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Effect of in vitro digestion on hydrophilic and lipophilic antioxidant capacity of commonly consumed fruits and vegetables

Auswirkung der In-vitro-Verdauung auf die hydrophile und lipophile antioxidative Kapazität von häufig verzehrten Obst- und Gemüsesorten

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Summary

Raw consumed fruits and vegetables are the main sources of dietary antioxidants as bioactive food components. The aim of this study was to compare the hydrophilic and lipophilic antioxidant activity of fruits and vegetables after in vitro digestion. For this purpose, the hydrophilic and lipophilic fractions of these foods were isolated and then in vitro digestion was performed. The Folin-Ciocalteu method, a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay, and a Trolox equivalent antioxidant capacity (TEAC) assay were used herein. A comparison of the antioxidant activities before and after in vitro digestion revealed that digestion appeared to increase the antioxidant capacity of the lipophilic fractions, whereas no such increase occurred in the hydrophilic fractions. For the hydrophilic fraction, the total phenolic content of the tested fruits and vegetables was in the range of 0.02–0.44 g kg^{-1} after gastric digestion, and it was 0.01–0.34 g kg^{-1} after duodenal digestion. For the lipophilic fraction, it ranged from 0.01 to 5.07 g kg^{-1} after gastric digestion and 0.06 to 3.97 g kg^{-1} after duodenal digestion. For the hydrophilic fraction, the total antioxidant capacity of the tested fruits and vegetables was in the range of 18.7–31.5% after gastric digestion and it was 21.0–39.9% after duodenal digestion. For the lipophilic fraction, it ranged from 26.8 to 93.7% after gastric digestion and 49.0 to 92.2% after duodenal digestion. The majority of the fruits and vegetables tested showed significantly increased antioxidant activities after in vitro digestion, when compared to their initial value.

Keywords: Antioxidant, digestion, fruit, hydrophilic, lipophilic, vegetable

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Introduction

Oxidative stress has been implicated in a number of diseases, including cardiovascular dysfunction, cancer, diabetes, neurodegenerative diseases, endothelial cell dysfunctions, and several autoimmune diseases linked to the degenerative process of ageing (Juranic et al., 2005; Kaur et al., 2009; Krajka-Kuzniak et al., 2009; Alesiani et al., 2010). When the antioxidant systems of the human body are not sufficient to meet its needs, the intake of antioxidants from external sources is strongly needed (Jarrett, 2008). An organism can obtain antioxidants from external sources, either in natural form, such as from fruits or vegetables, or in synthetic form, such as nutritional supplements. Therefore, in recent years, natural antioxidants have received considerable interest from nutritionists, food manufacturers, and consumers.

Fruits and vegetables are rich sources of potentially bioactive compounds known as phytochemicals. One purported effect of certain phytochemicals present in plant foods is combating oxidative stress in the body by maintaining the balance between oxidants and antioxidants (Scalbert et al., 2005). Although fruits and vegetables have powerful antioxidant phytochemicals, the accessibility of these compounds differs for various reasons. Factors affecting the bioaccessibility of polyphenols are their release from the food matrix, particle size, hydrophilic/lipophilic balance depending on their glycosylation and interactions within the gastrointestinal tract (Kulesza et al. 2020). To maximize the benefits of these potential health-promoting ingredients, knowledge about the breakdown of food components during digestion is essential. Because the possible efficacy of food metabolites on human health is mainly determined by the bioavailability of these molecules. The in vitro digestion model allows for mimicking the physiological process occurring in the gastrointestinal system of humans by simulating the food transit time, pH, and specific enzymes. This system is widely used to study the bioaccessibility and bioavailability of phytochemicals of fruits and vegetables (Hur et al. 2011; Lee et al. 2016). Studies that offer in vitro digestion model simulating both the gastric and duodenal phases, involve the addition of digestive enzymes at biologically relevant pH and temperatures. The total antioxidant capacity is measured by one or more biochemical assays before and after the gastric phase, and then again after the duodenal phase of the digestion model to determine the stability of antioxidants throughout the digestion process (Wootton-Beard et al. 2011).

Consequently, it was aimed to determine the total phenolic content and total antioxidant capacity of the commonly consumed fruits and vegetables that constitute a major component of the human diet. In addition, it was aimed to observe whether these foods maintained their hydrophilic and lipophilic antioxidant capacity following gastric and duodenal digestion. For this purpose, the hydrophilic and lipophilic fractions of these foods were we isolated and in vitro digestion was performed to analyze the stability of their total antioxidant capacity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Trolox equivalent antioxidant capacity (TEAC) assays, as well as the Folin-Ciocalteu method for total polyphenols.

Materials and Methods

Chemicals and samples

The compounds 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, a water-soluble analogue of vitamin E), 2,20-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), and the Folin-Ciocalteu phenol reagent were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Methanol, ethanoic acid, hydrochloric acid, tetrahydrofuran, potassium persulfate, sodium ethanoate and sodium carbonate were all of analytical grade and were obtained from Merck (Darmstadt, Germany). Deionised water was used throughout the experiments. Fruit and vegetable samples were purchased at markets in Konya, Turkey and they were locally sourced from Turkey. The samples were acquired at the maturity level that was required for consumption based on the softness, colour and specific aspects of each fruit and vegetable. The samples were purchased in April 2014.

Sample preparation

The fresh vegetables were washed clean with deionized water, and the parts for analysis were separated. The peel and seed were discarded and only the pulp used for the extract preparation (Table 1). Immediately afterwards, the separated fruit and vegetable parts were ground into fine particles with a special grinder. Hydrophilic and lipophilic components of the vegetables were extracted as previously reported, with minor modifications (Li et al., 2009). Briefly, 8 g of sample were extracted with 50 ml of tetrahydrofuran in a shaking water bath (50 Hz, 37°C) for 30 min. The mixture was then centrifuged at 4200 g for 15 min, and the supernatant was collected. The extraction was repeated once with 5 ml of tetrahydrofuran under the same conditions, and the two supernatants were combined into the 'lipophilic fraction'. Subsequently, the residue was extracted twice using a mixture of methanol, ethanoic acid and water (50:3.7:46.3, v/v/v) (50 ml each time) in a shaking water bath (50 Hz, 37°C) for 30 min and those two supernatants were combined into the 'hydrophilic fraction'.

