



**INVESTIGATING THE PHYSICOCHEMICAL PROPERTIES AND
IN VITRO BIOACCESSIBILITY OF PHENOLICS AND ANTIOXIDANT
CAPACITY OF ROOIBOS HERBAL TEA BEVERAGE**

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Received / Geliş: 07.06.2017; Accepted / Kabul: 25.09.2017; Published online / Online baskı: 15.11.2017

Suna, S. (2017). Investigating the physicochemical properties and in vitro bioaccessibility of phenolics and antioxidant capacity of rooibos herbal tea beverage. *GIDA* (2017) 42 (6): 682-692 doi: 10.15237/gida.GD17050

ABSTRACT

In this research, production of novel beverages was planned and evaluation of physicochemical properties, total phenolics, antioxidant capacity, and bioaccessibility of products were aimed. Accordingly, 1% rooibos extract, citric and ascorbic acids, natural lemon flavor were added all types of beverages. Sucrose, agave, aspartame and acesulfame-K were respectively added to the beverages coded as RS, RA and RSW. Mixtures were plate filtered, filled into 200 mL glass bottles and pasteurized at 98 °C for 15 min. Total phenolic content and antioxidant capacity with FRAP, CUPRAC and DPPH assays were determined in beverages and rooibos extract. An *in vitro* model simulating gastrointestinal (GI) digestion system was also adapted to assess the bioaccessibility of phenolics and antioxidant capacity. Total phenolics were determined more bioaccessible in RS (405.02±4.57 mg GAE/100 mL) while the highest bioaccessibilities of antioxidant capacity was obtained from RA with CUPRAC (7.19%) and FRAP (1.82%) resulting with the highest functionality.

Key words: Rooibos, herbal tea beverage, bioaccessibility, antioxidant capacity

**ROOİBOS ÇAYI İÇECEĞİNİN FİZİKOKİMYASAL ÖZELLİKLERİ
İLE FENOLİK MADDE VE ANTIÖKSİDAN KAPASİTE YÖNÜNDEN
BİYOALINABİLİRLİĞİNİN ARAŞTIRILMASI**

ÖZ

Bu çalışmada yeni içecek çeşitlerinin üretilmesi ve bu ürünlerin fizikokimyasal bileşimlerinin yanı sıra, toplam fenolik madde miktarı ve antioksidan kapasiteleri ile biyoalınabilirliklerinin ortaya konulması hedeflenmiştir. Bu amaçla içerisinde %1 rooibos ekstraktı bulunan içeceklerin her birine, sitrik ve askorbik asit ile doğal limon aroması eklenmiştir. Ayrıca RS, RA ve RSW olarak kodlanan içeceklere sırasıyla sükröz, agave, aspartam ve asesülfam-K ilave edilmiştir. Karışımlar plakalı filtreden geçirilerek, 200 mL'lik cam şişelere doldurulmuş, taç kapakla kapatılmış ve 98°C'de 15 dk pastörize edilmiştir. İçecek çeşitleri ve rooibos ekstraktında toplam fenolik madde analizi yapılmış, antioksidan kapasiteleri ise FRAP, CUPRAC ve DPPH yöntemleri ile tayin edilmiştir. Bununla birlikte, toplam fenollerin ve antioksidan kapasitenin biyoalınabilirliklerinin belirlenmesi için *in vitro* sindirim modeli uygulanmıştır. Toplam fenollerin biyoalınabilirliği RS içeceğinde en yüksek olarak saptanırken (405.02±4.57 mg GAE/100 mL), antioksidan özellik gösteren bileşenlerin biyoalınabilirliği %7.19 (CUPRAC) ve %1.82 (FRAP) oranları ile en yüksek olarak RA örneğinden elde edilmiştir.

