Chemical composition and antiproliferative potential of dried wild apple and pear tea before and after in vitro simulated digestion

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Abstract: Decoctions obtained from dried apple and pear fruits were subjected to in vitro digestion in the presence of a food matrix in order to determine changes in the polyphenol content and antiproliferative activity. The total phenolic and total proanthocyanidins contents were determined using spectrophotometrical methods, and the phenolic compounds were analyzed by RP-HPLC/DAD before and after digestion. Chlorogenic acid and phlorizin dihydrate were the major identified compounds. The addition of a food matrix immediately decreased the contents of individual and total phenolics. After digestion, they were slightly elevated but still lower than in the initial samples. Antiproliferative activity was investigated on human epithelial carcinoma cell line (HeLa), human colon carcinoma (LS174) and human foetal lung fibroblast (MRC-5) cells. The exhibited growth inhibition was not correlated with the content of phenolics in the tested samples, indicating that it could not be explained solely by their content. With the exception of the apple decoction effect on HeLa cells, the digestion process resulted in significant increases in the antiproliferative activity.

Keywords: bioaccessibility; phenolics; food matrix; fruit.

INTRODUCTION

Epidemiological studies demonstrated that diets rich in fruits could be linked with decreased mortality caused by certain types of cancer, mostly due to the additive and synergistic effects of bioactive compounds such as polyphenols, vitamins, minerals and fibre.1

Apples (Malus sp., Rosaceae) and pears (Pyrus sp., Rosaceae) are widely consumed fruits in Europe. There are various reports on the health-promoting

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1315
effect of phenolic compounds present in these fruits, and as a result of a recent study, there is a new claim “an apple or pear a day helps keep strokes at bay”. Thanks to recent advances in food processing technologies, the application of different drying techniques in order to reduce the moisture content have made achievable the prolongation of fresh fruit shelf life. Dried fruits serve as important healthy snacks since they are available during the whole year. In addition, compared to fresh fruits, the dried ones are much easier for storage and transport. These products can be freely incorporated into other foods and recipes, and they present a healthy substitute for salty or sugary snacks. Dried apples and pears are ingredients for tea infusions, popular due to their fruity fragrance, flavour, absence of caffeine and low adstringent and bitter taste.

Generally, six major polyphenolic groups are found in various apple varieties, i.e., hydroxycinnamic acids, flavan-3-ols, procyanidins, anthocyanins, flavonols and dihydrochalcones. Similarily, four classes of polyphenolic compounds are reported to be present in pears, i.e., phenolic acids, flavonols, flavan-3-ols and anthocyanins. There are numerous literature data regarding antiproliferative potential of apples and pears, as well as compounds present in them. One of the limiting factors for the valuable effects of polyphenolics is their bioavailability, which depends on their release from the food matrix, as well as their stability during digestion and the efficiency of their transepithelial passage. Prerequisite for their health effects is the quantitative liberation during the digestion process.

Although there are a large number of papers reporting the chemical composition and biological activity of apples and pears, there is a scarcity of reports dealing with apple and pear fruit teas. Moreover, literature data concerning changes in the polyphenolic compounds of apple and pear during the digestion process are limited. The aim of this research was to assess the stability and functional properties of polyphenols present in decoctions of dried wild apple and pear fruits using an in vitro model of simulated digestion in the presence of the food matrix.

**EXPERIMENTAL**

*Plant material and extraction procedure*

Wild pear and apple fruits were collected on the mountain Suvobor in central Serbia. After rinsing with water, they were manually cut into pieces and dried in a laboratory oven at 40 °C overnight. Preparation of the teas was realized in two traditional ways (as a decoction or infusion) according to Ph. Jug IV, with slight modifications. For decoction, plant material (10 g) was added to 100 mL of distilled water, heated in a steel kettle, and allowed to boil for 30 min. Afterwards, the tea was filtered through filter paper and filled with distilled water up to 100 mL. For infusion, 10 g of plant material was added to 100 mL of boiling distilled water in a steel kettle and allowed to cool down to room temperature. After 30 min, the tea was filtered through filter paper and filled with distilled water up to 100 mL. All extraction procedures were performed in triplicate.
In vitro gastrointestinal digestion

