Separation, identification and quantification of GTE catechins were achieved by HPLC. Antioxidant properties were measured by the method of radicals coloured by 2,2-diphenyl-1-picrylhydrazyl (DPPH). Sensory quality of breads was determined using a hedonic test. The most stable catechins were as follows: epigallatocatechin (EGC) < < epigallatocatechin gallate (EGCG) < epicatechin gallate (ECG), and detectable amounts increased with an increase in the level of GTE added to RB. The antioxidant effectiveness decreased in the order 1.1% GTE > 0.8% GTE > 0.5% GTE > C. Sensory characteristics, such as scores for aroma, flavour and overall acceptability, decreased significantly (in comparison to C) when RB was prepared with 1.1% GTE. Rye bread with 1.1% GTE was good in terms of antioxidant properties. However, due to changes in sensory properties, the level of GTE powder incorporated to bread should be limited to 0.8% or bread making technology with high levels of GTE should include addition of flavour-masking additives.

Keywords

rye bread; green tea extracts; sensory evaluation; polyphenol content; antioxidant capacity

In recent years, the general population of the industrialized world has demonstrated an increased interest in the role of foods with an addition of physiologically active plant ingredients (i.e., phytochemicals) or ingredients of animal origin (i.e., zoochemicals) in the well-being and life prolongation, as well as the prevention of initiation, promotion and development of cancer, cardiovascular diseases, osteoporosis and obesity. Those foods are defined as functional foods [1–3].

Bread is arguably one of the oldest functional foods developed by humans [4]. Owing to recommendations that bread, and especially whole-grain rye bread, should be an integral part of the diet, it seems that this product can be used as a matrix possibly capable of supplying functional ingredients to particular groups of people. Many nutrients have been added to bread, such as minerals, fibre, vitamins or some essential oils containing natural antioxidants [5].

Green tea contains a number of biologically active compounds, which include epicatechin (EC), epigallatocatechin (EGC), epicatechin gallate (ECG) and epigallatocatechin gallate (EGCG) [6–8]. These compounds, collectively referred to as green tea catechin polyphenols, exhibit antioxidant properties as free radical scavengers [9]. Recent epidemiological studies showed that green tea catechin polyphenols may reduce the risk of a variety of different diseases [10–13] and consumed on a daily basis may be conductive to maintenance of good health and long life [14, 15]. Green tea catechin polyphenols account for 35% of dry weight in green tea [6, 7].

Numerous human intervention studies on green tea demonstrate a significant increase in

Joanna Bajerska, Jan Jeszka, Department of Human Nutrition and Hygiene, Poznan University of Life Sciences, Wojska Polskiego 31, 60-624 Poznan, Poland.
Sylwia Mildner-Szkudlarz, Artur Szwengiel, Institute of Food Science and Nutrition, Poznan University of Life Sciences, Wojska Polskiego 31, 60-624 Poznan, Poland.

Correspondence address
Joanna Bajerska, tel. 0048 61 846 60 56, fax: 0048 61 848 73 32, e-mail: joanna.bajerska@up.poznan.pl

plasma antioxidant capacity in humans after consumption of moderate amounts from 1 to 6 cups of this beverage per day [8, 16]. For example, ERBA and co-workers indicated that 8-week consumption of two cups of green tea beverages daily (containing approximately 250 mg of total catechins) with a balanced controlled diet contributed to improvement of overall antioxidant status [17]. SERAFINI et al. showed that plasma total antioxidant capacity values of subjects who drank green tea (a single dose of 300 ml of green tea beverage) rose at as early as after 30 min. After black tea and red wine ingestion; the peaks appeared 20 min later [18].

Thus, preparation of baked products with green tea extract (GTE) may prove a valuable means of enhancing polyphenol consumption and improve the antioxidant status in Western consumers. However, marketing success of this new functional product will be achieved when the product is characterized by an attractive taste and smell, with stable polyphenol and catechin contents, is easy to eat, and has a positive effect in the prevention of several stress oxidative-dependent diseases (e.g. atherosclerosis, Parkinson's disease, heart disease and Alzheimer's disease) [1, 5]. Moreover, new products might be alternatives for consumers who seek health benefits of both rye bread and green tea [3].

The effect of GTE addition on the quality of obtained food products and catechin stability in these products was studied only on frozen and unfrozen white bread [19–21], sponge cakes [22] and biscuits [23], but no such data are available for addition of green tea to bread made from rye flour. Moreover, baking technology of rye bread is different from that of wheat bread. A majority of rye breads is prepared as sourdough fermented bread, baked from wholemeal rye [24, 25]. The fermentation process during breadmaking provides some additional benefits for consumers, e.g. increase in the solubility of pentosans in bread, which is optimal at a lower pH, reduction in the enzymatic activity which improves dough process ability [24]. KATINA et al. indicated that the sourdough fermentation process can stabilize or increase levels of various bioactive compounds (e.g. polyphenols) in rye bread [25].