In vitro digestion procedure

The in vitro digestion procedure was performed as described by Ryan et al. (2008). Briefly, 10 ml of the lipophilic and hydrophilic fractions from the samples were transferred to clean bottles and mixed with 20 ml saline solution containing 140 mol·l-1 NaCl and 5 mol·l-1 KCl. The sample was acidified to pH 2.0 with 1 ml of a porcine pepsin preparation (0.04 g pepsin in 1 ml 0.1 mol/l HCl), and incubated at 37°C in a shaking bath at 50 Hz for 1 h. Following this in vitro gastric digestion, 8 ml of sample were retained. The pH of the samples was increased to pH 5.3 using 0.9 mol/l sodium bicarbonate solution, followed by the addition of 1 ml of bile salts and pancreatin solution (0.8 g glycodeoxycholate in 20 ml saline, 0.8 g taurocholate in 20 ml saline, and 1.6 g pancreatin in 20 ml saline). The pH of each sample then was increased to pH 7.4 using 1 mol/l NaOH. The sample was then incubated for 2.5 h at 37°C, in a shaking bath at 50 Hz, to complete the duodenal phase of the in vitro digestion process. After the duodenal phase, 2 ml of each sample were stored at -20°C. The samples were analysed within 2 weeks.

Determination of total phenolic content

Total phenolic content (TPC) was measured using the Folin-Ciocalteu colorimetric method, as described previously (Ryan et al. 2008). Sample extracts (0.2 ml) were prepared for total phenolic content measurement by mixing with 4.8 ml of distilled water. Folin-Ciocalteu reagent (0.5 ml, 1:3 dilution) was added, and then the mixture was incubated at room temperature for 30 min. TPC was de-

termined following the addition of 1 ml of 35% sodium carbonate to the mixture with 1 h of subsequent incubation at room temperature. The absorbance was measured at 765 nm using a mini-spectrophotometer (Shimadzu UV-1240, Osaka, Japan). Gallic acid was used as the standard for a calibration curve, and the results were expressed as grams of gallic acid equivalents (GAE) per kilogram of fresh weight of fruit and vegetables. All determinations were performed three times (n = 3).

Determination of 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined according to the method of Yu et al. (2002). This method is based on the ability of antioxidants to scavenge DPPH free radicals. Briefly, 100 ml of either sample extract or standard were added to 0.9 ml of buffer (3.3 µg hydroxymethyl aminomethane hydrochloride (Tris–HCI) in water) and 2 ml of DPPH reagent (39.4 µg DPPH reagent in methanol) and vortexed vigorously. The mixture was incubated in the dark for 30 min at room temperature, and the discolouration of DPPH was measured relative to a 'blank' at 517 nm. Ethanol (100%) was used as control. Percentage of inhibition of DPPH absorbance was calculated according to following equation:

$$I = \frac{A_0 - A}{A_0} \times 100$$

where A_0 is absorbance of control and A is absorbance of sample. All determinations were performed in triplicate (n = 3).

Determination of trolox equivalent antioxidant capacity

The antioxidant capacity of each sample was determined using the procedure described by Miller and Rice-Evans (1996) with a few modifications. Decolourisation of the 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation by sample extract, was measured spectrophotometrically at 734 nm, relative to a Trolox standard. In the present study, metmyoglobin was used to generate the ABTS radical cation in potassium phosphate-buffered saline (PBS), pH 7.4. Metmyoglobin (400 mM, final concentration) was mixed with ABTS (5.0 mM, final

TABLE 1: *Total fenolic content of tested fruits and vegetables hydrophilic and lipophilic fractions before and after in vitro digestion.*