To examine the effects of digestion in the presence of a food matrix on the phenolic composition, a previously reported in vitro digestion method was applied. In the present experiments, an infant formula (Juvitana, Swisslion Product d.o.o. Indija, Serbia) of defined composition was used to simulate a food matrix. According to the manufacturer’s specification, it contained 20% turkey meat, 25% boiled corn paste, 10% boiled potato paste, 5% rice flour, 0.1% NaCl and 39.9% water. The digestive juices used during the in vitro digestion were prepared according to a previously reported method. The composition of the synthetic digestive juices is presented in Table S-I of the Supplementary material to this paper. All the enzymes used for in vitro digestion were purchased from Sigma–Aldrich (USA). The digestion started with the oral phase by mixing 6 mL of artificial saliva with 4.5 g of infant formula (food matrix) and 4.5 mL of prepared decoctions followed by gentle stirring for 5 min at 37 °C. The mixture was then transferred to 50 mL conical flasks and 3 mL of gastric juice was added. Flasks were then agitated in a rotary shaker at 37 °C (1 h, 55 rpm). After one hour, the mixture was supplemented with 9 mL of gastric juice, the pH was adjusted to 2.0 and mixture was incubated under the same conditions for 1 h. In the next step, feeding conditions in the upper part of the duodenum were imitated and 12 mL of intestinal juice and 6 mL of bile juice were added. The pH was adjusted to 5.4 and the incubation was then continued for a further hour at 37 °C and 55 rpm. After completed digestion, the chyme was centrifuged for 5 min at 2750 g and the obtained supernatant was filtered through a 22-μm hydrophilic polyethersulfone membrane. Samples were kept at –80 °C until further analyses.

The whole procedure was repeated for 4.5 g of infant formula mixed with 4.5 mL of distilled water (instead of decoction) and subjected to digestion under the same conditions as digested sample in order to obtain digested matrix. For the preparation a control for decoction digestion or control for matrix digestion, decoctions or infant formula were immediately mixed with all components at 0 °C and pH was adjusted to a final value of 5.4, centrifuged at 2750 g for 5 min at 0 °C. The obtained supernatants were filtered through 22-μm filter and kept at –80 °C until further analysis. Decoction dilution was prepared by mixing 4.5 mL with 40.5 mL of distilled water (instead of digestive enzyme cocktail) to reach the final volume after digestion and the pH was adjusted to 5.4. The diluted sample was then filtered through 22-μm filter and kept at –80 °C until further analysis.

Total phenolic content

Determination of total phenolic content (TPC) was conducted in agreement with a previously reported method. Samples (20 μL) were combined with distilled water (1580 μL), Folin–Ciocalteu reagent (100 μL) and 20% Na2CO3 (300 μL) and incubated at room temperature. After 2 h, the absorbance at 765 nm was measured. The content of total phenolics was calculated using a standard gallic acid curve and are expressed as milligrams of gallic acid equivalents per 1 mL of sample (mg GAE 100 mL⁻¹). All determinations were conducted in triplicate.

Total proanthocyanidin content

The content of total proanthocyanidins (TPR) in samples was determined spectrophotometrically using the p-(dimethylamino)cinnamaldehyde (p-DMACA) method with slight modifications. The fruit teas and digested fractions (100 and 500 μL, respectively) were mixed with 80 μL of p-DMACA reagent and a drop of glycerol. The total volume was made up to 2 mL with methanol and after 7 min, the absorbance at 640 nm was read against methanol as the blank. The contents of proanthocyanidins in the samples are expressed as mg
of catechin equivalents per 100 mL of sample (mg CE 100 mL⁻¹). All the determinations were conducted in triplicate.