In this study, the polyphenol contents, catechin stability, antioxidant properties and sensory profiles were analysed in rye breads with different levels of green tea extracts.

MATERIAL AND METHODS

Preparation of breads

Bread samples were prepared using a two–phase method (sour – dough). Sourdough was prepared using 68 g of 2000 type rye flour (Diamant, International Polska, Grodzisk Wielkopolski, Poland), 102 ml of water and 0.34 g of freeze-dried LV4 starter cultures (*Saccharomyces chevalieri*, *Lactobacillus casei* and *Lactobacillus brevisi*; Lesaffre International, Marcq-en-Baroeul, France). Then the sourdough was fermented for 24 h at a controlled temperature of 28 °C.

The materials used for the preparation of control bread were 0.5 kg rye flour (350 g and 82 g of rye flour types 720 and 2000, respectively, (Diamant), contained 223 ml water, 7.5 g fine salt and 15 g instant yeast (*Saccharomyces cerevisiae,* Lesaffre International). Test rye bread samples were prepared with 0%, 0.5%, 0.8%, and 1.1% green tea powder per 100 g flour and, in this way, the following samples of this bread were produced: control rye bread (C), 0.5% GTE, 0.8% GTE and 1.1% GTE rye bread.

Ingredients were slowly mixed for 1 min followed by intense mixing to optimum consistency. Next, a dough piece was fermented for 30 min at a temperature of 32 °C. After the fermentation process, dough was molded three times for 5 min and then a dough piece was fermented for another period of 30 min. The dough was then placed in a non-stick baking pan, and then proofed at 30 °C for 15 min. The dough was then baked at 210 °C for 15 min at 75% relative humidity, to produce bread. In this way after 1 day storage in a haversack at ambient temperature, bread was subjected to instrumental measurements and sensory tests. Other samples of rye breads were mechanically cut into slices of 10 mm thickness with a bread slicer (Zelmer, Rzeszow, Poland). The central part of slices was lyophilized (Heto, Thermo Fisher Scientific, Waltham, Massachusetts, USA), ground and stored at –20 °C until polyphenol, HPLC and chemical analyses (i.e. ash, fat, protein, dietary fibre).

Plant extract

Tea (*Camelia sinensis* L.) leaf extracts were prepared for the study from Japan Sencha Fukuju Green Tea, which were bought at a specialty tea store (The House of Tea, Poznan, Poland). Green tea aqueous extracts were prepared according to the method presented by GRAMZA et al. [26] Aqueous extracts were prepared by boiling ground tea (100 g) in double-distilled water (ddH_2O; 1000 ml), followed by stirring for 15 min at 70 °C

(the procedure was repeated three times). Collected extracts were centrifuged after filtration (2700×*g* for 15 min) and then freeze-dried under vacuum (Multi Branch Trade and Manufacturing Company "Elena", Zelazkow, Poland). Aqueous GTE was prepared in a way that total catechins ≥15% (w/w, HPLC determination), polyphenol content ≥50% (w/w) and (–)-EGCG ≥9.0% as a quality marker of GTE powder.

Extraction of tea polyphenols and caffeine from bread crumbs

Tea polyphenols from bread crumbs was extracted using a method described by WANG and ZHOU [19] with minor modifications. One gram of the freeze-dried and ground sample was accurately weighed. The sample was extracted in 40 ml of an aqueous solution with 70% methanol, 29.7% water and 0.3% formic acid. The extraction was carried out in a water bath at 70 °C for 45 min with mechanical shaking. The aqueous layer was obtained by filtration and its volume was made up to 50 ml with the same solvent.

Total polyphenol contents

The level of total polyphenols in green tea and rye bread extracts was determined, and results were expressed as catechin equivalents (CE) in $mg \cdot g^{-1}$ of rye bread according to a method described by SINGLETON and ROSSI [27].

