Research Article

Engin Celep, Selin Akyuz, Yigit İnan and Erdem Yesilada*

Stability of phenolic content of some herbal infusions and their antioxidant activity following in vitro digestion

Abstract: Herbal infusions are among the most widely consumed beverages throughout the world. Their popularity arises due to not only their pleasant aroma and taste, but also their high phenolic content and antioxidant activity. A vast number of in vitro studies revealed their high phenolic content, together with strong antioxidant activity. However, the majority of them seem to ignore some important physiological parameters, such as bioavailability or bioaccessibility. The present study was planned to evaluate the stability of phenolic content and antioxidant activities of seven most widely preferred herbal infusions. A stimulated version of gastrointestinal infusion was added to the study for comparative purposes. The results indicated the loss of both phenolic content and antioxidant activity of herbal infusions after being submitted to digestion.

Keywords: Herbal infusion; Antioxidant activity; Phenolic content; In vitro digestion.

Introduction

Free radical formation is an inevitable consequence of aerobic life due to biochemical processes or as a result of environmental factors. They are known to induce notable damage to biological systems, resulting in many important chronic diseases such as diabetes, Alzheimer’s, etc. Consequently, it is of major importance to enhance the antioxidant capacity of the body. On that account, it is highly recommended to promote the dietary antioxidant intake. Consuming an antioxidant-rich diet is critical for maintaining the overall health and well-being of an individual [1, 2].

Herbal teas are common beverages that are prepared by brewing of different organs such as leaves, flowers, seeds, fruits or roots of plant species other than the leaves of *Camellia sinensis* L. Their popularities tend to increase each day due to their preferable aroma and taste, together with their potential to exert positive effects on health. A remarkable interest in determining
the antioxidant capacities and total phenolic contents of foodstuffs along with medicinal plants has been emerging. Herbal teas are considered as a major substitute to dietary antioxidants with regards to vegetables, fruits, spices, etc. [3]. The results of a growing number of recent studies revealed the antioxidant capacities of several herbal infusions, so far [4–6]. Consequently, herbal teas arouse strong consumer interest.

Although there is a countless number of in vitro studies about the antioxidant capacities of herbal teas, a majority of them ignores the physicochemical conditions that the bioactive molecules have to face throughout the gastrointestinal tract. Antioxidant molecules are expected to be absorbed through the intestinal membrane so as to induce their pharmacological activity. In vitro simulation models of gastrointestinal digestion have been widely conducted in order to assess the bioavailability of the bioactive molecules. They are referred as alternatives to clinical or in vivo tests, even though they do not include every steps of natural digestion. They are carried out to mimic mechanical action, enzymatic activity and modified pH value, which occur during digestion. For this purpose, digestive enzymes, bile salts are used together with adjusting the pH and temperature of the test medium. In addition, a dialysis step is finally performed to simulate the intestinal permeability [7].

Seven mostly consumed herbal teas (elderberry, bilberry, raspberry, strawberry, rooibos and pomegranate) – (Vaccinium myrtillus, Rosa canina, Rubus idaeus, Sambucus nigra, Fragaria vesca, Aspalathus linearis and Punica granatum) – together with green tea (Camelia sinensis) were tested in this study in order to perform a comparative evaluation of these herbal infusions. Since antioxidant activity depends on different parameters, various tests with different mechanisms were conducted for a full assessment.

Materials and methods

Chemicals

All chemicals, enzymes and standards used in the experiments were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All chemicals were of analytical grade.

Plant material

The leaves of green tea and rooibos, the fruits of elderberry, bilberry, raspberry, strawberry and rosehip, and the peels of pomegranate were provided by Doğadan Bitkiçayıları Co. Infusions were prepared according to procedures performed by the public. Two gram of plants materials were packed into standard tea bags, and infused with 150 mL of water at 80°C in closed beakers. After 5 min, tea bags were removed from the beakers and infusions were left to settle for cooling at room temperature. Later, the infusions were lyophilized.

In vitro simulation of gastrointestinal digestion

The simulation of gastrointestinal digestion was performed according to a two-step method described by McDougall et al. [8] with slight modifications [7]. The simulated stomach solution was composed pepsin enzyme and salt NaCl, pH adjusted to two. After the incubation process at 37°C in a shaking water bath for 2 h, the mixture was immediately placed in an ice bath and then an aliquot was taken as “post-gastric” sample (PG) and stored at −20°C. The remaining mixture was placed in glass beaker and a segment of cellulose dialysis tubing (molecular weight cut off 12 kDa) containing sufficient NaHCO₃ to neutralize the titratable acidity was placed inside the beaker. Subsequently, pancreatin enzyme and bile salts were added to the medium and the solution was incubated under the same conditions for additional 2 h. Following the incubation process, the solution left outside the dialysis tubing was taken as the OUT sample representing material that remained in the gastrointestinal tract (colon available) and the solution that managed to diffuse into the dialysis tubing was taken as the IN sample (serum-available).