Sample name	Hydrophilic fraction [g·kg⁻¹]			Lipophilic fraction [g·kg⁻¹]		
	Fresh sample	After gastric digestion	After duodenal digestion	Fresh sample	After gastric digestion	After duodenal digestion
Kale (*Brassica oleracea* L.)	0.83 ± 0.02	0.19 ± 0.02[a]	0.15 ± 0.01[b]	2.12 ± 0.03	2.48 ± 0.02[a]	1.68 ± 0.01[b]
Dill (*Anethum graveolens*)	1.06 ± 0.04	0.29 ± 0.01[a]	0.19 ± 0.01[b]	3.10 ± 0.04	3.75 ± 0.00[a]	2.00 ± 0.04[b]
Parsley (*Petroselinium hortense*)	0.73 ± 0.02	0.22 ± 0.02[a]	0.13 ± 0.02[b]	1.36 ± 0.02	1.56 ± 0.01[a]	1.76 ± 0.11[b]
Purslane (*Portulaca oleracea*)	0.25 ± 0.03	0.02 ± 0.02[a]	0.02 ± 0.02[b]	4.18 ± 0.03	3.19 ± 0.00[a]	3.32 ± 0.01[b]
Cress (*Lepidium sativum*)	0.45 ± 0.04	0.14 ± 0.01[a]	0.04 ± 0.00[b]	1.15 ± 0.04	1.44 ± 0.01[a]	1.58 ± 0.00[b]
Mint (*Mentha piperita*)	0.66 ± 0.03	0.17 ± 0.03[a]	0.11 ± 0.01[b]	3.94 ± 0.02	3.10 ± 0.05[a]	1.17 ± 0.13[b]
Cucumber (*Cucumis sativus*)	0.18 ± 0.04	0.04 ± 0.01[a]	0.01 ± 0.01[b]	0.86 ± 0.01	0.01 ± 0.00[a]	0.06 ± 0.02[b]
Green pepper (*Capsicum annuum*)	0.35 ± 0.02	0.09 ± 0.01[a]	0.05 ± 0.01[b]	2.15 ± 0.01	2.20 ± 0.00[a]	1.33 ± 0.05[b]
Lettuce (*Lactuca sativa*)	0.18 ± 0.01	0.03 ± 0.01[a]	0.01 ± 0.01[b]	0.17 ± 0.02	0.20 ± 0.01[a]	0.25 ± 0.02[b]
Carrot (*Daucus carota*)	0.09 ± 0.02	0.05 ± 0.01[a]	0.34 ± 0.03[b]	1.10 ± 0.01	1.01 ± 0.01[a]	0.35 ± 0.02[b]
Sour cherry (*Prunus cerasus*)	0.26 ± 0.01	0.17 ± 0.03[a]	0.05 ± 0.01[b]	3.15 ± 0.03	3.26 ± 0.02[a]	2.93 ± 0.02[b]
Pear (*Pyrus communis*)	0.35 ± 0.01	0.14 ± 0.01[a]	0.01 ± 0.01[b]	2.83 ± 0.02	3.43 ± 0.01[a]	3.17 ± 0.01[b]
Plum (*Prunus domestica* L.)	0.29 ± 0.02	0.17 ± 0.02[a]	0.02 ± 0.01[b]	3.40 ± 0.02	3.42 ± 0.07	3.56 ± 0.04
Peach (*Prunus persica*)	0.36 ± 0.03	0.04 ± 0.02	0.04 ± 0.01	1.68 ± 0.02	2.28 ± 0.00[a]	2.39 ± 0.11[b]
Pomegranate (*Punica granatum*)	0.27 ± 0.03	0.19 ± 0.04	0.08 ± 0.01[b]	3.17 ± 0.03	3.27 ± 0.01[a]	3.42 ± 0.02[b]
Tomato (*Lycopersicon esculentum*)	0.13 ± 0.02	0.10 ± 0.04	0.08 ± 0.02[b]	2.86 ± 0.02	2.68 ± 0.11	1.34 ± 0.05[b]
Pumpkin (*Cucurbita moschata*)	0.33 ± 0.03	0.19 ± 0.02[a]	0.08 ± 0.00[b]	2.79 ± 0.02	2.91 ± 0.14	3.81 ± 0.05[b]
Apple (*Malus domestica*)	0.29 ± 0.02	0.34 ± 0.03	0.07 ± 0.02[b]	3.56 ± 0.10	3.46 ± 0.12	3.25 ± 0.24
Orange (*Citrus sinensis*)	0.26 ± 0.01	0.24 ± 0.07	0.05 ± 0.03[b]	2.12 ± 0.01	1.51 ± 0.01[a]	1.37 ± 0.02[b]
Tangerine (*Citrus reticulata*)	0.05 ± 0.01	0.24 ± 0.04	0.08 ± 0.02	1.17 ± 0.02	1.64 ± 0.02[a]	0.27 ± 0.06[b]
Melon (*Cucumis melo*)	0.14 ± 0.02	0.15 ± 0.03	0.05 ± 0.01[b]	2.98 ± 0.11	2.15 ± 0.51[a]	1.18 ± 0.03[b]
Watermelon (*Citrullus lanatus*)	0.03 ± 0.01	0.10 ± 0.02	0.02 ± 0.01	1.03 ± 0.02	0.81 ± 0.00[a]	0.16 ± 0.00[b]
Quince (*Cydonia oblonga*)	0.98 ± 0.03	0.32 ± 0.02	0.18 ± 0.07[b]	5.07 ± 0.14	4.94 ± 0.18	3.97 ± 0.03[b]
Radish (*Raphanus sativus*)	0.31 ± 0.02	0.19 ± 0.07	0.08 ± 0.01[b]	1.33 ± 0.03	2.98 ± 0.05[a]	1.72 ± 0.06[b]
Persimmon (*Diospyros kaki* L.)	0.45 ± 0.01	0.44 ± 0.09	0.11 ± 0.00[b]	2.89 ± 0.02	3.60 ± 0.02[a]	3.22 ± 0.05[b]
Grape (*Vitis vinifera*)	0.23 ± 0.02	0.21 ± 0.05	0.11 ± 0.01[b]	3.42 ± 0.03	0.74 ± 0.03[a]	0.81 ± 0.10[b]
Strawberry (*Fragaria ananassa*)	0.16 ± 0.01	0.12 ± 0.02	0.08 ± 0.01[b]	3.85 ± 0.02	0.79 ± 0.01[a]	1.02 ± 0.02[b]
Red pepper (*Capsicum annuum*)	0.53 ± 0.08	0.14 ± 0.01[a]	0.13 ± 0.01[b]	2.33 ± 0.03	3.14 ± 0.06[a]	2.95 ± 0.03[b]

[a,b]: P<0.05. [a]: Significant differens (P<0.05) prior compared with gastric digestion. [b]: Significant differens (P<0.05) prior compared with duodenal digestion. Values represented with mean ± standard deviation of the means of three independent experiment (n=3).

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concentration) in 5.0 mM PBS, and the reaction was initiated by the addition of hydrogen peroxide (0.1 mM, final concentration). The ABTS radical cation solution thus obtained was diluted with PBS (1/1 v/v) to give an absorbance of 0.8 at 734 nm. A solvent blank was recorded for each experiment. The measurements were recorded on all samples at the point when a sharp decrease in absorbance was observed in the 2.5 mM Trolox solution. These absorbance readings reflected the ABTS radical cation scavenging capacity and were plotted against the concentration of the antioxidant. The Trolox equivalent antioxidant capacity (TEAC) value represents the ratio between the slope of the linear plot of the ABTS·+ radical cation scavenging by the samples, to the slope of the plot for the ABTS radical cation scavenging by Trolox. Results are expressed as millimoles of Trolox equivalent antioxidant capacity per kilogram of fresh weight.