Determination of green tea catechin contents

The HPLC analyses of green tea catechin contents in GTE were performed on a Waters Alliance HPLC System 2695 (Milford, Massachusetts, USA) equipped with an X-Terra RP18 5 μm column (Milford) according to the method described by ANDRADE et al. [28] with minor modifications. Gradient elution was carried out using the following solvent system: mobile phase A consisting of methanol, and mobile phase B consisting of water and acetic acid 19 : 1 (v : v). The flow rate was 1 $ml \cdot min^{-1}$ throughout the analyses. The linear gradient elution system was: 2% A to 38% B for 60 min, hold at 62% A for 6 min and returning to 2% A, after another period of 2 min. The detection was performed with a PAD detector Waters 2996 in the range of 219–540 nm. Green tea catechin quantification was carried out on the basis of absorbances at 280 nm recorded in chromatograms relative to external standards of catechins. Sample injection volume was 10 μl. Column temperature was set at 40 °C. All reagents and standards used in the experiment were of analytical grade, supplied by Merck (Darmstadt, Germany) or Fluka (Buchs, Switzerland). Identification of each catechin in GTE and rye breads was made by comparing the retention time and UV spectrum with those of the standards of (–)-EGC, (–)-EGCG, (–)-EC, (–)-gallocatechin gallate (GCG), (–)-ECG, (–)-catechin gallate (CG), (–)-gallocatechin (GC) and (+)-catechin (C). Standards were freshly prepared for each series of analyses.

Chemical analysis

Bread samples were analysed for dry matter, ash (AOAC, Method 930.22, 926.03, 926.04), protein (AOAC, Method 926.04, 950.36, 920.87) and fat contents (AOAC, Method 922.06, 935.38) using analytical methods recommended by AOAC. [29–35]. Dietary fibre was analysed by a method described by ASP [36].

Sensory evaluation

Samples of rye breads (C, 0.5% GTE, 0.8% GTE; 1.1% GTE) were served to 52 panelists, made up of the staff and students of the Poznan University of Life Sciences, who were familiar with sensory attributes - taste, texture, colour, aroma and acceptability of the samples. A 10-point hedonic scale was designed to measure the degree of preference of samples. The samples were presented in identical containers, coded with 3-digit random numbers, served simultaneously to make it possible for panelists to re-evaluate a sample. The categories were converted to numerical scores ranging from 0 to 10, with 0 as the lowest and 10 as the highest.

The antioxidant properties

Radical scavenging activity of plant extracts against stable DPPH (2,2-diphenyl-2-picrylhydrazylhydrate) was determined spectrophotometrically according to the method described by HU et al. [37]. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. Changes in colour (from purple to light yellow) were measured at 515 nm on a UV/visible light spectrophotometer (Spekol 11; Jena Analytik, Jena, Germany). The DPPH radical solution of 0.0768 $mg \cdot ml^{-1}$ was freshly prepared in methanol, stored in a flask covered with aluminium foil and kept in the dark at 4 °C between measurements. Trolox was dissolved in ethanol to produce a solution at 0.63 $mg \cdot ml^{-1}$. Then it was diluted to five different concentrations with methanol as a reference standard. An aliquot of sample extract (0.1 ml) at different concentrations was added to 3.9 ml of DPPH solution. Before measurements, each sample was incubated in the dark for 15 min. The decrease in absorbance for each sample was

measured at 515 nm. Methanol was used to set zero on the spectrophotometer. A blank sample containing the same amount of methanol and DPPH solution was prepared and measured.

Statistical analysis

The results are given as means and the standard deviation of three replicates. All data were analysed using Statsoft Software (version 8.0; Statsoft, Tulsa, Oklahoma, USA). Differences between samples (A, B, C, D or A, B, C adjusted to control bread) were determined using one-way ANOVA. If a significant F ratio was obtained, Tukey's HSD was used to locate differences between means; the significance level was set at $P < 0.05$.

RESULTS AND DISCUSSION

Chemical composition of the green tea extract

Contents of major catechins and caffeine in GTE are compiled in Tab. 1. Aqueous GTE used in this study contained four times less catechins than extracts added to wheat bread in the experiment conducted by WANG et al., where total catechin contents were 73% and 60% of these extracts [19]. It is well known that polyphenols are better extracted in hydroalcoholic solutions, which explains why there were significantly higher amounts of tea catechins in the ethanolic extract used by WANG et al. However, according to some reports on sporadic cases of liver disorders (acute hepatitis or hepatocellular necrosis) after ingestion of green tea supplements based on hydro-alcoholic extracts from green tea leaves [38], it seems advisable in view of health safety to prepare functional foods using aqueous GTE.

Chemical composition of bread

The chemical composition of control rye breads (without green tea extracts) is shown in Tab. 2. The energy value of this type of bread was (19.4 ± 0.7) kJ·g^{-1}.

