P51: EVALUATION OF ANTIOXIDANT CAPACITY AND TOTAL POLYPHENOL CONTENT OF BREAD

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Abstract – The aim of this study was to determine the total polyphenol content and antioxidant capacity of bread prepared with a tea fraction enriched in polyphenols. The best performance regarding bread extraction procedure was achieved with 80% aqueous methanol. The total polyphenol content and DPPH radical scavenging activity in the enriched bread were 4.8 mg GAE/g and 6.7 mg Trolox/g, respectively (results expressed on the sample dry matter basis). During bread shelf-life a reduction in TPC by 5% was obtained, whereas no change was observed in the antioxidant capacity.

Keywords: total polyphenol content, antioxidant capacity, bread, tea

1. INTRODUCTION

Cereals are consumed as dietary staple food by almost 95% of the population [1]. The highest amount of bioactive compounds is found in the outer layers of grain (bran and aleurone layer) and includes phenolic compounds, flavonoids, phytosterols and tocopherols [2, 3]. Phenolic acids are known to contribute to the antioxidative potential of cereals, being associated with the nutritional and health benefits [4].

White flour has a lower potential antioxidant activity than flour containing the outer layers of the wheat grain and germ. Among cereal products, wheat bran and whole meal buckwheat were found to have the greatest total antioxidant capacity [5]. Accordingly, the antioxidant capacity of white bread is low because of the removal of bran layers and germ, rich in dietary antioxidants, which occur during the milling of wheat grain. Thus, there is a great demand for the use of natural antioxidants in bakery products.

Fruits, tea, vegetables or medicinal plants are potential sources of natural antioxidants [6]. Tea contains bioactive compounds such as catechins (flavan-3-ols), flavonols, theaflavins, thearubigins, theanine, gallic acid, vitamins and minerals [7]. Tea catechins and polyphenols have shown health benefits and antioxidant functions [8, 9].

This study aims to evaluate the antioxidant potential of white bread enriched with a polyphenols enriched fraction obtained previously from decaffeinated black tea [10]. Total polyphenol content (TPC) was quantified by Folin-Ciocalteu method with gallic acid as standard and antioxidant capacity was evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay with Trolox as reference.

2. EXPERIMENTAL

2.1. Materials

The polyphenols enriched fraction in aqueous form was obtained from decaffeinated black tea dust [10]. TPC content was 11.2 mg gallic acid equivalents (GAE)/mL, while antioxidant capacity was 15.8 mg Trolox/mL. The ingredients used for bread making were from a local market.

2.2. Chemicals

Folin-Ciocalteu's phenol reagent, gallic acid, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and DPPH (2,2-diphenyl-1-picrylhydrazyl) were purchased from Sigma-Aldrich (St. Louis, USA), while sodium carbonate was from Carl Roth (Karlsruhe, Germany).

2.3. Preparation of bread

Wheat flour (100 g) was mixed with the polyphenols-enriched fraction (60 mL), where salt (2 g) and yeast (3 g) were previously dissolved. Dough was mixed (Diosna mixer, Germany) for 6 min, followed by a resting step for 30 min. Then, pieces of dough were put into the baking shapes and placed in a proofer (MCE Meccanica, Italy) for 45 min. The loaves were baked in an oven (Mondial Forni, Italy) at 220 °C for 25 min. The bread samples were cooled to room temperature. Afterward, crumb samples were frozen (at – 20 °C), lyophilized and ground in a mill to obtain sample powder. Control bread was prepared under the same conditions, employing water instead of the tea fraction.

2.4. Sample extraction procedure

Powdered samples (0.2 g) were extracted on a vortex (Heidolph Instruments GmbH & Co., Germany) at room temperature for 100 min with 10 mL of the following solvents: water, methanol/water (80/20) and methanol. Then, the extracts were separated by centrifugation at 9000 rpm for 30 min (Eppendorf, model 5804R, Hamburg, Germany). The fresh extracts were used for the measurement of TPC and antioxidant capacity.

2.5. Determination of total polyphenol content (TPC)

The TPC of the bread extracts was determined with the Folin-Ciocalteu spectrophotometric method [10]. Briefly, 500 µL of each extract was shaken for 10 sec with 5 mL of Folin-Ciocalteu reagent (diluted with water 1:15). After the mixture was incubated in the dark for 10 min at room temperature, 500 µL of 20% Na_2CO_3 was added and the mixture was incubated again for 20 min. The absorbance was measured at 755 nm using a V-550 model UV-Vis spectrophotometer (JASCO Corporation, Japan). Gallic acid was used as a standard and a calibration curve was plotted with a range of concentration from 0 to 0.175 mg/mL. The equation of the gallic acid calibration curve was $A = 8.1103 \cdot c + 0.0192$ and the correlation coefficient was 0.9999. Analyses were carried out in triplicate and data were reported as mean values expressed in terms of mg gallic acid equivalents per g of the sample dry matter basis (mg GAE/g d.m.).