Statistical Analysis

All experiments were performed in triplicate, and the results are expressed as mean values ± standard deviation. In order to investigate the differences in the antioxidant capacities and phenolic content between different fruit and vegetable fractions, statistical analyses were carried out, including analysis of variance (ANOVA) and Two Independent Sample Tests (Mann-Whitney U Tests). Statistical analyses were performed

Results and discussion

Total phenolic content of the samples

The different fruit and vegetable samples possessed diverse total phenolic contents (Table 1). Before digestion, the measurable phenolic contents were in the range of 0.03–1.06 g kg^{-1}, 0.17–5.07 g kg^{-1}, and 0.98–6.05 g kg^{-1} (expressed as GAE) for the hydrophilic fraction, the lipophilic fraction, and the total sample, respectively. Statistically significant differences were observed between the phenolic contents of the lipophilic and hydrophilic fractions, with the total phenolic contents of the lipophilic fractions being much higher than those of the hydrophilic fractions ($P < 0.001$). Similarly, studies that have analyzed various fruits and vegetables and shown that the antioxidant capacities and polyphenol contents in lipophilic fractions were higher than those in hydrophilic fractions (Deng et al., 2013; Liu et al., 2018).

The total measurable phenol content of some fruits, such as apple and grape, were similar to those reported by Karadeniz et al. (2005) and Wu et al. (2004), but different than those reported of Takebayashi et al. (2013). In the present study, values different than those reported in previous studies were obtained. The total measurable phenolic content of green pepper, red pepper, and cucumber was lower than those reported by Deng et al. (2013). These differences might have been attributed to various factors, such as the cultivars examined, the season, and pre- and post-harvest conditions that can affect the chemical composition of plant foods. Following the gastric phase of the in vitro digestion model, there was a significant increase ($P < 0.05$) in the total measurable phenol content of 15 fruits and vegetables. After the duodenal phase of digestion, the total measurable phenolic content of 4 fruits and 6 vegetables (pear, peach, pomegranate, persimmon, purslane, cress, lettuce, pumpkin, radish, and red pepper) was significantly increased ($P < 0.05$) when compared with their initial measurable phenolic content. In contrast, the total measurable phenolic contents of 17 fruits decreased. The chemical environment of the gastrointestinal tract might affect the release of phenols. In a similar study conducted by Chen et al., (2014) after the gastric phase of the in vitro digestion the total phenolic content of pear, red grape, citrus (Hunan), and loquat increased, whereas the total phenolic content of apple, peach, orange, plum, nectarine, citrus (yellow) and grapefruit decreased. Moreover, the duodenal phase of the in vitro digestion increased the total phenolic content of apple, grape (black, green, and red), nectarine, peach, watermelon, and pear, while it decreased in plum and grapefruit. It has been reported that in vitro digestion causes a 16-fold reduction in the phenolic content of blueberries (Şensu et al, 2021). It was determined that in vitro digestion reduced the total phenolic content of apple, orange, grape, pomegranate, and kiwifruit juices by 7.8%–35% (Quan et al., 2018). In a review, it was indicated that most in vitro studies have reported that the gastric phase maintains the stability of phenolics; however, the intestinal phase may cause a decrease in the amount of phenolics (Wojtunik-Kulesza et al., 2020). These results support the findings of the current study.

Contrary to gastric conditions, pancreatic conditions lead to the degradation of red grape phenolic acids and resveratrol, but not catechin and quercetin (Tagliazucchi et al., 2010). According to Tagliazucchi et al. (2010), about 15% of polyphenols were degraded during the transition from the acidic gastric environment to the alkaline intestinal environment.

2,2-diphenyl-1-picrylhydrazyl radical scavenging activity

Since both lipophilic and hydrophilic components contribute significantly to the antioxidant capacity of plant foods, it is recommended that both fractions should be analyzed (Li et al., 2009). Total antioxidant capacity (TAC), as measured by the DPPH assay, is shown in Table 2. The DPPH radical inhibition values varied from 2.3% to 10.8%, and from 40.8% to 91.7% in the hydrophilic fraction and lipophilic fractions, respectively. The samples with the highest lipophilic DPPH radical inhibition values ranked as follows: sour cherry, pomegranate, quince, kale, plum (DPPH radical inhibition values of 91.7%, 88.1%, 87.4%, 86.9%, and 85.4%, respectively). Cucumber and lettuce had the lowest DPPH radical inhibition values among the tested samples. The differences in antioxidant capacities between lipophilic and hydrophilic fractions were statistically analyzed. In DPPH radical inhibition assays, the antioxidant capacities of the lipophilic fractions were higher than those of the hydrophilic fractions ($P < 0.05$).

After the gastric phase of the in vitro digestion of the lipophilic fractions, the DPPH radical inhibition values of 11 fruits and 5 vegetables were significantly increased ($P < 0.05$). Pomegranate, strawberry, grape, and persimmon (kaki) exhibited the highest antioxidant capacities, followed by quince and sour cherry. Cucumber had the lowest DPPH radical inhibition value following the gastric phase of digestion. Chen et al. (2014) also found significant increasement in DPPH value of fruits especially grape, pear, apple, citrus, and peach after the gastric phase of the digestion. In contrast to the gastric phase of digestion, the DPPH radical inhibition value of 15 fruits and vegetables significantly decreased ($P < 0.05$) following the duodenal phase of the in vitro digestion. After the duodenal phase of digestion, the total DPPH radical inhibition value of

the lipophilic fractions from 13 vegetable and 8 fruit samples significantly increased ($P < 0.05$), compared to their initial DPPH assays. In contrast, the DPPH assay of kale, pomegranate, and quince decreased after in vitro duodenal digestion. Interestingly, the radical scavenging capacity of 18 samples increased following the gastric digestion phase and decreased following the duodenal digestion phase, although there were some exceptions to this trend (kale, cucumber, lettuce, carrot, sour cherry, pear, peach, tomato, radish). In the same way it was reported a significant reduction in DPPH value of almost whole fruit (except red grape) after duodenal phase by Chen et al. (2014). Sollano-Mendieta et al. (2021) found a significant decrease in the DPPH value of 12 plum ecotypes after the gastric phase and an increase after the intestinal phase. Moreover, in this study, the DPPH radical inhibition of the hydrophilic fraction improved, but lipophilic fraction declined after the intestinal phase in the plum samples.