Anahtar kelimeler: Rooibos, bitki çayı içeceği, biyoalınabilirlik, antioksidan kapasite

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INTRODUCTION

Natural herbal extracts have gained importance due to their bioactive components in functional beverages. Between the herbs marketed worldwide, rooibos has taken attention with its consumption as an ingredient in ready to drink beverages and a quantity of food products like ice tea, yoghurt, instant cappuccino and bread (Joubert and de Beer, 2011). Rooibos (*Aspalathus linearis* (Burm.f.) R.Dahlgren) is an endemic South African plant which is cultivated mainly for herbal tea production. Rooibos has been marketed especially in Germany, the Netherlands, the United Kingdom, the United States of America and Japan (Walters et al., 2017). Caffeine-free and anti-ageing properties played an important role on its distribution around the world. Health beneficial effects of rooibos were reported for the abatement of allergies, asthma, infantile colic and dermatologic disorders (Joubert et al., 2008). Additionally, beneficial effects of rooibos especially its anti-diabetic properties is focused on its major flavonoid dihydrochalcone aspalathin. While fresh leaves of rooibos contain polyphenols as flavanols, dihydrochalcones and aspalathin, processed leaves and stem include benzoic and cinnamic acid (Muller et al., 2016). Additionally, anti-cancer properties of rooibos demonstrated in rat liver, oesophagus, and skin carcinogenesis models, have been attributed to their polyphenolic compounds (Marnewick et al., 2009; Sissing et al., 2011).

As it is known, health effects of polyphenols depend on the consumed amount and their bioaccessibility, which is defined as the amount of an ingested food constituent that is available for absorption in the gut after digestion (Palafox-Carlos et al., 2011). In this point, it is necessary to know the bioaccessibility of antioxidants because phytochemicals must be previously available to exert their biological activities (Costa et al., 2014). There is very limited information about the bioaccessibility changes of phenolics and antioxidant capacity of herbs or herbal drinks.

Tea is traditionally prepared by brewing the fresh or dried leaves, stems, roots or seeds with boiled water or using ready to infuse commercial tea

bags. However, brewing methods and parameters of plant species differ from each other and improper applications might minimize the expected benefits and lead to deleterious health effects. The main objective of this study was to produce novel herbal tea beverages to benefit from the nutritional and functional properties of rooibos. Together with physicochemical properties, bioaccessibilities of total phenolic content and antioxidant capacity of the beverages were investigated. Moreover, standardizing the production process of a new functional rooibos herbal tea beverage, preventing the mistakes applied in traditional techniques and producing a microbiologically safe value added product were aimed.

MATERIAL AND METHOD

Chemicals

All reagents used were in analytical grade. TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) and bile salts were purchased from Fluka (Switzerland). Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), neocuproine (2,9-dimethyl-1,10-phenanthroline), DPPH (2,2-diphenyl-2-picrylhydrazyl), methanol, sodium carbonate, gallic acid, oxalic acid and sodium hydroxide were purchased from Sigma Aldrich (Germany). Pepsin, pancreatin, iron (III) chloride hexahydrate, Folin-Ciocalteu reagent, 2,6-dichlorophenol indophenol, copper (II) chloride, ammonium acetate and hydrochloric acid were supplied from Merck (Germany).

Materials

Rooibos (*Aspalathus linearis*) leaves were purchased from an importer company in dried form. Natural lemon flavor was obtained from Aromsa Company (Kocaeli, Turkey) and organic agave syrup (The LifeCo) was acquired from market.

Methods

Production of herbal tea beverages

Herbal tea beverages were produced in a pilot scale. Initially, 1% plant material placed in a synthetic cloth bag was infused with boiling water. Then the obtained extract was cooled down to room temperature, used as the main ingredient of

the beverages and coded as RE (Rooibos extract). RE, citric acid, ascorbic acid and natural lemon flavor were added to all types of the beverages differing from the addition of sucrose for the beverage coded as RS (Sucrose added herbal tea beverage), natural sweetener agave for RA (Agave added herbal tea beverage) and aspartame and acesulfame–K for RSW (Sweetener added herbal tea beverage). Mixtures were then plate filtered (Plate filter 60X60 CFP, Zambelli Enotech, Italy), filled into 200 mL glass bottles and pasteurized at 98 °C for 15 min after sealing with crown caps. Pasteurization was chosen as the conservation method according to the beverages pH value (3.42–3.55). In general for acidic products, pH < 4.5, pathogenic organisms do not cause a problem and only a mild heat treatment is required for stabilising the product (Richardson, 2004). Afterwards, bottles were cooled and stored at room temperature until analyzed. Taste and flavor balance were taken into consideration in the recipes of the beverages. Moreover, for RSW, substituted aspartame and acesulfame–K in the place of sugar were calculated according to the sweetness value of the sweeteners. The quantities of the ingredients used in beverage production could not be given because the products are patented (2012/09534).