2.6. DPPH assay

To determine the antioxidant capacity of the bread extracts, the DPPH radical scavenging method was performed according to a previous study [10], with certain modifications. 400 µL of each extract was added to 6 mL freshly prepared $DPPH^{\bullet}$ methanolic solution (0.04 mg/mL). After vortexing, the mixture was allowed to stand in the dark at room temperature for 30 min. The absorbance was then measured at 517 nm using the JASCO spectrophotometer. The Trolox solution prepared in aqueous methanol was used for the calibration curve within the concentration rage from 0 to 0.6 mmol Trolox/L. The equation of the Trolox calibration curve was $A = - 1.4393 \cdot c + 1.0842$ and the correlation coefficient was 0.9998. All measurements were performed in triplicate. Results were expressed as mg Trolox equivalent per g of the sample dry matter basis (mg TE/g d.m.).

3. RESULTS AND DISCUSSION

Solvents, such as methanol, ethanol, ethyl acetate, acetone or dimethylformamide and with different amount of water, were used for extraction of phenolic compounds from fruits, vegetables or cereals [11, 12]. Selection of the most efficient solvent for phenolic compounds extractions depends on the type of food matrix [13].

In this study, for extracting phenolic compounds from the prepared bread, methanol, water and their combination were used. TPC and antioxidant capacity of polyphenol-enriched bread are summarized in Table 1. The type of extracting solvent influenced the measured polyphenolic content and antioxidant capacity of bread. The mixture with 80% methanol, extracted most of the phenolic compounds present in bread. The polarity of the solvent for extraction influences the solubility of phenolics [12].

Table 1. Effect of solvent type on TPC and antioxidant capacity of polyphenol-enriched bread.

Solvent	TPC	AC
100% water	1.60 ± 0.10	3.73 ± 0.06
100% methanol	2.09 ± 0.18	4.41 ± 0.16
80% methanol	4.81 ± 0.10	6.70 ± 0.09

TPC, total polyphenol content (mg GAE/g d.m.); AC, antioxidant capacity (mg TE/g d.m.)
Each value is expressed as mean ± standard deviation (n = 3).

Table 2 illustrates the TPC and antioxidant capacity values for the dough, fresh and aged polyphenol-enriched bread, respectively, using 80% aqueous methanol for sample extraction.

Table 2. Changes in TPC and antioxidant capacity of polyphenol-enriched bread.

Sample	**TPC**	**AC**
Dough	6.83 ± 0.15	7.05 ± 0.2
Fresh bread	4.81 ± 0.10	6.70 ± 0.09
Aged bread	4.60 ± 0.10	6.75 ± 0.14

TPC, total polyphenol content (mg GAE/g d.m.); AC, antioxidant capacity (mg TE/g d.m.)
Each value is expressed as mean ± standard deviation (n = 3).

Addition of polyphenols fraction from tea increased the phenolic content and antioxidant capacity of bread compared with control, which had TPC of 0.49 mg GAE/g d.m. and antioxidant capacity of 1.4 mg TE/g d.m. The presence of catechins, theaflavins and thearubigins from tea contributes to the increase in antioxidant capacity. Tea polyphenols are powerful antioxidants and free radical scavengers [14]. Moreover, the antioxidant capacity is correlated with the TPC of tea [14].

During bread baking, polyphenolic compounds undergo thermal degradation. It was found that around 84% of the total green tea catechins were retained in bread after baking when green tea extract was added to dough [15].

In this study, baking resulted in decreased TPC and antioxidant capacity of bread when compared to dough. Thus, TPC was reduced by 30% and antioxidant capacity by 5% (Table 2).

After bread storage at room temperature for 6 days, a reduction in TPC by almost 5% was noted, whereas no change was observed in the antioxidant capacity (Table 2).

4. CONCLUSIONS

Addition of polyphenols fraction from tea enhanced the antioxidant capacity and TPC of white wheat bread. The combination of methanol/water, as compared with water and methanol, seems to be more effective for the phenolic compounds extraction procedure from bread. 80% methanol was the best solvent for extracting phenolics. When polyphenols fraction was incorporated into bread dough, it was found that 30% of the total polyphenol content was loss during baking.

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