Identification of the phenolic compounds by HPLC-DAD analysis

The obtained decoctions, digested fractions and controls were centrifuged for 10 min at 4000 g. The supernatants were subjected to solid phase extraction on C18 columns (BAKERBOND spe, 1000 mg capacity), obtained from J.T. Baker, Deventer, The Netherlands. Columns conditioning was achieved with 5 mL of water, followed by 5 mL of 0.5 % formic acid (v/v). After that step, 5 mL of supernatant solutions of the digested samples and controls were applied to the column and the bound material was eluted with 2 volumes of 0.5 % formic acid in water and finally with 5 mL of 0.5 % formic acid in acetonitrile (v/v). The fractions were collected and concentrated for further HPLC analysis. The chemical analyses of the obtained decoctions, digested fractions and controls were performed on an Agilent series 1200 RR HPLC instrument with a DAD detector, on a reverse phase Lichrospher RP-18 (Agilent) analytical column (250 mm x 4 mm i.d., 5 µm particle size). The mobile phase consisted of solvent A (1 % of formic acid in water) and solvent B (acetonitrile). The fractions were separated using gradient elution according to the following scheme: 5–15 % B 0–5 min; 15–20 % B 5–8 min; 20 % B 8–12 min; 20–30 % B 12–15 min; 30 % B 15–17 min; 30–35 % B 17–20 min; 35 % B 20–22 min; 35–100 % B 22–25 min. The flow was adjusted to 1 mL min⁻¹, and the detection wavelengths were set at 290, 350 and 520 nm. The standards, chlorogenic acid, phlorizine dihydrate, hyperoside, quercitrin and isquercitrine were purchased from Extrasynthese (Cedex, France). All experiments were repeated in triplicate and the results are expressed as mean value ± standard deviation in µg mL⁻¹ of sample.

Cell lines

Cervix adenocarcinoma cell line (HeLa), human colon carcinoma (LS174), and a non-cancerous cell line, MRC-5 (human embryonic lung fibroblast) were grown in RPMI-1640 medium (Sigma). All lines were from the ATCC (American Type Culture Collection) catalogue of human cell lines. The media were supplemented with 10% foetal bovine serum, 3 mmol/L L-glutamine and 1% penicillin-streptomycin (Sigma).

Treatment of the cell lines

The cell lines were treated according to a previously reported method. The target cells HeLa (2000 cells per well), LS174 (7000 cells per well) and non-cancerous MRC-5 (5000 cells per well) were seeded into wells of a 96-well flat-bottomed microtitre plate. Twenty-four hours later, after cell adherence, different concentrations of the investigated teas and digested samples were added to the wells. Exceptions were the control cells to which only a nutrient medium was added. The chosen concentrations range was 1–200 µg ml⁻¹. The final concentration of DMSO solvent did not exceeded 0.5 %, which is non-toxic to the cells. All concentrations were set up in triplicate. Nutrient medium with the corresponding concentrations of the investigated samples, but without cells, was used as a blank, also in triplicate. The cultures were incubated for 72 h.

Determination of cell survival

The effect of the prepared samples on the survival of the cancer cell was determined using the microculture tetrazolium test (MTT) according to Mosmann with modification by Ohno and Abe. 72 h after addition of the compounds. Briefly, 20 mL of MTT solution (5 mg mL⁻¹ phosphate-buffered saline) was added to each well. The samples were incubated for a further 4 h at 37 °C in a humidified atmosphere of 95 vol.% air/5 vol.% CO₂. Then 100 µL of
100 g L$^{-1}$ sodium dodecyl sulphate was added to the extract of the insoluble product formazan, resulting from MTT dye conversion by viable cells. The number of viable cells in each well was proportional to the intensity of the light absorbance, which was read in an enzyme-linked immunosorbent assay (ELISA) plate reader at 570 nm. The absorbance ($A$) was measured at 570 nm 24 h later. Cell survival rate (%) was calculated using the following equation:

$$\text{Cell survival} = 100 \left( \frac{A_c - A_b}{A_c - A_b} \right)$$

where $A_c$ is absorbance of the control cells grown only in nutrient medium, $A_s$ - absorbance of the tested sample and $A_b$ - absorbance of the blank.

The $IC_{50}$ value is defined as the concentration of an agent inhibiting cell survival by 50 % compared with a vehicle-treated control. As positive control, cis-diamminedichloroplatinum (cis-DDP) was used. All experiments were performed in triplicate.

Statistical analysis

All results were calculated from triplicate measurements and expressed as the mean value ± standard deviation. Differences between the group means and their significance were verified using one-way ANOVA. Statistically significant effects were further analyzed and means were compared using Bonferroni test. A level of $p < 0.05$ was taken as statistically significant.