ANALYSES

Determination of some physical and chemical properties of herbal tea beverages

Dry matter content of dried rooibos leaves was determined by oven drying method while RA-500 model KEM refractometer was used for the determination of total soluble solid content (brix) (AOAC, 1990). The pH was measured by using a Sevencompact pH/Ion Mettler Toledo pH meter and titratable acidity was determined with potentiometric method (Cemeroğlu, 2007). Shimadzu UV 1208 model spectrophotometer was used for the determination of ascorbic acid content, which was determined by using a 2,6 dichlorophenol indophenol dye (Cemeroğlu, 2007). The color of the beverages were measured by using a HunterLab Colour Analyzer (MSEZ4500L; HunterLab, Virginia, USA) and expressed as L^* , a^* and b^* values (Bakker et al.,

1986). All analyses were performed in three replicates

In vitro digestion procedure

In order to assess the functional properties of the beverages, water extracts and physiological extracts were investigated for the determination of antioxidative capacity and total phenolic content. Due to the fact that, beverages were produced from rooibos extract (RE), which was obtained with the infusion of dried plant material in water, analyzed amounts were taken directly from RE and the beverages (RS, RA, RSW) for water extracts (Sengul et al., 2014). This method was chosen to support gastrointestinal digestion. Additionally, extracts obtained by an *in vitro* digestion method (physiological extract) were used for the determination of the same analyses. Bioaccessibility was also calculated as the percentage of total phenolic content and antioxidant capacity (Vitali et al., 2009). For the determination of physiological extracts, an *in vitro* digestion enzymatic extraction method that mimics the conditions in the gastrointestinal tract was used as described by Glahn et al. (1998) with slight modifications. Briefly, 10 mL of distilled water and 0.5 mL of pepsin (20 g/L in 0.1 mol/L HCl) were added to 1 mL of sample, pH was adjusted to 2 by using 5 mol/L HCl and sample was incubated at 37°C in a shaking water bath for 1 h. Simulation of gastric digestion was stopped by the addition of 1 M NaHCO₃ (to adjust pH to 7.2). 2.5 mL of bile/pancreatin solution (2 g/L of pancreatin and 12 g/L of bile salt in 0.1 M NaHCO₃) and 2.5 mL of NaCl/KCl (120 mmol/L NaCl and 5 mmol/L KCl) were added to the sample and simulation of intestinal digestion was conducted for the following 2 h. Samples were centrifuged at 3500 rpm for 10 min and the supernatant was used for the analyses.

Determination of phenolic content

Folin-Ciocalteu spectrophotometric method was used for the determination of total phenolic content (Singleton and Rossi, 1965; Mahdavi et al., 2010). Gallic acid solution was used for the calibration of the standard curve ($R^2=0.9835$) and the results were expressed as gallic acid equivalents (GAE).

Determination of antioxidant capacity

For the determination of antioxidant capacity, many methods are developed and it is recommended to use several of them together in order to determine the *in vitro* available antioxidant capacity. The methods used in this research were 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, ferric reducing antioxidant power (FRAP) assay and cupric ion reducing antioxidant capacity (CUPRAC) assay. Results of these assays were calculated as trolox equivalent and given as “ $\mu\text{mol trolox/mL}$ ”.

DPPH assay of total antioxidant capacity

Antioxidant capacity of herbal tea beverages was measured using a modified version of the Katalinic et al. (2006). Firstly, 0.1 mL sample was added to 3.9 mL of 6×10^{-5} M methanolic solution of DPPH radical and vortexed (Vortex Mixer Classic, Velp Scientifica, Italia) for 15-30 s. The reaction was allowed to proceed in the dark at room temperature for 30 min, and the absorbance was then measured at 515 nm. A trolox calibration curve ($R^2=0.9974$) was conducted by measuring the reduction in absorbance of the DPPH solution.

FRAP assay of total antioxidant capacity

According to Benzie and Strain (1996), 3 mL of daily prepared FRAP reagent (incubated at 37°C) was mixed with 300 μL of distilled water and 100 μL of the test sample. Then samples were incubated at 37°C for 30 min. At the end of incubation, absorbance was measured immediately at 595 nm. The FRAP reagent was prepared by mixing 25 mL of 0.3 mol L^{-1} acetate buffer (pH 3.6), 2.5 mL of 20 mmol L^{-1} $\text{FeCl}_3 \times 6 \text{ H}_2\text{O}$ and 2.5 mL 10 mmol L^{-1} TPTZ solution in 40 mmol L^{-1} HCl. A trolox calibration curve ($R^2=0.9896$) was used for expressing the results.