Quantitation of bioactive metabolites

The total phenolic contents were measured with Folin-Ciocalteu method along with minor modifications spectrophotometrically [9]. The results were expressed as milligram equivalents of gallic acid (GAE/g dry extract). The total flavonoid content was determined according to a previously published method [10], and the results were given as milligram quercetin equivalents per g sample.

Total antioxidant capacity

Phosphomolybdenum method with minor modifications was used to determine the total antioxidant capacity of every sample [11]. The results were expressed as milligram ascorbic acid equivalents (AAE) in 1 g sample.
Free radical scavenging activity

Free radical scavenging activity was determined by a previously published method using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) [7]. The results were expressed as milligram ascorbic acid equivalents (AAE) in 1 g sample.

Metal reducing capacity

The cupric ion reducing capacity of samples were calculated according the method of Apak et al. [12]. The results were expressed as milligram ascorbic acid equivalents (AAE) in 1 g sample.

Statistical analysis

All experiments were performed in triplicate. The results in each test were expressed as the mean ± standard deviation. Statistical comparisons were made using ANOVA followed by Tukey-Kramer post hoc test for multiple comparisons. Statistically significant difference was defined as p < 0.05.

Results

Total phenolic and flavonoid contents of the samples

Total phenolic and flavonoid contents of seven herbal infusions together with green tea were presented in Tables 1 and 2, respectively. As expected, the nondigested green tea infusion had the highest total phenolic content (174.16 ± 2.44). Surprisingly, pomegranate peel infusion had nearly the same amount of phenolic content in IN fraction and more in OUT fraction than green tea. Besides, total flavonoid content of pomegranate is much higher than both digested and nondigested fractions of green tea. Our results disclosed significant decline (p < 0.05) in both

Table 1: Total phenolic contents of herbal teas before and after in vitro gastrointestinal digestion.a

| Samples     | NDa | PG     | INA | OUT            |
|-------------|-----|--------|-----|----------------|
| Green tea   | 174.16 ± 2.44 | 81.20 ± 1.26c | 36.14 ± 0.88c,d | 44.28 ± 1.85c,d,e |
| Elderberry  | 71.28 ± 1.27  | 43.62 ± 1.03c  | 16.68 ± 0.71c,d | 33.75 ± 0.23c,d,e |
| Bilberry    | 31.23 ± 2.16  | 20.54 ± 0.63c  | 12.56 ± 0.73c,d | 13.76 ± 0.64c,d,e |
| Raspberry   | 49.73 ± 1.51  | 34.38 ± 1.92c  | 15.30 ± 0.55c,d | 34.51 ± 1.36c,d,e |
| Strawberry  | 57.67 ± 1.37  | 33.33 ± 1.35c  | 15.67 ± 0.23c,d | 24.95 ± 0.49c,d,e |
| Rosehip     | 80.23 ± 2.41  | 42.75 ± 0.43c  | 12.87 ± 0.08c,d | 34.21 ± 0.72c,d,e |
| Rooibos     | 47.55 ± 0.69  | 34.35 ± 0.10c  | 10.34 ± 0.26c,d | 18.23 ± 0.39c,d,e |
| Pomegranate | 89.65 ± 2.59  | 44.77 ± 0.74c  | 34.52 ± 1.38c   | 53.79 ± 0.91c,d,e |

aResults were expressed as the mean of triplicates ± standard deviation (SD) and as milligram gallic acid equivalents (GAE) in 1 g sample.

The abbreviations for samples are ND, non-digested; PG, postgastric; IN, serum available; OUT, colon available. c p < 0.05 (compared to ND sample). d p < 0.05 (compared to PG sample). e p < 0.05 (compared to IN sample).

Table 2: Total flavonoid contents of herbal teas before and after in vitro gastrointestinal digestion.a

| Samples     | NDa | PG     | INA | OUT            |
|-------------|-----|--------|-----|----------------|
| Green tea   | 42.71 ± 0.86  | 20.64 ± 0.78c | 13.14 ± 0.25c,d | 39.39 ± 1.03c,d,e |
| Elderberry  | 40.94 ± 1.12  | 16.07 ± 0.74  | 10.37 ± 0.61c,d | 30.90 ± 0.47c,d,e |
| Bilberry    | 40.44 ± 0.79  | 16.88 ± 0.27  | 13.32 ± 0.11c,d | 24.67 ± 0.26c,d,e |
| Raspberry   | 41.46 ± 0.54  | 16.08 ± 0.32  | 12.31 ± 0.36c,d | 20.64 ± 0.88c,d,e |
| Strawberry  | 34.95 ± 1.35  | 28.54 ± 0.59c  | 10.46 ± 0.24c,d | 15.29 ± 0.31c,d,e |
| Rosehip     | 39.83 ± 0.84  | 18.13 ± 0.26c  | 13.26 ± 0.28c,d | 19.50 ± 0.54c,d,e |
| Rooibos     | 49.32 ± 0.89  | 35.16 ± 1.59c  | 12.84 ± 1.78c,d | 30.61 ± 1.21c,d,e |
| Pomegranate | 99.62 ± 3.18  | 61.36 ± 3.42c  | 31.54 ± 1.26c,d | 66.08 ± 2.89c,d,e |