RESULTS AND DISCUSSION

Total phenolic and proanthocyanidin content

The chemical composition of fruit teas is closely related to their biological activity. The first part of the present investigation was focused on the determination of the effect of the food matrix on the stability of polyphenolic compounds during in vitro digestion. In order to perform this quantification, the content of total phenolic compounds (TPC) and total proanthocyanidins (TPR) in fruit teas, digested fruit teas as well as the control of teas digestion were studied (Table I). Both groups of compounds were significantly higher in the apple samples compared to the pear samples. In addition, the decoctions contained higher TP and TPR contents compared to the infusions. Due to this, decoctions were chosen for further study.

### TABLE I. TP and TPR content in apple and pear samples and the food matrix subjected to in vitro digestion; n.d. – not detected. Means with different letters are significantly different ($p < 0.05$)

<table>
<thead>
<tr>
<th>Sample</th>
<th>TPC / mg GAE 100 mL$^{-1}$</th>
<th>TPR / mg CE 100 mL$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple decoction</td>
<td>190.61±6.92$^a$</td>
<td>71.82±3.75$^a$</td>
</tr>
<tr>
<td>Pear decoction</td>
<td>84.18±4.21$^b$</td>
<td>63.43±2.91$^a$</td>
</tr>
<tr>
<td>Apple digested sample</td>
<td>38.93±1.89$^c$</td>
<td>n.d.</td>
</tr>
<tr>
<td>Pear digested sample</td>
<td>37.28±1.58$^c$</td>
<td>n.d.</td>
</tr>
<tr>
<td>Apple digested sample control</td>
<td>26.91±0.83$^d$</td>
<td>n.d.</td>
</tr>
<tr>
<td>Pear digested sample control</td>
<td>25.88±0.71$^d$</td>
<td>n.d.</td>
</tr>
<tr>
<td>Apple infusion</td>
<td>112.76±4.8$^e$</td>
<td>28.55±1.36$^c$</td>
</tr>
<tr>
<td>Pear infusion</td>
<td>45.28±2.12$^c$</td>
<td>10.62±0.39$^d$</td>
</tr>
<tr>
<td>Matrix</td>
<td>21.19±0.75$^d$</td>
<td>n.d.</td>
</tr>
<tr>
<td>Matrix control</td>
<td>18.54±0.51$^d$</td>
<td>n.d.</td>
</tr>
</tbody>
</table>
The total phenolics existing in food do not always reflect the amount that can be absorbed and metabolized in the human body. It is well known that bioavailability of phenolic compounds is influenced by numerous factors, such as the interaction with food matrix. In the present investigation, in control samples immediately after addition of food matrix and the digestive enzyme cocktail, the amounts of TPC were reduced by 85.88 and 69.26% in case of apple and pear decoctions, respectively. This indicates that the constituents of the food matrix rapidly bound a high percent of soluble phenolic compounds from the investigated samples through hydrogen bonding and hydrophobic interactions.

After simulated in vitro digestion, the TPC in the digested samples was slightly increased compared to the control samples. Still it was 80 and 56% lower in the case of apple and pear decoctions, respectively. This could be explained by the release of polyphenolic compounds from the complex with the food matrix components during time. This is in accordance with results of a previous in vitro study, in which a decrease of TPC in extracts of four commercial apple cultivars (Jonaprinz, Jonagold, Golden and Mutzu) was observed after simulated in vitro digestion. Similarly, Cilla et al. showed that the TPC decreased by at least 47% in digested fruit beverages compared to undigested ones.

A change in TPC during digestion was also observed for the food matrix. Namely, the amount of total phenolics increased in the digested matrix (21.19 mg GAE 100 mL–1) compared to the digestion of the control matrix (18.54 mg GAE 100 mL–1). Thus, it could be concluded that some of the phenolic compounds present in the digested samples originated from the food matrix.

The amount of TPR was higher in the apple samples compared to the pear samples. The TPR content significantly decreased after digestion and it could not be quantified in the digested samples or in the control of the digested samples. Similarly, Fernandez and Labra investigated the effect of in vitro simulated digestion on the stability and content of proanthocyanidins from grape skin and seeds. They showed that up to 80% of the total proanthocyanidins were significantly degraded during the pancreatic digestion. In addition, the results of the present study compared well with those obtained in previous research with chokeberry juice, where the amount of TPR immediately decreased 10-fold after addition of a food matrix and a digestive enzyme cocktail. In the matrix and matrix control samples, proanthocyanidins were not detected, which is in accordance with the declaration for the used infant formula.