CUPRAC assay of total antioxidant capacity

Estimation of cupric ion reducing antioxidant capacity was conducted according to the method of Apak et al. (2008). 1 mL 1×10^{-2} M CuCl_2^+ + 1 mL 7.5×10^{-3} M neocuproine + 1 mL 1 M NH_4Ac were added to $x \text{ mL } 10^{-3}$ M antioxidant neutral solution + $(1-x) \text{ H}_2\text{O:VT} = 4 \text{ mL}$; and the final absorbance was measured at 450 nm after 30 min

Calculation of antioxidant capacity was done as trolox equivalents ($R^2=0.9987$).

Sensory analyses

Herbal tea beverages were organoleptically evaluated for their quality attributes, such as colour, odour, appearance and taste by using a ranking test with 6 panelists (Altuğ and Elmacı, 2011). Panelists granted points to the samples from the best preferred to least preferred beverage. Due to number of the panelists and the beverages, samples were statistically evaluated. According to this method, the sample taken below 8 point was accepted as preferred, above 16 point were accepted as rejected and when the sample was granted points between 8-16, this means there was no significant difference ($P<0.05$).

Statistical analysis

The experiment was conducted in a completely randomized design with three replications. The results were statistically evaluated by one-way analysis of variance (ANOVA) using the JMP software package version 6.0 (SAS Institute Inc. NC, 27513). When significant differences were found ($P < 0.05$), the Least Significant Difference (LSD) test was used to determine the differences among means.

RESULTS AND DISCUSSION**Physicochemical properties**

Dry matter content of dried rooibos leaves was measured as $92.87 \pm 0.04 \text{ g/100g}$ (7.13 g/100g as moisture). This result was appropriate according to Turkish Standards Institution limits which were subjected as maximum 10 g/100g moisture for dried herbs (Anonymous, 2014).

Physicochemical properties of RE was determined as $0.09 \pm 0.00 \text{ g/100g}$ for total soluble solid content, 7.63 ± 0.02 for pH, $0.77 \pm 0.10 \text{ mg/100mL}$ for ascorbic acid content and 0.00 g/100mL for titratable acidity. Color of the RE was recorded as 1.32 ± 0.01 , 7.65 ± 0.04 and 2.28 ± 0.02 respectively for L^* , a^* and b^* values. Physicochemical properties of the beverages were shown in Table 1. All data in tables are expressed as means \pm standard deviations ($n = 3$).

Table 1. Physicochemical properties of rooibos herbal tea beverages

Sample	Total soluble solid content (g/100g)	Titratable acidity (g/100mL)**	pH	Ascorbic acid (mg/100mL)	Color		
					<i>L</i> *	<i>a</i> *	<i>b</i> *
RS	4.03±0.05 ^a	0.06±0.00	3.42±0.01 ^c	1.24±0.35 ^b	31.63±0.13 ^b	14,25±0,07 ^b	47.37±0.22 ^a
RA	3.16±0.05 ^b	0.06±0.00	3.55±0.01 ^a	1.12±0.62 ^b	26.16±0.11 ^c	17.25±0.07 ^a	42.53±0.14 ^b
RSW	0.12±0.02 ^c	0.06±0.00	3.52±0.01 ^b	2.59±0.27 ^a	33.30±0.02 ^a	12.70±0.01 ^c	47.16±0.02 ^a

** : Citric acid

Mean values within a column with unlike superscript letters were significantly different ($P < 0.05$)

Data are expressed as means \pm standard deviations (n=3).

RS: sucrose added herbal tea beverage, RA: agave added herbal tea beverage, RSW: sweetener added herbal tea beverage

Total soluble solid content of RE was analysed as 0.09 g/100g. This value was determined as 4.03, 3.16 and 0.12 g/100g for RS, RA and RSW respectively, as the result of different sucrose amounts in formulations. Analysis results between total soluble solid content of the beverages were significantly different ($P < 0.05$).