aResults were expressed as the mean of triplicates ± standard deviation (SD) and as milligram quercetin equivalents (QE) in 1 g sample.

The abbreviations for samples are ND, non-digested; PG, postgastric; IN, serum available; OUT, colon available. c p < 0.05 (compared to ND sample). d p < 0.05 (compared to PG sample). e p < 0.05 (compared to IN sample).
Results were expressed as the mean of triplicates. The lowest amount of contents was observed in IN fractions in each sample.

Antioxidant capacities of the samples

The total antioxidant capacities of samples were given in Table 3. The capacity of nondigested green tea (1074.3 ± 2.16) seemed to be slightly higher than pomegranate (100.97 ± 2.72), while IN fraction of pomegranate (81.46 ± 2.51) was nearly twice as high as that of green tea (44.54 ± 1.87). Nevertheless, the results of DPPH scavenging test, given in Table 4, indicated free radical activity of IN fraction of green tea (251.52 ± 2.72) is higher than pomegranate (155.07 ± 3.89). Furthermore, elderberry (175.29 ± 3.14) also showed better activity than pomegranate. The results of CUPRAC assay was given in Table 5.

### Table 3: Total antioxidant capacities of herbal teas before and after in vitro gastrointestinal digestion. *

| Samples       | ND  | PG    | IN    | OUT   |
|---------------|-----|-------|-------|-------|
| Green tea     | ±   | 44.54 ± 1.87 |       | 91.46 ± 2.53 |
| Elderberry    | ±   |       |       |       |
| Bilberry      | ±   |       |       |       |
| Raspberry     | ±   |       |       |       |
| Strawberry    | ±   |       |       |       |
| Rosehip       | ±   |       |       |       |
| Rooibos       | ±   |       |       |       |
| Pomegranate   | ±   |       |       |       |

*Results were expressed as the mean of triplicates ± standard deviation (SD) and as milligram ascorbic acid equivalents (AAE) in 1 g sample. The abbreviations for samples are ND, non-digested; PG, postgastric; IN, serum available; OUT, colon available. *p < 0.05 (compared to ND sample)."p < 0.05 (compared to IN sample).

### Table 4: DPPH radical scavenging activities of coffee samples before and after in vitro gastrointestinal digestion. *

| Samples       | ND  | PG    | IN    | OUT   |
|---------------|-----|-------|-------|-------|
| Green tea     | ±   | 251.52 ± 2.72 |       | 419.42 ± 7.15 |
| Elderberry    | ±   |       |       |       |
| Bilberry      | ±   |       |       |       |
| Raspberry     | ±   |       |       |       |
| Strawberry    | ±   |       |       |       |
| Rosehip       | ±   |       |       |       |
| Rooibos       | ±   |       |       |       |
| Pomegranate   | ±   |       |       |       |

*Results were expressed as the mean of triplicates ± standard deviation (SD) and as milligram ascorbic acid equivalents (AAE) in 1 g sample. The abbreviations for samples are ND, non-digested; PG, postgastric; IN, serum available; OUT, colon available. "p < 0.05 (compared to ND sample)."p < 0.05 (compared to IN sample).

### Table 5: Cupric ion reducing capacities (CUPRAC) of coffee samples before and after in vitro gastrointestinal digestion. *

| Samples       | ND  | PG    | IN    | OUT   |
|---------------|-----|-------|-------|-------|
| Green tea     | ±   | 337.93 ± 3.71 |       | 530.79 ± 1.62 |
| Elderberry    | ±   |       |       |       |
| Bilberry      | ±   |       |       |       |
| Raspberry     | ±   |       |       |       |
| Strawberry    | ±   |       |       |       |
| Rosehip       | ±   |       |       |       |
| Rooibos       | ±   |       |       |       |
| Pomegranate   | ±   |       |       |       |

*Results were expressed as the mean of triplicates ± standard deviation (SD) and as milligram ascorbic acid equivalents (AAE) in 1 g sample. The abbreviations for samples are ND, non-digested; PG, postgastric; IN, serum available; OUT, colon available. "p < 0.05 (compared to ND sample)."p < 0.05 (compared to IN sample).
They indicated that green tea again was the most active infusion among other herbal infusions.