Concentrations of polyphenols and tea catechins in rye breads prepared with different levels of GTE are presented in Tab. 3. The highest total polyphenol contents of (6632.6 ± 87.9) mg·kg^{-1} (expressed as catechin equivalent) was found in dry matter of bread made with 1.1% green tea powder. Also the highest caffeine content was found in bread prepared with 1.1% GTE (618.8 ± 2.6 mg·kg^{-1} of dry matter). Contents of seven catechins in rye bread samples significantly increased with the increasing level of GTE. The C and CG were detected only at higher values of GTE added to rye bread, while GC was not detected in any rye bread samples. LIU et al. substituted cake flour with 10%, 20% and 30% green tea powder, respectively, and obtained almost five times higher amounts of green tea catechins in sponge cakes [22].

Stability of tea catechins should also be taken into consideration for commercial applications of tea polyphenols as functional ingredients. The stability of green tea catechins in rye breads after the baking process is presented in Tab. 4. It was reported that breadmaking process was optimal for the stability of tea catechins and caffeine. After breadmaking process, (–)-EGC was the most stable catechin. Regardless of the amount of GTE added in the baking process, the percentage loss of this catechin was 6–7% (in corresponding amounts determined in GTE powder). Furthermore, it can be seen from Tab. 4 that (–)-EGCG and (–)-ECG amounts increased with an increasing level of GTE added to rye bread. Average loss of EGCG and ECG contents during breadmaking process was 18% and 35%, respectively.

Tab. 1. Green tea catechin and caffeine contents in GTE powder.

Component	Content based on dry weight [%]
Epigallocatechin-3-gallate	9.6 ± 0.3
Epigallocatechin	4.1 ± 0.1
Epicatechin-3-gallate	2.5 ± 0.1
Epicatechin	0.4 ± 0.02
Gallocatechin gallate	0.2 ± 0.01
Catechin	0.2 ± 0.01
Catechin gallate	1.5 ± 0.04
Gallocatechin	0.1 ± 0.002
Total catechins	17.2 ± 0.4
Caffeine	7.6 ± 0.1

Each value is expressed as mean ± standard deviation (n = 3).

Tab. 2. Chemical composition of bread.

Nutrient	Control rye bread
Dry matter [%]	53.3 ± 2.4
Ash [%]	1.8 ± 0.3
Protein [%]	8.8 ± 0.2
Lipid [%]	0.2 ± 0.01
Dietary fibre [%]	17.1 ± 0.5
Energy [kJ·g^{-1}]	19.4 ± 0.7

Values given are averages of three samples. Each value is expressed as mean ± standard deviation.

Tab. 3. Total polyphenols, catechin and caffeine contents in bread with different GTE levels.

Component [mg·kg^{-1}]	0.5% GTE	0.8% GTE	1.1% GTE
Total polyphenols*	2826.5 ± 77.0^{A}	3943.9 ± 78.3^{B}	6632.6 ± 87.9^{C}
Caffeine	263.7 ± 2.6^{A}	417.3 ± 2.4^{B}	618.8 ± 2.6^{C}
(–)-Epigallocatechin-3-gallate	386.7 ± 2.5^{A}	629.3 ± 2.5^{B}	945.0 ± 5.0^{C}
(–)-Epigallocatechin	192.7 ± 7.5^{A}	309.7 ± 9.5^{B}	432.0 ± 6.0^{C}
(–)-Epicatechin-3-gallate	58.0 ± 2.0^{A}	128.0 ± 3.3^{B}	238.0 ± 7.8^{C}
(–)-Epicatechin	12.7 ± 0.6^{A}	19.0 ± 2.0^{B}	28.2 ± 1.6^{C}
(–)-Gallocatechin gallate	2.8 ± 0.4^{A}	8.7 ± 0.8^{B}	14.2 ± 2.3^{C}
(+)-Catechin	n. d.	6.0 ± 2.0^{A}	9.5 ± 0.5^{B}
(–)-Catechin gallate	n. d.	n. d.	5.0 ± 0.5
(–)-Gallocatechin	n. d.	n. d.	n. d.

Individual values were adjusted to control bread (without GTE content). Each value is expressed as mean ± standard deviation (n = 3). The data were calculated as mg·kg^{-1} dry matter of rye bread. Means with different capital letters differ significantly at $P < 0.05$.
* - expressed as catechin equivalents, n. d. – not detected.

Tab. 4. Stability of major tea catechins in rye breads with different GTE contents.

Component [%]	0.5% GTE	0.8% GTE	1.1% GTE
(–)-Epigallocatechin-3-gallate	79.0 ± 1.39	80.0 ± 1.59	87.0 ± 1.72
(–)-Epigallocatechin	93.0 ± 1.25	93.0 ± 1.63	94.0 ± 1.67
(–)-Epicatechin-3-gallate	46.0 ± 0.38	63.0 ± 0.94	85.0 ± 1.40
Caffeine	68.0 ± 1.67	67.0 ± 1.39	72.0 ± 1.40

Values given are averages of three samples. Each value is expressed as mean ± standard deviation.