Chemical characterization

Expansion of knowledge on the bioavailability of plants metabolites is essential for an understanding of their health effect. The impact of a food matrix on the bioavailability of polyphenols has not been examined in detail. Interact-
ions between food components and polyphenols (such as binding to proteins and polysaccharides) can exist and these interactions may modify their absorption.23

The quantification of phenolic compounds in apple and pear decoctions and their digested fractions was performed using an HPLC-DAD method, and results are presented in Table II. The decoction profiles encompassed hydroxycinnamic acid, flavonols and dihydrochalcones. The addition of the food matrix affected the content of individual phenolics in a similar way as was the case with TPC and TPR. After simulated in vitro digestion, the concentration of phenolic compounds was slightly (but not significantly) increased compared to the control samples, although their contents were still much lower than in the apple and pear decoctions. Previous results24 indicated that during the digestion process, the amount of phenolic compounds may be metabolized into structurally different forms with different chemical and biological properties. In the present study, after examination of the HPLC-DAD chromatogram for the digested samples at 290, 350 and 520 nm, no new peak that might suggest the formation of new derivatives was detected.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Chlorogenic acid</th>
<th>Phlorizine dihydrate</th>
<th>Hyperoside</th>
<th>Quercitrin</th>
<th>Isoquercitrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple decoction</td>
<td>133.97±4.31^a</td>
<td>43.65±1.09^a</td>
<td>1.57±0.04^a</td>
<td>13.82±0.42^a</td>
<td>1.71±0.06^a</td>
</tr>
<tr>
<td>Pear decoction</td>
<td>42.23±1.12^b</td>
<td>nd</td>
<td>tr</td>
<td>4.97±0.11^b</td>
<td>0.83±0.01^b</td>
</tr>
<tr>
<td>Apple digested sample</td>
<td>12.39±0.33^c</td>
<td>2.91±0.16^b</td>
<td>tr</td>
<td>3.52±0.09^e</td>
<td>tr</td>
</tr>
<tr>
<td>Pear digested sample</td>
<td>6.07±0.18^d</td>
<td>nd</td>
<td>nd</td>
<td>tr</td>
<td>nd</td>
</tr>
<tr>
<td>Apple digested sample</td>
<td>13.00±0.41^b</td>
<td>2.06±0.10^b</td>
<td>tr</td>
<td>3.39±0.08^c</td>
<td>tr</td>
</tr>
<tr>
<td>control</td>
<td>6.23±0.16^d</td>
<td>nd</td>
<td>tr</td>
<td>tr</td>
<td>nd</td>
</tr>
<tr>
<td>Matrix digestion control</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>LOD</td>
<td>0.02</td>
<td>0.10</td>
<td>0.03</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>LOQ</td>
<td>0.10</td>
<td>0.50</td>
<td>0.20</td>
<td>0.30</td>
<td>0.40</td>
</tr>
</tbody>
</table>

According to previous results,25 chlorogenic acid is one of the dominant polyphenol compounds in apple fruit with an average concentration 9.02 mg in 100 g of fresh apple. In addition, it is one of the predominant compounds in pear fruit.26

During the drying process, the content of chlorogenic acid was significantly reduced compared to that in fresh fruit.27 In the present study, chlorogenic acid was also the main phenolic compound in amounts of 133.97 and 42.23 µg mL⁻¹ for apple and pear decoctions, respectively. After gastric and pancreatic digestion, the
content of chlorogenic acid was 90.75 and 85.63 % lower in the case of apple and pear decoctions, respectively. The obtained results are in accordance with previous literature data. According to Boaventura et al.,\textsuperscript{20} the content of chlorogenic acid in yerba mate tea was decreased by 67 % after \textit{in vitro} digestion, while in the case of sea fennel, it was approximately 82 % lower after gastric digestion.\textsuperscript{28} Dupas \textit{et al.}\textsuperscript{29} showed that chlorogenic acid in its native form is poorly absorbed in the body when ingested in a realistic food matrix. Previous reports showed isomerisation of chlorogenic acid, an ester of caffeic acid with quinic acid, and even its hydrolysis into its free acid in both simulated and human duodenal juices.\textsuperscript{30}

Among the phenolic compounds in the apple decoction, phlorizine dihydrate was the most affected by the addition of a food matrix and digestion. On the other hand, in previous studies,\textsuperscript{30,31} the digestion process did not induce any significant change in the content of phloretin derivatives. The obtained differences might be explained by the different experimental conditions used in these studies. Common to all previous assays is the complete gastric digestion in the absence of a food matrix and, therefore, they do not allow appropriate comparison with the results obtained in the present study.