Titratable acidity values of the beverages were adjusted after the analysis results of market survey in similar beverages. Titratable acidity of RE was analysed as 0.00 g/100mL whereas beverages titratable acidity were all determined 0.06 g/100mL which were not found significantly different ($P > 0.05$). İncedayı (2017) reported titratable acidity as 0.22 g/100mL in a carbonated linden herbal tea beverage which were found higher than our results because of the different formulation and the material.

pH of RE was analysed as 7.63 while this value ranged from 3.24 to 3.55 in in beverages as a result of acid addition to the formula ($P < 0.05$). Phelan and Rees (2003) determined similar pH values of some commercial tea beverages like blackcurrant, ginseng and vanilla as 3.45, echinacea and raspberry as 3.49 and traditional lemon as 3.69.

Ascorbic acid was added to the beverages as an antioxidant source and preservative with a contribution to the acidity (Kitchens and Owens,

2007; Riachi and De Maria, 2015). At the same time, it provided the formation of taste-flavor balance together with citric acid. Ascorbic acid content of RE was analysed as 0.77 mg/100 mL. As a result of ascorbic acid addition to the formula, ascorbic acid content of the beverages were found higher than RE, which was ranged from 1.12 to 2.59 mg/100 mL. The highest ascorbic acid content was determined in RSW with 2.59 mg/100 mL. Results of ascorbic acid analysis were found lower than the added amount and this reduction could be explained by the loss during heat treatment (Lešková et al. 2006). Costa et al. (2012) reported the ascorbic acid content as 7.20 mg/100 mL in a beverage prepared with 0.75% rooibos red tea leaves (*Aspalathus linearis*). As a result of the diversity in production method, our data was differed from this result. Additionally, the differences between the beverages were found significant ($P < 0.05$).

According to the results of the statistical analysis, the overall color parameters for the beverages were found significantly different ($P < 0.05$). The highest *L** (brightness), *a** (redness) and *b** (yellowness) values were determined from RSW, RA and RS beverages respectively. Color of RE was determined as 1.32, 7.65 and 2.28 for *L**, *a** and *b** values respectively.

Bioaccessibility of total phenolics

Phenolic compounds play an important role regarding antioxidant effects and defensive action in plants or the human body (Boo et al., 2012). But during GI digestion, polyphenols might either interact with other food constituents (e.g., chelation of ions), be further degraded (such as anthocyanins in the small intestine), or metabolized, such as by hydrolysis via deglycosylation or cleavage by esterases. These structural changes could affect both their further uptake and their bioactivity (Bouayed et al., 2011). Additionally, bioaccessibility of polyphenols are affected by the chemical composition of the food, its release from the food matrix, interactions with other food constituents and the presence of

suppressors or cofactors (Parada and Aguilera, 2007). Besides, it is revealed that, non extractable food polyphenols might become bioactive in human gut when it is released from the food matrix by the action of digestive enzymes in the small intestine and bacterial degradation in the large intestine (Jenner et al., 2005).

RE showed the highest total phenolic content with 14800.90 ± 123.77 mg GAE/100 mL, and 15926.30 ± 782.23 mg GAE/100 mL in physiological extract. Bioaccessibility of total phenolic content of RE was calculated 107.58% as the percentage of total phenolic content. Total phenolic contents and the bioaccessibilities of the beverages (RS, RA, RSW) were given in Table 2.

Table 2. Bioaccessibility of total phenolic contents of herbal tea beverages

Sample	TP (mg GAE*/100 mL)	TP Physiological extract (mg GAE*/100 mL)	TP Bioaccessibility (%)**
RS	371.08 ± 2.25^b	405.02 ± 4.57^b	109.15
RA	479.55 ± 2.90^b	504.12 ± 11.60^b	105.12
RSW	9419.76 ± 129.20^a	9038.84 ± 122.09^a	95.97

Mean values within a column with unlike superscript letters were significantly different ($P < 0.05$)

Data are expressed as means \pm standard deviations (n=3).

*GAE: gallic acid equivalent

**Bioaccessibility was calculated as the percentage of total phenolic content

RS: sucrose added herbal tea beverage, RA: agave added herbal tea beverage, RSW: sweetener added herbal tea beverage

Santos et al. (2016) studied the effects of different extraction time (5, 7.5, 10 min) and temperature (65°C, 75°C, 85°C) applications on total phenolics of red rooibos (*Aspalathus linearis*) and displayed the results between 1623 ± 151 mg GAE/100g for 65 °C 5 min and 2493 ± 114 mg GAE/100g for 85°C 10 min. Joubert and de Beer (2012) revealed total polyphenols of rooibos infusion as 25.78 ± 1.12 g GAE/100g soluble solids. Magcwebeba et al. (2016) reported total polyphenols of rooibos methanol and aqueous extracts as 35.07 ± 3.44 mg GAE/100 g and 25.05 ± 2.84 mg GAE/100 g extracts respectively.