WANG et al., when adding 0.05%, 0.1% and 0.15% GTE, respectively, to bread dough (prepared from white flour) in bread making process indicated that 17% of (–)-EGCG and 34% (–)-EGC were lost in bread, compared with their corresponding amounts in dough. However, only 9% of (–)-ECG was lost in breads [19]. In our study, stability of green tea catechins was EGC > > EGCG > ECG, while in the WANG's study it was ECG > EGCG > EGC [19]. WANG et al. concluded that catechin losses could be due to the combined effect of oxidation, isomeration/epimerization and degradation of tea catechins during various breadmaking stages [19]. On the other hand, yeasts added to bread dough assimilate oxygen during the mixing process, thus oxidation of catechins induced by active oxygen must play a very minor role [19]. It is well know that degradations and epimerization of tea catechins in aqueous systems depend strongly also on pH values [19, 39]. According to ZHU et al., tea catechins were stable in acid solutions at pH values < 4, exhibited intermediate stability in the pH range from 4 to 8, and were highly unstable at pH > 8 [40]. CHEN et al., when evaluating green tea catechin contents in tea drinks, observed that those components were relatively stable at pH 3 and 4, but they were readily degraded at pH 5 and 6 during autoclaving [41]. To prepare rye bread, the two phase fermentation method (sour and dough) was applied, which tends to provide a final product with pH level < 5 (pH from 4.81 to 4.84 for each sample of bread). It seems that the thermal process can be the main cause of degradation of tea catechins in rye bread. Our breads were baked at 210 °C for 15 min, where core temperature of the crumb remained above 80 °C for approximately 10 min, which could provide sufficient energy for catechin epimerization.

As estimated, one slice of rye bread (40 g) with a green tea extract (0.5% and 1.1% GTE) provided between 18.7 mg and 47.6 mg total tea catechins. The amount of EGCG and caffeine contents ranged from 11.0 mg to 26.9 mg and from 7.2 mg to 17.6 mg per one slice of fresh rye bread with 0.5% and 1.1% GTE, respectively. WANG et al. [19] showed that concentrations of green tea catechins in one slice of wheat bread (53 g) with

0.05%, 0.1% and 0.15% GTE powder ranged from 10.2 mg to 27.6 mg. Thereby, in our results total catechin contents in one slice of rye bread (40 g) were two times higher than those obtained by WANG et al. [19].

Antioxidant properties

Antioxidant properties of four rye breads with or without GTE addition are presented in Fig. 1. The results showed that adding green tea greatly enhanced antioxidant properties of rye breads. DPPH of four types of bread ranged from 1.4 mmol·g^{-1} to 18.4 mmol·g^{-1}. Thus, antioxidant properties expressed as scavenging ability towards DPPH radicals were in the descending order: 1.1% GTE > 0.8% GTE > 0.5% GTE > C, and the improved antioxidant properties of green tea rye breads were due to the incorporation of green tea catechins. In the literature there are numerous studies on the in vitro antioxidant activity of tea catechins against various radicals, such as hydroxyl, superoxide, peroxyl and DPPH [42–44]. In the past decade, the free radical scavenging ability of tea catechins was studied by many researchers. It was reported that three adjacent hydroxyl (OH) groups at position C-3 and C-4 and C-5 in (–)-EGCG, (–)-GCG and (–)-EGC are more effective for scavenging free radicals than the two adjacent OH groups at C-3 and C-4 in (–)-ECG, (–)-EC, and (–)-CG. In our study, (–)-EGC was more stable, followed by (–)-EGCG and (–)-ECG, which can improve antioxidant properties of rye breads with GTE powder.

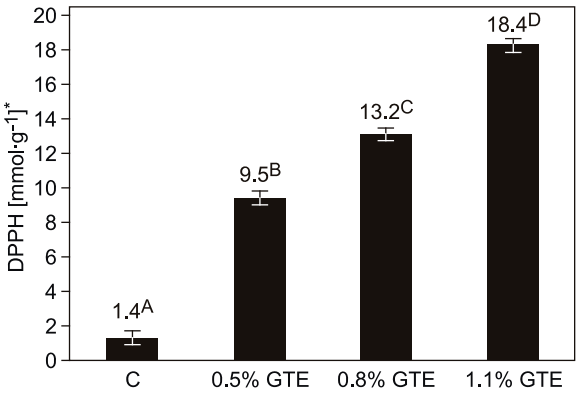

Fig. 1. Total antioxidant capacities of rye breads.

Antioxidant capacity expressed as DPPH in mmol of Trolox equivalents per 1 g. Means with different capital letter differ significantly at $P < 0.05$.