Discussion

The increased free radical formation as a result of lifestyle factors or environmental reasons is a major concern targeting the overall health status of individuals. This phenomenon causes the worldwide interest on dietary antioxidants and their possible health-protecting effects.

Phenolics found in plants possess powerful antioxidant activity in various distinct mechanisms as a result of their ideal chemical structure [13]. A growing body of studies exhibited their free-radical scavenging, lipid peroxidation inhibiting and metal-chelating properties. Consumption of phenolic-rich diets have been attributed to a reduction in the incidences of life-threatening conditions such as cardiovascular diseases, cancer, diabetes, etc. [14].

Tea and herbal infusions are regarded as one of the major sources of phenolic compounds in our daily diet. Besides, with the exception of green tea, they do not contain caffeine. This condition leads to a vast number of researches about the in vitro antioxidant capacities of herbal infusions. These studies give valuable data, especially for comparative purposes. Nevertheless, a majority of these studies overlook the digestion process these infusions undergo. It is an undeniable truth that the bioavailability of antioxidant compounds following their ingestion plays a major role in their pharmacological activities. The extensive research pointing out the bioavailability of such compounds should also be taken into account. Investigating the phenolic content and antioxidant capacity of herbal infusions after they undergo a simulated digestion process seems to be a more realistic approach.

The present study was aimed to analyze the stability of phenolic content and antioxidant capacities after being submitted to in vitro digestion. In spite of all its limitations compared to natural digestion, this method provides the screening of a variety of samples, and is usually applied to mimic the physicochemical alterations observed in the gastrointestinal tract [8]. In addition, cellulosic dialysis membrane as a model imitating the intestinal brush border was used to confirm the bioaccessibility of antioxidant compounds and to determine their effect on colon health. The study also included the non-digested samples in order to compare them with the digested forms. The concentrations of non-digested samples were adjusted according to those of digested samples.

Our findings indicate significant decreases ($p < 0.05$) in both total phenolic and total flavonoid content, which are in positive correlation with results presented by Marhuenda et al. [15]. They reported a significant reduction in total phenolic contents of homogenates of raspberry and strawberry. The results of a similar study revealed as well the same decline in phenolic content in the IN fraction of raspberry extracts [16]. The alterations of pH value in digestion medium, particularly basic pH of the intestines, might be the cause of these dramatic decreases. Besides, the digestive enzymes found in the GI tract might also participate in this decrease due to their positive effect on the release of phenolic compounds from the sample matrix. The chemical structure of the phenolics appeared to be unstable and readily hydrolyzed [8, 17]. Several authors emphasized the same decrease in the total phenolic contents of different plant extracts [18]. All of these results mark the poor bioavailability of phenolic compounds. In addition, the results of our study revealed that a large portion of the phenolic substances found in herbal infusions were not absorbed and remained in OUT fraction, which represents “colon available” materials. Recent studies suggest that dietary polyphenols induce positive effects on the microbial activity of colon [19].

A decrease in the total antioxidant capacity of all herbal infusions was observed following in vitro digestion. Pomegranate infusion showed the highest antioxidant capacity in serum available (IN) fractions, surpassing green tea. Consequently, the results of nondigested samples might be interpreted as misleading to some extent. Supporting our notion, Marhuenda et al. [15] noted as well that the highest initial antioxidant activity did not lead to highest activity after in vitro digestion.

Nonetheless, free radical scavenging and copper reducing activity of bioaccessible IN fractions of green tea were found to be the highest. This difference may arise from the differences in the method used for analyzing the activities. It can be assumed that herbal infusion prepared from the peels of pomegranate exerts its antioxidant activity through the inhibition of lipid peroxidation or chain breaking reactions. Another assumption is that free radical scavenging molecules in pomegranate infusion are less bioavailable than those in green tea. On the other hand, another factor causing these alterations is possibly the formation of two chiral enantiomers with different reactivity due to pH-induced racemization [20]. The general increments in all herbal infusion may arise from the loss of phenolic concentrations in samples. They were degraded by the pH rise in the medium, causing an overall loss in the antioxidant capacity following in vitro digestion [21]. It also should be noted that antioxidant
molecules may interfere with other ingredients in the samples such as sugars, minerals or volatile compounds resulting in changes of their bioactivity [18, 22].

Conclusion

This study is believed to be the first one comparatively evaluating the stability of antioxidant capacity of herbal infusions consumed widely in Turkey, along with green tea. In spite of an enormous number of studies about the same parameters overlooking the physiological changes, this one is supposed to give new information about the bioavailability of antioxidant molecules in samples prepared just the same way by the public. Nevertheless, it should be noted that further studies are required for a full evaluation.

Conflict of interest statement: The authors declare that there is no conflict of interests regarding the publication of this article.

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