Oh et al. (2013) also determined total phenolic content of the water extracts (5%) of rooibos as 38.66 mg GAE/g tea. Our results are in disagreement with literature data despite the

differences in extraction method and concentration. Among samples, RSW had the highest amount in total phenolics as a result of the higher amount of RE addition to the formula. However, the highest bioaccessibility ratio (109.15 ± 1.24) was obtained from RS.

Previous studies revealed *in vitro* increment of total phenolics, which is in agreement with our results. Henning et al. (2014) studied the effects of *in vitro* digestion on total phenolic content and antioxidant activity in some dietary supplements and determined the ratio of total phenolic content (mg GAE/L) of nondigested (water extracts) and digested extracts. They reported the total phenolics maintained after gastrointestinal digestion of resveratrol dietary supplement as 139.67 %. Related with our results, total

procyanidin and total flavone contents of the food and the digested samples were determined in a study respectively as 716-3191, 258-559 $\mu\text{g/g}$ for cocoa liquor and 376-870, 299-438 $\mu\text{g/g}$ for cocoa powder (Ortega et al., 2009). All of these results confirm that different formulations and then food matrix have an influence on the release of total phenols and, therefore, they affect the bioaccessible fraction.

Antioxidant capacity

Antioxidant capacity of RE was analysed with FRAP, CUPRAC and DPPH methods and

determined respectively as 44.53 ± 6.07 $\mu\text{mol trolox/mL}$, 11.75 ± 0.14 $\mu\text{mol trolox/mL}$ and 8.83 ± 0.07 $\mu\text{mol trolox/mL}$. For the physiological extracts, these values were recorded as 0.95 ± 0.10 $\mu\text{mol trolox/mL}$ and 0.70 ± 0.03 $\mu\text{mol trolox/mL}$ in FRAP and CUPRAC assays while it could not be analyzed in DPPH method. Correspondingly, bioaccessibility of the antioxidant capacity was calculated as 2.13% and 5.96% in FRAP and CUPRAC assays. Bioaccessibilities of the antioxidant capacity of the beverages (RS, RA, RSW) were also given in Table 3.

Table 3. Bioaccessibility of antioxidant capacities of herbal tea beverages

Sample	FRAP ($\mu\text{mol trolox/mL}$)	FRAP Physiological extract	FRAP Bioaccessibility (%)**	CUPRAC ($\mu\text{mol trolox/mL}$)	CUPRAC Physiological extract	CUPRAC Bioaccessibility (%)**	DPPH ($\mu\text{mol trolox/mL}$)*
RS	48.66 ± 11.30	0.71 ± 0.00^b	1.52 ± 0.37	6.90 ± 0.14^b	0.28 ± 0.01^b	4.05 ± 0.18	7.79 ± 0.75
RA	49.16 ± 8.31	0.87 ± 0.07^a	1.82 ± 0.45	8.53 ± 0.32^a	0.61 ± 0.13^a	7.19 ± 1.75	8.21 ± 0.33
RSW	49.71 ± 8.23	0.81 ± 0.10^{ab}	1.66 ± 0.33	6.89 ± 0.23^b	0.12 ± 0.03^b	1.73 ± 0.60	7.76 ± 0.59

Mean values within a column with unlike superscript letters were significantly different ($P < 0.05$)

Data are expressed as means \pm standard deviations ($n=3$).

* DPPH Bioaccessibility of the beverages could not be analyzed and shown in the table

**Bioaccessibility was calculated as the percentage of total antioxidant capacity

RS: sucrose added herbal tea beverage, RA: agave added herbal tea beverage, RSW: sweetener added herbal tea beverage