Bearing in mind quercetin and its derivatives, previous results showed that aglycones and glucosides can be absorbed in the small intestine, while phenolics with a rhamnose, arabinose or xylose moiety must reach the colon and be hydrolyzed before absorption.\textsuperscript{23} The content of quercetin derivatives in investigated apple and pear decoctions ranged from 0.83 to 13.82 μg mL\textsuperscript{-1}, while they were detected only in traces in the digested samples and controls. Hollman and Katan\textsuperscript{32} reported that the absorption of quercetin is more efficient after ingestion of onions rich in quercetin glucosides than after ingestion of apples (only 30 %) containing both glucosides and other glycosides.

\textbf{Antiproliferative activity}

Increased consumption of fruit and vegetables is recommended as a key component in a healthy diet for the prevention of certain types of cancer.\textsuperscript{34} Owing to the high availability of apples and pears and their consumption among European nations, they could be considered as an important source of chemopreventive compounds. It was stated that regular consumption of one or more apples per day may reduce the risk for lung and colon cancer.\textsuperscript{34} Previously, Cetkovic \textit{et al.}\textsuperscript{35} showed strong cytotoxic effect of apple pomace extracts on HeLa cell line with \textit{IC}\textsubscript{50} values below 10 mg mL\textsuperscript{-1}. Moreover, apple peel extract obtained from organic Gala apples exhibited a significant decrease in the growth and clonogenic survival of human prostate carcinoma (CWR22Rnu1 and DU145) and breast carcinoma (Mcf-7 and Mcf-7:Her18) cells.\textsuperscript{8} In a study conducted by Sun \textit{et al.},\textsuperscript{9} an apple extract showed relatively potent antiproliferative activity on HepG2 human liver cancer cells with median effective dose (\textit{EC}\textsubscript{50}) of 49.37 mg/mL, while a
Pear extract showed no antiproliferative activity under the employed experimental conditions.

Recent studies indicated that polyphenols are the main phytochemicals with antiproliferative property in higher plants. However, the intake of apple and pear fruits and their products does not automatically mean increased phenolics concentration in plasma. To exert pharmacological activity, they should be bioavailable, and their bioavailability is determined on the release from the food matrix during the digestion process and subsequent intestinal absorption. In this study, wild apple and pear decoctions were evaluated before and after in vitro digestion for their antiproliferative activity on human epithelial carcinoma cell line (HeLa), human colon cancer (LS174) cells and human foetal lung fibroblast (MRC-5) cells. The inhibition of cells proliferation and the cytotoxic effects of the investigated samples are presented in Table III. The results indicated that the exhibited growth inhibition was not correlated with the content of phenolic compounds in the tested samples, indicating that it could not be explained solely by their content.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cell line</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HeLa</td>
<td>LS174</td>
</tr>
<tr>
<td>Apple decoction</td>
<td>26.91±0.22a</td>
<td>41.01±0.55a</td>
</tr>
<tr>
<td>Pear decoction</td>
<td>37.94±1.04b</td>
<td>69.94±1.82b</td>
</tr>
<tr>
<td>Apple digested sample</td>
<td>56.21±2.02c</td>
<td>25.71±0.12c</td>
</tr>
<tr>
<td>Pear digested sample</td>
<td>26.19±0.07a</td>
<td>25.97±0.32c</td>
</tr>
<tr>
<td>Apple digested sample control</td>
<td>57.54±1.26c</td>
<td>27.25±0.21c</td>
</tr>
<tr>
<td>Pear digested sample control</td>
<td>28.16±0.99a</td>
<td>25.33±0.31c</td>
</tr>
<tr>
<td>Matrix</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
<tr>
<td>Matrix digestion control</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>0.72±0.13d</td>
<td>2.64±0.26d</td>
</tr>
</tbody>
</table>

Generally, with the exception of the effect of the apple decoction on HeLa cells, digestion process resulted in significant increases in antiproliferative activity. Namely, inhibition of the proliferation of HeLa cells in the presence of apple decoction after in vitro digestion decreased approximately 2-fold in comparison to the non-digested sample, with the IC₅₀ value decreasing from 56.21 to 26.91 μg mL⁻¹.