Sensory evaluation of breads

As it was already mentioned, marketing success of new functional products will be ensured when the product, apart from stable polyphenol/catechin contents, will also be characterized by an attractive taste and aroma. Aroma, colour, texture, flavour and general acceptability of control bread and breads with different percentages of GTE were evaluated using a ten-point hedonic scale (Tab. 5).

All sensory results ranged from 5.4 to 7.1, indicating that the four breads were moderately acceptable. No statistically significant differences between the control and breads fortified with GTE were found in colour and texture attributes. It seems that the dark color of rye flour had a masking effect on consumers' colour acceptability, regardless of the level of GTE powder addition to rye bread.

Both flavour and general acceptability scores of 1.1% GTE bread decreased significantly ($P < 0.05$), as compared to the control. LU et al. formulated sponge cake by replacing cake flour with high doses (10%, 20% and 30%) of green tea powder and declared that scores for sensory characteristics were the lowest when sponge cake was prepared with the highest (30%) addition of green tea powder. At the same time, this type of sponge cake was slightly bitter, which according to LU et al. should be attributed to the caffeine content in green tea powder [22]. SCHARBERT and HOFMANN reported that tea catechins, especially (–)-EGCG, are responsible for astringent

Tab. 5. Sensory evaluation of control and rye breads with different GTE levels.

Samples	Characteristics				
	Aroma	Colour	Texture	Flavour	General acceptability
Control	6.7 ± 2.1^{A}	6.7 ± 2.0	7.0 ± 2.0	7.1 ± 1.9^{A}	6.8 ± 2.0^{A}
0.5% GTE	5.9 ± 2.6^{AB}	6.7 ± 1.4	6.3 ± 2.2	6.3 ± 2.2^{AB}	5.9 ± 2.4^{AB}
0.8% GTE	5.8 ± 2.3^{AB}	7.0 ± 1.7	7.0 ± 1.7	6.5 ± 2.1^{AB}	6.3 ± 2.0^{AB}
1.1% GTE	5.4 ± 2.4^{B}	6.5 ± 2.1	6.6 ± 1.9	5.8 ± 1.9^{B}	5.7 ± 1.9^{B}

Values are expressed as means ± standard deviation. Number of panelists $n = 52$. Values in each column denoted with different letters differ significantly at $P < 0.05$.

taste [45]. WANG et al. showed that the more GTE (from 0.15% to 0.5%) was added to wheat bread, the more intensive astringency was detected [21]. However, in our study an inferior flavour was perceived significantly only at the highest GTE powder level (1.1%), which could be explained by the masking effect of flavour-taste bouquet produced during the sourdough fermentation process.

CONCLUSION

A novel formulation of rye bread produced with green tea as a source of polyphenols was developed. Rye bread containing 1.1% of GTE per 100 g of rye flour was the best source of green tea catechins and was good in terms of antioxidant properties. However, due to changes in sensory properties, the level of GTE powder introduced to rye bread should be limited to 0.8%, or the breadmaking technology with a high level of GTE powder should include a green tea flavour-masking additive, such as flaxseed.

Acknowledgments

We gratefully acknowledge research grant No. NN312331635 from the Polish State Committee for Scientific Research.