The different values obtained from the three assays are a consequence of the evidence that the test materials (i.e. DPPH, Cu^{2+} and Fe^{3+}) quenched/reduced by beverages react according to different mechanism and kinetics (Jeszka-Skowron et al., 2015). The differences between FRAP and DPPH antioxidant capacities were not found significant ($P > 0.05$) whereas results from CUPRAC assay were significantly different ($P < 0.05$) as presented in Table 3. Santos et al. (2016) determined antioxidant capacity of rooibos teas in the range between 63-73 % inhibition for DPPH and 2947-3380 mg ascorbic acid equivalent/100g for FRAP assays. Pellegrini et al. (2003) analysed antioxidant activity of some commercial beverages like ice tea, green tea, coffee (espresso) and expressed the results as 2.28, 6.01 and 36.54 mmol trolox/kg with TEAC and 7.43, 18.00 and 129.38 mmol Fe^{2+} /kg with FRAP methods. Joubert and de Beer (2012) reported total antioxidant capacity of rooibos infusion as

1777 ± 114 $\mu\text{mol trolox equivalents/g}$ soluble solids with DPPH method. According to the results of another study, FRAP antioxidant activity of methanolic and aqueous extracts were reported as 3.04 ± 0.19 mmol trolox equivalent/g and 2.24 ± 0.18 mmol trolox equivalent/g respectively (Magcwebeba et al., 2016). Our results were consistent with literature data.

Bioaccessibility of antioxidant capacities were determined higher in the order of CUPRAC (5.96%) and FRAP (2.13%) assays in RE. In accordance with these results, the highest bioaccessibility ratios were obtained from RA respectively in CUPRAC and FRAP assays (Table 3). Likewise, RA had the highest antioxidant capacity with 8.53 ± 0.32 $\mu\text{mol trolox/mL}$ and 8.21 ± 0.33 $\mu\text{mol trolox/mL}$ both in CUPRAC and DPPH assays (Table 3). A possible reason of varying bioaccessibility of antioxidant capacity values could be associated with several factors

related to the process conditions, chemical interactions with other phytochemicals, biomolecules present in the food and also the protocols used for the measurements (Parada and Aguilera, 2007). In agreement with our data, Henning et al. (2014) reported a 21.5% and 8.1% decrement of TEAC (trolox equivalent antioxidant capacity) in green tea and grape seed samples during *in vitro* simulated digestion. In another study, Değirmencioglu et al. (2016) concluded DPPH bioaccessibilities of fermented vegetable juices between 16-32%. In spite of the fact that polyphenols supply major antioxidant potency of the samples, our results displayed that digestion may alter antioxidant properties depending on the variations in polyphenol content (Henning et al., 2014). Besides it is known that, structural changes after gastrointestinal digestion affect both further polyphenol uptake and result in a significant loss of the antioxidant activity (Rodríguez-Roque et al., 2013).

Results of sensory analyses

The result of the sensorial analysis was depicted in Figure 1.

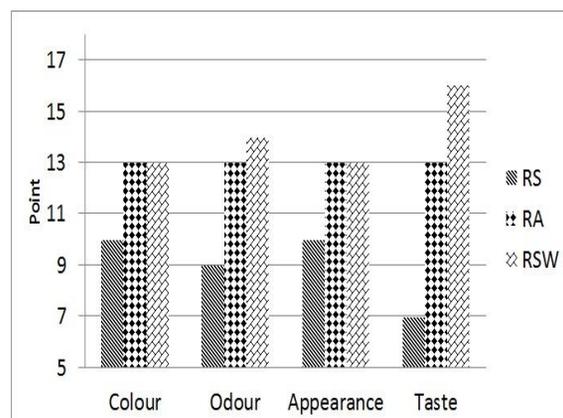


Figure 1. Sensorial properties of rooibos herbal tea beverages

Samples were tested for color (it should have typical rooibos tea colour), odour (it should have typical rooibos tea odour and not have any strange odor), appearance (it should be clear and not contain any particles) and taste (it should have typical rooibos tea taste and not have any strange taste). During the analysis, RE was served to the panelists to inform them about the typical properties of rooibos. There were no significant

differences in colour, odour and appearance values between samples. For taste criteria, RS was the most favorite one while RA and RSW were accepted by the panelists ($P < 0.05$).

CONCLUSION

According to the results of our study, agave added herbal tea beverage was thought to be the most nutritional and beneficial beverage among our products depending on the highest bioaccessibilities of antioxidant capacity determined with FRAP and CUPRAC assays and total antioxidant capacity analysed with DPPH method. Total phenolics were also found more bioaccessible in sugar added beverage. In overall evaluation, all of the samples were accepted by the panelists, supporting the goal of our study which is to produce novel and functional ready to drink herbal tea beverages.

Acknowledgement

Products in this study were patented by Turkish Patent Institute (2012/09534).

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