The decline in the content of bioactive compounds was probably the cause of the decrease in the inhibition of the proliferation of the tested cancer cell lines. In the cases of the effect of the apple decoction on LS174 cells, and of the pear decoction on HeLa and LS174 cells, the situation was different. Lower IC₅₀...
values were recorded with the digested samples in comparison to the non-digested ones. The decrease in total phenolic content did not result in a lowering of the exhibited antiproliferative potentials. There are numerous reports on increasing bioactivity of plant extracts after simulated \textit{in vitro} digestion. The authors mainly reported increasing free radical scavenging capacities. Chen \textit{et al.} showed that wild raspberry extract produced after digestion had a pronounced protective effect against acrylamide induced cytotoxicity compared with that produced before digestion.

It could be speculated that during digestion, a high proportion of apple and pear decoctions are transformed into other unknown and/or undetected compounds with different chemical and pharmacological properties. Furthermore, numerous data suggest that phenolic compounds exert a pro-oxidative activity at higher concentrations. For example, for caffeic acid there are results that it can be carcinogenic at 2 \%, tumour promoter at 0.5 to 1 \% and anticancerogenic at 0.05 to 0.5 \%.

In both cases, the difference in the activity of the digested samples and control digestion samples was small, indicating that phenolic compounds entrapped in the food matrix showed small contributions to the exerted activity. Since the food matrix is also a source of phenolic compounds, its effect on the proliferation of HeLa, LS174 and MRC5 cell lines was also investigated. No effect was observed for the food matrix or for its digested sample. The investigated samples showed no cytotoxic effects on normal MRC-5 cell line at concentration lower than 200 μg mL\textsuperscript{-1}.

CONCLUSIONS

\textit{In vitro} simulated digestion in the presence of a food matrix induced significant decrease in the content of phenolic compounds in apple and pear decoctions. On the other hand, the antiproliferative effect was not correlated with the content of phenolic compounds in the tested samples, indicating that it could not be explained solely by their content. However, further research is needed to investigate the bioavailability and biological effects of these bioactive compounds in humans.

SUPPLEMENTARY MATERIAL

The additional data are available electronically at the pages of journal website: http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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ИЗВОД
ХЕМИЈСКИ САСТАВ И АНТИПРОЛИФЕРАТИВНО ДЕЛОВАЊЕ ЧАЈА ОД ДИВЉЕ ЈАБУКЕ И КРУШКЕ ПРЕ И НАКОН СИМУЛИРАНЕ ДИГЕСТИЈЕ IN VITRO

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У овом раду анализиране су измене у садржају полиfenола и антипролиферативној активности дескотка добијених од сувих плодова јабуке и крушке. Ове промене испитиване су након in vitro дигестије у присуству компоненти матрикса хране. Садржај укупних фенола и проантоксизанидина одређен је применом спектрофотометријских метода, док је састој појединачних фенола анализиран применом RP-HPLC/DAD технике пре и након дигестије. Хлорогенска киселина и фlorizин дибихидрат била су доминантна идентификована једињења. Додатак матрикса хране аутоматски је довео до смањења садржаја укупних и појединачних полиfenола. Након дигестије њихов садржај је био нижа, али је и даље био нижи у односу на садржај у почетним узорцима. Антипролиферативна активност испитиваних узорака анализирана је на две туморске хелијске линије (HeLa и LS174). Покazaна инхибиција раста туморских ћелија није била у корелацији са садржајем фенолних једињења у испитиваним узорцима, указујући на то да се она не може објаснити само њиховим садржајем. Са изузетком ефекта дескотка плодова јабуке на раст HeLa ћелија, након дигестије узорака дошло је до значајног повећања њихове антипролиферативне активности.

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