Studies carried out on mulberry (Liang et al., 2012) and gooseberry (Chiang et al., 2013) also found that digestion enhanced the availability of antioxidants in these fruits, wherein the digested fruits showed a higher TAC. However, due to pH, anthocyanins are largely transformed into non-red forms and are degraded (97%). Similar results were obtained for vitamin C (>95% degradation), leading to a decrease in the TAC after digestion. In line with this, Bermúdez-Soto et al. (2007) found an increase in the number of certain polyphenols after the gastric phase, yet the pancreatic digestion phase caused a decrease in the level of these antioxidants. Moreover, the interaction of phenolics with other dietary compounds released during digestion (e.g., dietary fibre or proteins) is known to affect their solubility and availability, and thus their antioxidant potential (Bouayed et al. 2011). Simulated gastrointestinal digestion affects the stability of the antioxidant capacity of phenolic compounds. Synergistic effects of nutrients, chemical reactions promoting the oxidation and polymerization of bioactive compounds, enzymatic actions causing molecular transformations, and polyphenol oxidase activity and different pH conditions are the main factors (Ketnawa, 2021).

Trolox equivalent antioxidant capacity analysis

The different fruit and vegetable samples possessed diverse TEAC values (Table 3). The TEAC values before diges-

TABLE 2: *Total antioxidant capacity values for tested fruits hydrophilic and lipophilic fractions before and after in vitro digestion.*

Sample name	Hydrophilic fraction [%]			Lipophilic fraction [%]		
	Fresh sample	After gastric digestion	After duodenal digestion	Fresh sample	After gastric digestion	After duodenal digestion
Kale (*Brassica oleracea* L.)	4.4 ± 0.4	27.6 ± 2.1^{a}	31.7 ± 2.0^{b}	87.4 ± 1.2	83.8 ± 2.7	89.3 ± 0.3^{b}
Dill (*Anethum graveolens*)	4.2 ± 0.3	31.5 ± 4.9^{a}	39.9 ± 2.3^{b}	68.9 ± 1.5	81.7 ± 2.7^{a}	72.9 ± 0.2^{b}
Parsley (*Petroselinium hortense*)	4.0 ± 1.2	19.6 ± 1.3^{a}	26.0 ± 1.8^{b}	57.7 ± 0.9	75.0 ± 0.4^{a}	66.2 ± 1.7^{b}
Purslane (*Portulaca oleracea*)	2.9 ± 0.5	18.7 ± 0.9^{a}	25.2 ± 1.4^{b}	65.9 ± 0.8	84.8 ± 0.7^{a}	72.5 ± 1.4^{b}
Cress (*Lepidium sativum*)	3.3 ± 0.9	20.9 ± 1.2^{a}	25.3 ± 1.3^{b}	65.1 ± 1.5	82.1 ± 0.5^{a}	78.5 ± 2.1^{b}
Mint (*Mentha piperita*)	4.4 ± 0.9	28.7 ± 1.2^{a}	30.5 ± 2.4^{b}	66.6 ± 2.3	74.0 ± 2.1^{a}	74.0 ± 2.6^{b}
Cucumber (*Cucumis sativus*)	2.3 ± 0.5	19.3 ± 1.6^{a}	23.0 ± 1.4^{b}	42.1 ± 0.2	26.8 ± 0.2^{a}	49.0 ± 0.7^{b}
Green pepper (*Capsicum annuum*)	3.2 ± 1.5	21.5 ± 1.6^{a}	22.4 ± 1.2^{b}	52.1 ± 0.5	66.4 ± 0.6^{a}	84.1 ± 1.3^{b}
Lettuce (*Lactuca sativa*)	2.3 ± 0.3	19.7 ± 1.9^{a}	22.8 ± 1.2^{b}	39.7 ± 1.1	77.1 ± 1.2^{a}	56.4 ± 0.7^{b}
Carrot (*Daucus carota*)	2.6 ± 0.5	19.0 ± 1.7^{a}	22.6 ± 1.4^{b}	83.6 ± 2.9	46.6 ± 0.2^{a}	85.2 ± 0.1
Sour cherry (*Prunus cerasus*)	4.0 ± 0.9	26.7 ± 1.3^{a}	27.9 ± 3.1^{b}	91.7 ± 2.8	89.4 ± 2.0	91.5 ± 0.4
Pear (*Pyrus communis*)	3.4 ± 0.6	23.7 ± 2.1^{a}	25.0 ± 1.9^{b}	58.5 ± 1.5	61.0 ± 1.0	88.8 ± 1.2^{b}
Plum (*Prunus domestica* L.)	3.2 ± 0.5	24.7 ± 1.1^{a}	26.0 ± 1.4^{b}	85.3 ± 1.2	88.5 ± 1.2	77.1 ± 1.0^{b}
Peach (Prunus persica)	2.6 ± 0.2	21.4 ± 0.8^{a}	23.6 ± 1.8^{b}	60.6 ± 0.8	68.4 ± 0.3^{a}	66.2 ± 0.2^{b}
Pomegranate (*Punica granatum*)	4.2 ± 1.2	28.4 ± 1.2^{a}	25.9 ± 1.4^{b}	88.1 ± 2.2	92.7 ± 2.0^{a}	88.8 ± 0.8
Tomato (*Lycopersicon esculentum*)	2.5 ± 0.5	19.5 ± 0.9^{a}	22.0 ± 1.5^{b}	45.2 ± 1.0	58.3 ± 0.8^{a}	51.6 ± 0.2^{b}
Pumpkin (*Cucurbita moschata*)	2.7 ± 0.6	18.7 ± 1.5^{a}	22.8 ± 1.0^{b}	43.1 ± 0.3	88.2 ± 0.2	76.3 ± 0.3^{b}
Apple (*Malus domestica*)	2.9 ± 0.2	23.6 ± 2.1^{a}	26.4 ± 1.5^{b}	58.1 ± 0.8	85.2 ± 1.5^{a}	78.0 ± 0.8^{b}
Orange (*Citrus sinensis*)	2.6 ± 0.5	19.5 ± 0.6^{a}	23.1 ± 1.7^{b}	44.5 ± 0.2	88.1 ± 0.2^{a}	61.1 ± 0.1^{b}
Tangerine (*Citrus reticulata*)	2.5 ± 0.8	19.4 ± 1.2^{a}	21.0 ± 0.8^{b}	47.1 ± 1.1	82.7 ± 0.5^{a}	59.9 ± 0.7^{b}
Melon (*Cucumis melo*)	2.4 ± 0.9	19.7 ± 1.6^{a}	22.2 ± 1.2^{b}	40.8 ± 0.1	82.0 ± 1.3^{a}	71.6 ± 0.5^{b}
Watermelon (*Citrullus lanatus*)	2.3 ± 0.1	18.7 ± 0.7^{a}	22.2 ± 1.4^{b}	47.1 ± 0.9	76.8 ± 0.6^{a}	67.5 ± 1.2^{b}
Quince (*Cydonia oblonga*)	4.6 ± 1.2	29.8 ± 2.1^{a}	36.7 ± 2.6^{b}	86.9 ± 1.4	90.3 ± 1.9^{a}	73.8 ± 0.7^{b}
Radish (*Raphanus sativus*)	2.9 ± 0.1	19.4 ± 0.2^{a}	23.1 ± 1.6^{b}	49.5 ± 0.8	46.1 ± 0.7^{a}	92.2 ± 0.5^{b}
Persimmon (*Diospyros kaki* L.)	4.7 ± 1.0	22.8 ± 1.9^{a}	25.4 ± 2.1^{b}	63.9 ± 2.7	92.4 ± 1.0^{a}	83.4 ± 0.6^{b}
Grape (*Vitis vinifera*)	3.5 ± 0.7	22.1 ± 1.7^{a}	25.6 ± 1.4^{b}	50.7 ± 2.9	93.5 ± 2.0^{a}	83.7 ± 0.5^{b}
Strawberry (*Fragaria ananassa*)	3.9 ± 0.4	22.0 ± 1.1^{a}	24.2 ± 1.5^{b}	72.8 ± 1.1	93.7 ± 1.8^{a}	78.4 ± 0.4^{b}
Red pepper (*Capsicum annuum*)	10.8 ± 1.2	19.3 ± 0.7^{a}	24.5 ± 2.1^{b}	50.7 ± 1.6	71.1 ± 0.2^{a}	63.6 ± 0.9^{b}