REFERENCES

1. Hasler, C. M.: Functional foods: benefits, concerns and challenge. A position paper from the American Council on Science and Health. The Journal of Nutrition, *132*, 2002, pp. 3772–3781.
2. Williamson, C.: Functional foods: what are the benefits? British Journal of Community Health Nursing, *14*, 2009, pp. 230–236.
3. Grajek, W. – Olejnik, A. – Sip, A.: Probiotics, prebiotics and antioxidants as functional foods. Acta Biochimica Polonica, *52*, 2005, pp. 665–671.
4. Alldrick, A. J.: The bakery: A potential leader in functional food applications. In: Functional Food News [online]. Issue No. 6, November 2007 [cit. 8 February 2010], <http://www.functionalfoodnet.eu/images/site/assets/5-bread.pdf>.
5. Siró, I. – Kápolna, E. – Kápolna, B. – Lugasi, A.: Functional food. Product development, marketing and consumer acceptance – a review. Appetite, *51*, 2008, pp. 456–467.
6. Balentine, D. A. – Wiseman, S. A. – Bouwens, L. C.: The chemistry of tea flavonoids. Critical Reviews in Food Science and Nutrition, *37*, 1997, pp. 693–704.
7. Wu, C. D. – Wei, G. X.: Tea as a functional food for oral health. Nutrition, *18*, 2002, pp. 443–444.
8. Cabrera, C. – Artacho, R. – Giménez, R.: Beneficial effects of green tea--a review. Journal of the American College of Nutrition, *25*, 2006, pp. 79–99.
9. Wiseman, S. A. – Balentine, D. A. – Frei, B.: Antioxidants in tea. Critical Reviews in Food Science and Nutrition, *37*, 1997, pp. 705–718.
10. Suzuki, Y. – Tsubono, Y. – Nakaya, N. – Suzuki, Y. – Koizumi, Y. – Tsuji, I.: Green tea and the risk of breast cancer: pooled analysis of two prospective studies in Japan. British Journal of Cancer, *90*, 2004, pp. 1361–1363.
11. Yung, L. M. – Leung, F. P. – Wong, W. T. – Tian, X. Y. – Yung, L. H. – Chen, Z. Y. – Yao, X. Q. – Huang, Y.: Tea polyphenols benefit vascular function. Inflammopharmacology, *16*, 2008, pp. 230–234.
12. Imai, K. – Nakachi, K.: Cross sectional study of effects of drinking green tea on cardiovascular and liver diseases. British Medical Journal (Clinical research ed.), *18*, 1995, pp. 693–696.
13. Thielecke, F. – Boschmann, M.: The potential role of green tea catechins in the prevention of the metabolic syndrome – a review. Phytochemistry, *70*, 2009, pp. 11–24.
14. Crespy, V. – Williamson, G.: A review of the health effects of green tea catechins in vivo animal models. The Journal of Nutrition, *134*, 2004, pp. 3431–3440.
15. Yang, C. S. – Landau, J. M.: Effects of tea consumption on nutrition and health. The Journal of Nutrition, *130*, 2000, pp. 2409–2412.
16. Rietveld, A. – Wiseman, S.: Antioxidant effects of tea: evidence from human clinical trials. The Journal of Nutrition, *133*, 2003, pp. 3285–3292.
17. Erba, D. – Riso, P. – Bordoni, A. – Foti, P. – Biagi, P. L. – Testolin, G.: Effectiveness of moderate green tea consumption on antioxidative status and plasma lipid profile in humans. The Journal of Nutritional Biochemistry, *16*, 2005, pp. 144–149.
18. Serafini, M. – Laranjinha, J. A. – Almeida, L. M. – Maiani, G.: Inhibition of human LDL lipid peroxidation by phenol-rich beverages and their impact on plasma total antioxidant capacity in humans. The Journal of Nutritional Biochemistry, *11*, 2000, pp. 585–590.
19. Wang, R. – Zhou, W.: Stability of tea catechins in the breadmaking process. Journal of Agricultural and Food Chemistry, *52*, 2004, pp. 8224–8229.
20. Wang, R. – Zhou, W. – Yu, H. H. – Chow, W. F.: Effects of green tea extract on the quality of bread made from unfrozen and frozen dough processes. Journal of the Science of Food and Agriculture, *86*, 2006, pp. 857–864.
21. Wang, R. – Zhou, W. – Isabelle, M.: Comparison study of the effect of green tea extract (GTE) on the quality of bread by instrumental analysis and sensory evaluation, Food Research International, *40*, 2007, pp. 470–479.
22. Lu, T. M. – Lee, C. C. – Mau, J. L. – Lin, S. D.: Quality and antioxidant property of green tea sponge cake. Food Chemistry, *119*, 2010, pp. 1090–1095.
23. Mildner-Szkudlarz, S. – Zawirska-Wojtasiak, R. – Obuchowski, W. – Gośliński M. J.: Evaluation of antioxidant activity of green tea extract and its effect on the biscuits lipid fraction oxidative stability.

