Radical scavenging activity of extruded corn gruels with addition of linden inflorescence

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Abstract: Antioxidant activity is one of the most desirable properties of natural compounds. Among these substances are phenolic compounds which exhibit excellent antiradical activity. The main aim of the present study was determination of the free radical scavenging activity of gruels with 5, 10 and 20% addition of linden inflorescence. The studies were based on two methods: TLC-bioautographic assay and spectrophotometric analysis using DPPH (2,2-diphenyl-1-picrylhydrazyl radical). The obtained results indicate that the radical scavenging properties of the extracts are positively correlated with the content of phenolic compounds in gruels and that a high-temperature extrusion process does not deactivate antioxidant polyphenolic compounds.

Keywords: DPPH radical scavenging activity, phenolic compounds, instant gruels, Tilia inflorescences.

1 Introduction

Linden inflorescence (Tilia inflorescences, species Tilia cordata Mill.) is a widely used medicinal plant possessing several pharmacological effects due to the presence of active compounds [1-4]. Extracts and infusions from this plant have been applied in treatment of symptoms of common cold, bronchitis and cough. Moreover, it has also been claimed to be effective as a diaphoretic for feverish colds and infectious diseases where a sweating cure is needed. The following are the most important pharmacological effects of secondary metabolites found in Tilia inflorescences: antispasmodic, stomachic, sedative and diuretic [3].

The main constituents of linden inflorescence include: flavonoids, mainly quercetin glycosides (rutin, quercitrin and isoquercitrin), kaempferol glycosides, tylioside and phenolic acids (caffeic, p-coumaric and chlorogenic acids). Apart from flavonoids, it is also rich in condensed tannins, polysaccharides and terpenoids [2-5].

The importance of dietary phenolic components for the prevention of some diseases and for health quality improvement has attracted much research attention in the last decade [6-9]. Polyphenols were found to exhibit a wide range of pharmacological properties, for example: neuroprotective, cardioprotective, anti-cancer and antimicrobial activities. Besides, polyphenols are effective natural food preservatives preventing oxidative deterioration and microbial contamination [8-10].

Antioxidant activity of plant extracts was found to correlate well with polyphenol content. Extracts rich in phenolic compounds can be used as medicines or preventive agents protecting humans and animals from the destructive action of free radicals [11-13]. Reactive oxygen species (ROS) have been implicated in many pathological conditions, including rheumatoid arthritis [14], hemorrhagic shock [15], cardiomyopathy [16] and gastrointestinal ischemia [17]. High concentration of ROS leads to destruction of cell membranes, proteins, and nucleic acids, which is dangerous because it may lead to carcinoma formation [18,19].

Therefore, plants rich in polyphenols are widely used as food components. One of the most interesting techniques for food production is extrusion-cooking [20,21]. Extrusion-cooking as a HTST (high temperature, short time) process seems to be one of the best methods for obtaining the maximum nutritive value of several...
plant materials. Prevention or reduction of nutrient destruction, together with improvements in starch or protein digestibility, is clearly of importance in most extrusion applications [21,22].

The aim of this work was to produce instant corn gruels with linden inflorescence using extrusion-cooking and to examine antioxidant activity of the products. Corn products are popular as a source of gluten-free carbohydrates, especially for consumers with celiac disease diet or infants and young children. Corn (maize), rice or buckwheat products play an important role in the nutrition of people with gluten intolerance and retarded digestive track. Those individuals cannot consume bakery, pasta or snack products made from commonly used raw materials, which mostly are rich in gluten [21]. Nowadays, consumers are looking for functional foods, that are both tasty and that also increase the body’s natural resistance, prevent or support therapies in selected diseases, increase physical efficiency and positively influence a person’s mental state. Linden inflorescence seems to be one of the most popular plants in infant and young children’s diets. Incorporation of this plant, connected with HTST treatment, is expected to achieve convenient and highly nutritious products with disease-preventing functions, which would be a consequence of various levels of linden inflorescence.

2 Experimental procedure

2.1 Chemicals and instruments

The chemicals used as standards were purchased from Sigma-Aldrich Fine Chemicals (St. Louis, MO, USA). The standards were caffeic acid (> 98%) and rutin (> 99%). Extracts used for experiments were prepared and purified with methanol and ethanol obtained from the Polish reagents (POCH, Gliwice, Poland). An ultrasonic bath (Bandelin Electronic, Sonorex RK 100H, Germany) was used for the extract preparation. Water was purified using a Millipore Direct-Q3 purification system (Bedford, MA, USA).

Testing based on the TLC-DPPH method was carried out on HPTLC silica gel 60 F254 plates (10 x 10 cm) (Merck, Darmstadt, Germany). Application of the extracts samples was carried out using a Desaga AS-30 applicator (Heidelberg, Germany). Measurement of antioxidant activity was performed spectrophotometrically using a GENESYS™ 20 UV-Vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) in a 1 cm quartz cell. All solvents and chemical reagents were of analytical purity grade.

2.2 Plant Materials

Herbal industrial “Kawon-Hurt” from Gostyń, Poland provided Tilia inflorescences (batch number 096/2011). Dry plant material was milled and sieved.