^{a,b}: $P<0.05$. ^{a}: Significant differens ($P<0.05$) prior compared with gastric digestion. ^{b}: Significant differens ($P<0.05$) prior compared with duodenal digestion. Values represented with mean ± standard deviation of the means of three independent experiment (n=3).

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tion were in the range of 0.12 mmol·kg^{-1} to 4.13 mmol·kg^{-1}, and 3.55 mmol·kg^{-1} to 18.28 mmol·kg^{-1} (expressed as Trolox equivalents) in the hydrophilic and lipophilic fractions, respectively. Dill, sour cherry, pomegranate, and strawberry possessed the highest TEAC values (18.28, 18.22, 17.24, and 17.05 mmol kg^{-1}, respectively).

Following the gastric phase of the in vitro digestion model, there was a significant increase ($P < 0.05$) in the TEAC values of the lipophilic fractions from 22 fruits and vegetables. After the duodenal phase of digestion, the TEAC values of the lipophilic fractions of 21 fruit and vegetable samples significantly increased ($P < 0.05$) when compared to their initial values. Compared with the gastric phase of digestion, the TEAC value for 20 fruits and vegetables significantly decreased ($P < 0.05$) after the duodenal phase of digestion. The TEAC values for carrot, orange, tangerine, strawberry, and red pepper decreased significantly following in vitro digestion when compared to their initial forms. Pomegranate and sour cherry had the highest antioxidant capacity following in vitro digestion. Additionally, compared to the initial TEAC value, pomegranate, pear, and grape exhibited the most remarkable increases upon in vitro digestion among the tested foods (1.49-fold, 1.34-fold, and 1.29-fold respectively). In a study in which 33 fruits were analyzed, it was shown that the antioxidant capacity of 26 fruits increased significantly after the gastric and duodenal phases of the in vitro digestion model. It was determined that plum had the highest antioxidant capacity before and after in vitro digestion. Moreover, the antioxidant capacity of pear (fragrant) increased by approximately 3.27-fold (Chen et al., 2014).