Journal of Food Science, *74*, 2009, pp. 362–370.
24. Michalska, A. – Ceglinska, A. – Amarowicz, R. – Piskula, M. K. – Szawara–Nowak, D. – Zielinski H.: Antioxidant contents and antioxidative properties of traditional rye breads. Journal of Agricultural and Food Chemistry, *7*, 2007, pp. 734–740.
25. Katina, K. – Arendt, E. K. – Liukonen, K. L. – Autio, K. – Flander, L. – Poutanen, K.: Potential of sourdough for healthier cereal products. Trends in Food Science and Technology, *16*, 2005, pp. 104–112.
26. Gramza-Michalowska, A. G. – Regula, J.: Use of tea extracts (*Camelia sinensis*) in jelly candies as polyphenols sources in human diet. Asia Pacific Journal of Clinical Nutrition, *16*, 2007, pp. 43–46.
27. Singleton, V. L. – Rossi, J. A. Jr.: Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture, *16*, 1965, pp. 144–158.
28. Andrade, P. B. – Mendes, G. – Falco, V. – Valentao, P. – Seabra, R. M.: Preliminary study of flavonols in port wine grape varieties. Food Chemistry, *73*, 2001, pp. 397–399.
29. AOAC No. 32.3.08 Official Method 930.22. Ash of bread, Final Action. In: Cunniff, P. (ed.): Official methods of analysis of AOAC International. 16th ed. Arlington, Virginia : AOAC International, 1995, p. 34.
30. AOAC No. 32.1.05 Official Method 926.03. Ash of flour, Direct Method, Final Action. In: Cunniff, P. (ed.): Official methods of analysis of AOAC International. 16th ed. Arlington, Virginia : AOAC International, 1995, p. 31.
31. AOAC No. 32.3.01 Official Method 926.04. Preparation of sample, Final Action. In: Cunniff, P. (ed.): Official methods of analysis of AOAC International. 16th ed. Arlington, Virginia : AOAC International, 1995, p. 31.
32. AOAC No. 32.3.13 Official Method 950.36. Protein in bread, Final Action. In: Cunniff, P. (ed.): Official methods of analysis of AOAC International. 16th ed. Arlington, Virginia : AOAC International, 1995, p. 34.
33. AOAC No. 32.1.22 Official Method 920.87. Protein (total) in flour, Final Action. In: Cunniff, P. (ed.): Official methods of analysis of AOAC International. 16th ed. Arlington, Virginia : AOAC International, 1995, p. 12.
34. AOAC No. 32.1.14 Official Method 922.06. Fat in flour, acid hydrolysis method, Final Action. In: Cunniff, P. (ed.): Official methods of analysis of AOAC International. 16th ed. Arlington, Virginia : AOAC International, 1995, p. 5.
35. AOAC No. 32.3.14 Official Method 935.38. Fat in bread flour, acid hydrolysis method, Final Action. In: Cunniff, P. (ed.): Official methods of analysis of AOAC International. 16th ed. Arlington, Virginia : AOAC International, 1995, p. 34.
36. Asp, N. G. – Johansson, C. G. – Hallmer, H. – Siljeström, M.: Rapid enzymatic assay of insoluble and soluble dietary fiber. Journal of Agricultural and Food Chemistry, *31*, 1983, pp. 476–482.
37. Hu, J. – Zhou, D. – Chen, Y.: Preparation and antioxidant activity of green tea extract enriched in epigallocatechin (EGC) and epigallocatechin gallate (EGCG). Journal of Agricultural and Food Chemistry, *25*, 2009, pp. 1349–1353.
38. Mazzanti, G. – Menniti-Ippolito, F. – Moro, P. A. – Cassetti, F. – Raschetti, R. – Santuccio, C. – Mastrangelo, S.: Hepatotoxicity from green tea: a review of the literature and two unpublished cases. European Journal of Clinical Pharmacology, *65*, 2009, pp. 331–341.
39. Wang, R. – Zhou, W. – Jiang, X.: Mathematical modeling of the stability of green tea catechin epigallocatechin gallate (EGCG) during bread baking. Journal of Food Engineering, *87*, 2008, pp. 505–513.
40. Zhu, Q. – Zhang, A. – Tsang, D. – Huang, Y. – Chen, Z.: Stability of green tea catechins. Journal of Agricultural and Food Chemistry, *45*, 1997, pp. 4624–4628.
41. Chen, Z.-Y. – Zhu, Q. Y. – Tsang, D. – Huang, Y.: Degradation of green tea catechins in tea drinks. Journal of Agricultural and Food Chemistry, *49*, 2001, pp. 477–482.
42. Nanjo, F. – Mori, M. – Goto, K. – Hara Y.: Radical scavenging activity of tea catechins and their related compounds. Bioscience, Biotechnology and Biochemistry, *63*, 1999, pp. 1621–1623.
43. Sang, S. – Cheng, X. – Stark, R. E. – Rosen, R. T. – Yang. C. S. – Ho, C. T.: Chemical studies on antioxidant mechanism of tea catechins: analysis of radical reaction products of catechin and epicatechin with 2,2-diphenyl-1-picrylhydrazyl. Bioorganic and Medicinal Chemistry, *10*, 2002 pp. 2233–2237.
44. Yilmaz, Y.: Novel uses of catechins in foods. Trends in Food Science and Technology, *17*, 2006, pp. 64–71.
45. Scharbert, S. – Hofmann, T.: Molecular definition of black tea taste by means of quantitative studies, taste reconstitution, and omission experiments. Journal of Agricultural and Food Chemistry, *53*, 2005, pp. 5337–5384.

Received 23 April 2010; revised 27 May 2010; accepted 31 May 2010.