2.3 Production of gruels

The Department of Food Process Engineering at the University of Life Sciences in Lublin was responsible for production of gruels used for experiments. The first step in obtaining the gruels included blending of corn grit and linden inflorescence with water ensuring a moisture content of 16%. The main components were blended with water in ratios of 99:1, 97:3, 95:5, 90:10 and 80:20. The obtained moistened materials were processed with a TS-45 single screw extruder (Metalchem, Gliwice). The following parameters were used: configuration of L/D = 12, diameter of the forming die $\phi = 3 \text{ mm}$, temperature 120–130°C and screw speed 125 rpm. Samples were ground in a iG5A laboratory grinder (TestChem, Poland) to particle size less than 1 mm [19]. The industrial processing has led to moisture content of 5.5–6.0%.

2.4 Extraction procedure

Extraction was carried out for corn grit and linden inflorescence as pure components of the experimental material and corn gruels with 1, 3, 5, 10 and 20% addition of linden inflorescence. The ultrasound-assisted solvent extraction was performed with 2g portions of material and 40 mL of solvent (80% aqueous ethanol) for 30 min at 60°C. The extraction was repeated three times for each sample [23]. Extracts were processed further: filtration, combination and evaporation to dryness. The residuals were dissolved in 10 mL of methanol. The procedure was repeated three times for each sample.

2.5 TLC–DPPH test

The TLC-DPPH test was used in two studies: screening for antioxidant activity of each of the extracts and analysis of the extracts for active compounds. The first part was based on a dot-blot test [24,25]. The main aim of this step
of the experiment was to select active extracts among extracts of corn grit, linden inflorescence and corn gruels with different contents of linden. The materials that yielded positive results in this test were investigated in the subsequent analysis. The second part of the measurement was focused on analysis of the chosen extracts towards active compounds and reference to standards (caffeic acid and rutin). The analysis was performed as follows: the active extracts and two mentioned standards in concentrations of 0.5 mg mL$^{-1}$ were applied to HPTLC plates with a Desaga S-30 applicator. The samples (10 μL of aliquots extracts) were applied spot-wise, with a distance of 7 mm between them, and a 15 mm distance from the lower left edge of the plate. The plates were developed in vertical chambers pre-saturated for 15 min with the optimized mobile phase consisting of acetonitrile:water:chloroform:formic acid (60:15:10:5, v/v/v/v). After this procedure, the plates were dried for 30 min. The TLC plates were immersed for 5 s in 0.1% (w/v) DPPH solution in methanol. Afterwards plates were kept in the dark for 30 min and scanned every 5 minutes for an hour. The test was repeated three times.

2.6 Image processing using Sorbfil TLC Videodensitometer program

The results of the TLC-DPPH test were documented by flat-bed scanning, saved as JPG documents at a resolution of 40 pixels cm$^{-1}$. For image analysis the Sorbfil TLC Videodensitometer software (Sorbpolymer, Russia) was used. The suitable width of each track line was set and the evaluation of the chosen track to measure peak area was performed by the Process Track command. The software evaluated a band in each track on a TLC image on the assumption that the size and the intensity of a bright spot depended on the quantity of a substance in the band. In order to change the video scan images into chromatograms, a rectangular selection tool was used to outline the tracks [26]. Rf and peak area were measured. The total areas under the peaks in one track (one extract) were compared with the area obtained for rutin.

2.7 Radical scavenging assay

The radical-scavenging activity of the analyzed extracts was determined spectrophotometrically against DPPH radical [24,25]. The concentration of DPPH used for the experiment was 0.1 mM (4 mg of the free radical and 100 mL of methanol). The solution was prepared directly before measurement. The first part of the experiment included measurement of the reference sample of DPPH solution. It was prepared by mixing 2.0 mL of the solution and 1.0 mL of methanol. The next step was to focus on measurement of extracts. Samples were prepared by mixing 2.0 mL of DPPH solution and 1.0 mL of the extracts. The measurement is based on change of absorbance which corresponds to free radical scavenging activity of the extracts. Each measurement was repeated three times at 517 nm at room temperature. The final result is the average of three replicates. The antioxidant activity can be calculated with the following formula:

$$% = \frac{A_0 - A_1}{A_0} \times 100\%$$

where, $A_0$ – absorbance of the reference sample, $A_1$ – absorbance of the sample with tested extracts.

3 Results and discussion

In the present work, antioxidant properties of ethanolic extract from corn gruels with different addition of linden inflorescence were evaluated. The gruels were produced using an extrusion-cooking process (The Experimental). The extraction of dried plants was performed in optimized conditions using sonication. This method was used because results of recent studies confirmed that ultrasound-assisted extraction was an effective, easy to operate, reliable and feasible method for extraction of polyphenols from a plant material [23,27-31]. The optimal solvent was selected among ethanol, methanol, 80% aqueous ethanol and 80% aqueous methanol. 80% aqueous ethanol was proven to be the best extractant for active antioxidant polyphenols.