Changes in the antioxidant activity of the fruits and vegetables following in vitro digestion are provided in Table 4. Following digestion, averages in the TAC assays over all fruits and all vegetables increased nearly 1.19-fold and 1.23-fold, and averages in the TEAC values of all of the fruits and vegetables increased by nearly 1.21-fold and 1.13-fold, respectively. There were no significant differences between the antioxidant capacities of the fruits and the vegetables. The preservation of the stability of the carotenoids and anthocyanins during the in vitro digestion process in the study of Tommonaro (2017) explained the preservation of the antioxidant capacity in the lipophilic fraction after digestion. In addition, a significant loss of antioxidant activi-

TABLE 3: *Trolox equivalent antioxidant capacity values for tested foods hydrophilic and lipophilic fractions before and after in vitro digestion.*

Sample name	Hydrophilic fraction [mmol·kg^{-1}]			Lipophilic fraction [mmol·kg^{-1}]		
	Fresh sample	After gastric digestion	After duodenal digestion	Fresh sample	After gastric digestion	After duodenal digestion
Kale (*Brassica oleracea* L.)	3.18 ± 0.25	3.85 ± 0.22^{a}	2.98 ± 0.15^{b}	14.31 ± 0.23	15.25 ± 0.68	16.14 ± 0.55^{b}
Dill (*Anethum graveolens*)	2.87 ± 0.05	3.33 ± 0.18^{a}	3.16 ± 0.18	18.28 ± 0.19	19.25 ± 0.75	18.96 ± 0.52
Parsley (*Petroselinium hortense*)	0.84 ± 0.01	1.25 ± 0.02^{a}	1.22 ± 0.03^{b}	5.91 ± 0.15	9.65 ± 0.56^{a}	8. 15 ± 0.35^{b}
Purslane (*Portulaca oleracea*)	1.01 ± 0.01	1.18 ± 0.02^{a}	1.03 ± 0.08	11.25 ± 0.51	15.12 ± 0.12^{a}	13.21 ± 0.51^{b}
Cress (*Lepidium sativum*)	1.03 ± 0.02	1.85 ± 0.03^{a}	1.69 ± 0.04^{b}	8.75 ± 0.08	11.28 ± 0.09^{a}	10.85 ± 0.39^{b}
Mint (*Mentha piperita*)	0.85 ± 0.01	0.98 ± 0.01^{a}	0.88 ± 0.02	7.36 ± 0.02	11.53 ± 0.15^{a}	9.83 ± 0.08^{b}
Cucumber (*Cucumis sativus*)	0.41 ± 0.01	0.66 ± 0.00^{a}	0.44 ± 0.03	3.55 ± 0.10	3.67 ± 0.11	4.87 ± 0.12^{b}
Green pepper (*Capsicum annuum*)	1.24 ± 0.03	1.52 ± 0.02^{a}	0.98 ± 0.01^{b}	8.17 ± 0.09	13.21 ± 0.84^{a}	11.84 ± 0.65^{b}
Lettuce (*Lactuca sativa*)	0.58 ± 0.02	0.63 ± 0.08^{a}	0.81 ± 0.02^{b}	5.19 ± 0.12	7.55 ± 0.08^{a}	6.98 ± 0.07^{b}
Carrot (Daucus carota)	0.41 ± 0.01	0.35 ± 0.01^{a}	0.88 ± 0.01^{b}	8.94 ± 0.04	8.21 ± 0.06^{a}	7.76 ± 0.03^{b}
Sour cherry (*Prunus cerasus*)	3.02 ± 0.21	2.58 ± 0.17^{a}	1.24 ± 0.13^{b}	18.22 ± 0.08	21.14 ± 0.23^{a}	19.18 ± 0.09^{b}
Pear (*Pyrus communis*)	0.85 ± 0.05	1.25 ± 0.18	0.80 ± 0.05	9.39 ± 0.18	11.87 ± 0.21^{a}	13.95 ± 0.17^{b}
Plum (*Prunus domestica* L.)	2.12 ± 0.01	1.98 ± 0.08	1.58 ± 0.11^{b}	15.18 ± 0.04	17.29 ± 0.15^{a}	16.38 ± 0.13^{b}
Peach (*Prunus persica*)	0.76 ± 0.01	0.88 ± 0.01^{a}	1.01 ± 0.02^{b}	11.48 ± 0.11	12.84 ± 0.42^{a}	14.12 ± 0.31^{b}
Pomegranate (*Punica granatum*)	4.13 ± 0.04	4.52 ± 0.12^{a}	3.46 ± 0.18^{b}	17.24 ± 0.14	20.25 ± 0.27^{a}	22.35 ± 0.20^{b}
Tomato (*Lycopersicon esculentum*)	1.16 ± 0.21	0.86 ± 0.02	0.96 ± 0.08	9.24 ± 0.11	11.57 ± 0.35^{a}	10.83 ± 0.12^{b}
Pumpkin (*Cucurbita moschata*)	0.91 ± 0.02	0.78 ± 0.03	0.35 ± 0.02^{b}	8.95 ± 0.06	12.25 ± 0.23^{a}	11.14 ± 0.15^{b}
Apple (*Malus domestica*)	1.22 ± 0.03	1.35 ± 0.14	1.08 ± 0.01^{b}	9.47 ± 0.07	12.52 ± 0.15^{a}	11.36 ± 0.18^{b}
Orange (*Citrus sinensis*)	0.31 ± 0.01	0.85 ± 0.08	0.56 ± 0.02^{b}	5.96 ± 0.02	5.22 ± 0.08^{a}	4.35 ± 0.03^{b}
Tangerine (*Citrus reticulata*)	0.28 ± 0.07	0.71 ± 0.06	0.33 ± 0.02^{b}	5.29 ± 0.08	5.03 ± 0.06^{a}	4.85 ± 0.04^{b}
Melon (*Cucumis melo*)	0.42 ± 0.06	0.39 ± 0.02	0.27 ± 0.01^{b}	4.48 ± 0.09	6.61 ± 0.05^{a}	5.84 ± 0.03^{b}
Watermelon (*Citrullus lanatus*)	0.14 ± 0.03	0.18 ± 0.01	0.08 ± 0.00^{b}	3.81 ± 0.06	6.02 ± 0.06^{a}	5.18 ± 0.04^{b}
Quince (*Cydonia oblonga*)	0.25 ± 0.04	1.42 ± 0.18^{a}	0.98 ± 0.07^{b}	11.50 ± 0.10	13.84 ± 0.61^{a}	12.83 ± 0.02^{b}
Radish (*Raphanus sativus*)	2.18 ± 0.01	1.88 ± 0.42	1.58 ± 0.18^{b}	13.43 ± 0.15	12.57 ± 0.04^{a}	13.25 ± 0.03
Persimmon (*Diospyros kaki* L.)	0.12 ± 0.02	0.38 ± 0.00^{a}	0.15 ± 0.05	9.74 ± 0.08	12.87 ± 0.09^{a}	13.14 ± 0.02^{b}
Grape (*Vitis vinifera*)	1.18 ± 0.12	1.25 ± 0.08	1.15 ± 0.08	12.47 ± 0.07	13.52 ± 0.12^{a}	16.18 ± 0.08^{b}
Strawberry (*Fragaria ananassa*)	1.24 ± 0.05	1.57 ± 0.02^{a}	1.02 ± 0.05^{b}	17.05 ± 0.19	19.23 ± 0.31^{a}	16.74 ± 0.23^{b}
Red pepper (*Capsicum annuum*)	1.31 ± 0.02	1.82 ± 0.05^{a}	1.19 ± 0.06^{b}	12.84 ± 0.10	12.90 ± 0.05	11.41 ±0.06^{b}

^{a,b}: $P<0.05$. ^{a}: Significant differens ($P<0.05$) prior compared with gastric digestion. ^{b}: Significant differens ($P<0.05$) prior compared with duodenal digestion. Values represented with mean ± standard deviation of the means of three independent experiment (n=3).

TABLE 4: *Comparison of the antioxidant capacity and phenolic contents of fruits and vegetables.*

Samples	Sample number	Total Phenol [g·kg⁻¹]	DPPH value [%]	TEAC value [mmol·kg⁻¹]
Vegetables (n=14)	Fresh	2.10 ± 1.18	64.0 ± 17.9	9.73 ± 3.95
	Gastric digestion	2.18 ± 1.16	68.7 ± 18.0	11.98 ± 3.77
	Duodenal digestion	1.66 ± 1.10	76.4 ± 10.0	10.99 ± 3.56
Fruits (n=14)	Fresh	2.88 ± 1.08	58.4 ± 15.1	10.94 ± 4.83
	Gastric digestion	2.52 ± 1.29	84.6 ± 9.9	12.02 ± 5.34
	Duodenal digestion	2.19 ± 1.33	72.3 ± 13.5	11.86 ± 5.32

ty was observed in tomato due to the hydrophilic fraction containing polar metabolites. In tomato hybrids, a marked loss of antioxidant capacity linked to the hydrophilic fraction was observed, and the lipophilic fraction, containing mainly carotenoids, showed increased antioxidant activity after gastric digestion (Tommonaro et al., 2017). Indeed, these data confirmed that the bioavailability of bioactive metabolites in fruits and vegetables is strictly linked to the digestive process.

Furthermore, the total measurable phenolic content of the foods tested showed a strong correlation with their total antioxidant capacities (R = 0.837 for DPPH method, and R = 0.820 for TEAC). Additionally, after digestion, the phenolic compounds were significantly correlated with the DPPH ($R = 0.939$, $P < 0.001$) and TEAC ($R = 0.922$, $P < 0.001$). The phenolic compounds made a significant contribution to the antioxidant capacity of the fruits and vegetables. The findings obtained from studies on different fruits and vegetables were also in this direction (Alberti et al., 2017; Stafussa et al., 2018; Rojas-Ocampo et al., 2021).

One of them is, used in vitro digestion model is particularly useful for digestion studies on simple foods and isolated or purified food components, but it is insufficient to detect interactions of compounds (Alminger et al. 2014). Its strength is that in vitro digestion and applied antioxidant capacity determination methods allow testing and comparing a large number of samples, as they are faster and cheaper. It should be noted that enzymes could play a role in polyphenol bioaccessibility by releasing phenolic compounds bound to dietary proteins. However, more data need to understand which polyphenols are affected by digestive enzymes and which are affected by the alkaline environment in the intestines in the antioxidant capacity changes.

Conclusion

In the present study, impact of in vitro digestion on the antioxidant capacity provided by lipophilic and hydrophilic fractions of commonly consumed 28 fruits and vegetables were determined. A comparison of the antioxidant activities before and after in vitro digestion revealed that digestion appeared to increase the antioxidant capacity of the lipophilic fractions, whereas no such increase occurred in the hydrophilic fractions. Also the values of the DPPH radical inhibition and TEAC were much higher in the lipophilic fractions than in the hydrophilic fractions. Even a marked loss of antioxidant activity at the hydrophilic fraction of some fruits and vegetables was observed, its seen that a considerable antioxidant capacity is available after the digestion process. The fruits and vegetables that exhibited significantly increased antioxidant activities after in vitro digestion when compared to their initial value, are grape, apple, pear, kale, and persimmon. The fruits and vegetables that showed the greatest antioxidant activities (based on a combined consideration of the results obtained by the DPPH assays and TEAC assays) were sour cherry, pomegranate, radish, kale, pear, and plum. These fruits and vegetables may become important dietary sources of natural antioxidants for the prevention of diseases linked to oxidative stress. Finally this study aims to provide information on antioxidant capacity by providing data concerning the bioaccessibility and bioavailability of antioxidants in a human system. Its seen that the bioavailability of antioxidant capacity of metabolites is strictly dependent on the digestion process; therefore more in-depth studies are necessary for understanding the effects digestion process.

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Conflict of interest

No potential conflict of interest was reported by authors.

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