The next step of the experiment was to select the antioxidant active samples during a dot-blot test [32]. Corn gruels with 1, 2 and 3% content of an analyzed plant did not exhibit free radical scavenging properties. The following samples gave positive results: extracts of linden inflorescence and corn gruels with 5, 10 and 20% addition of the material. These extracts were analyzed subsequently by means of TLC-DPPH. Besides extracts, caffeic acid and rutin were applied to the plates, as reference compounds. The analyzed extracts contain polyphenols, which are very polar compounds, and therefore silica gel was used as the stationary phase with eluent containing water. Under conditions used to develop the plate, the more polar compounds were strongly retained on the stationary phase and were characterized with lower Rf values (hydrophilic
interaction chromatography, HILIC) [33]. The results of the TLC–DPPH* test indicated that antioxidant potential of analyzed samples was positively correlated with the content of linden inflorescence in corn gruels and that it was changing with time. Free radical scavenging properties increased with the addition of linden inflorescence. This finding was based on a comparison of the activity of the separated spots in relation to rutin’s antioxidant properties (Table 1, Figs. 1a, 1b). The highest radical scavenging activity was observed for crude Tilia inflorescence extract followed by corn gruels with 20, 10 and 5% addition of this plant. The highest radical scavenging activity for all samples containing linden inflorescence, demonstrated using the TLC–DPPH test, was observed after 30 min. (Figs. 1a in relation to 1b). The values of the standard deviation (SD) as a measure of repeatability of the TLC–

| Time  | Activity in relation to rutin (area under the common peak/area under rutin peak) ± SD* |
|-------|------------------------------------------------------------------------------------------|
|       | Corn gruel | Linden inflorescence | Corn gruel with 5% addition of linden inflorescence | Corn gruel with 10% addition of linden inflorescence | Corn gruel with 20% addition of linden inflorescence |
| 15 min | 0.014 (± 0.0007) | 3.458 (± 0.050) | 0.16 (± 0.005) | 0.35 (± 0.014) | 0.75 (± 0.024) |
| 30 min | 0.057 (± 0.002) | 4.00 (± 0.120) | 0.42 (± 0.014) | 0.64 (± 0.028) | 1.63 (± 0.046) |
| 60 min | 0.080 (± 0.0003) | 3.91 (± 0.090) | 0.35 (± 0.011) | 0.62 (± 0.023) | 1.35 (± 0.036) |

*SD - standard deviation (n=3)

Figure 1: HPTLC the analysed extracts. System: silica/acetonitrile:water:chloroform:formic acid (60:15:10:5, v/v/v/v). Plates after development dried (30 min) and derivatised by immersion in 0.2% (w/v) DPPH* solution in chloroform, kept in the dark (30 min) and then scanned (after 15 min – 1a, and 30 min – 1b). 1 – extract of linden inflorescence (200 mg mL\(^{-1}\)), 2, 3, 4 – extract of gruels with addition 10, 5 and 20% of linden inflorescence respectively (200 mg mL\(^{-1}\)), 5 – caffeic acid (0.5 mg mL\(^{-1}\)), 6 – rutin (0.5 mg mL\(^{-1}\)).
Radical scavenging activity of extruded corn gruels with addition of linden inflorescence while product with a 10% addition of inflorescence revealed moderate free radical scavenging properties. However, corn gruel containing 20% linden scavenges free radicals very well.

The results obtained from all of the experiments demonstrate that antioxidant activity of the examined samples depends largely on the phenolic compounds present in the linden. Moreover, the study findings confirmed that a high-temperature extrusion process did not deactivate polyphenolic antioxidant compounds, which were present in raw *Tilia* *inflorescence*. Özer and coworkers [34] reported the total antioxidant activity value of nutritious snack food decreased slightly with an increase in screw speed and decrease in moisture content, while total phenolic values had insignificant changes after extrusion.

These natural substances exhibit numerous biological activities [8-10] which explains the high content of phenolic compounds in food. This phenomenon is particularly evident in the case of extruded products enriched in herbs, cereals, vegetables and fruits with high content of polyphenolic antioxidants [35,36]. The versatility of potential technological solutions allows using various raw materials and additives, and their composition will influence quality, pro-health properties, sensory, and nutritional characteristics of extruded products [35-37]. In the case of these types of food products, one of the most convenient methods of food production is extrusion-cooking. The method provides stable products with all nutritive components preserved or enhanced by the addition of natural, biologically active compounds [37]. It should be emphasized that the extrusion-cooking method is appropriate not only in terms of nutritional but also economic aspects.

4 Conclusions

The results indicate that the extrusion-cooking method is appropriate for functional food production. Instant gruels, prepared by the aforementioned method, have great potential to be a good source of natural antioxidants. The antioxidant activity is correlated with the linden content as shown by TLC and spectrophotometric assays. The study findings confirmed that the high-temperature extrusion process had no negative impact on antioxidant activity of polyphenolic compounds, which are present both in the raw products as well as gruels enriched with *Tilia* *inflorescence*. The results of the experiment showed that extrusion-cooking could be used for the production of a wide range of products containing antioxidant active polyphenols.

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