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Antioxidant capacity, total phenols and color profile during the storage of selected plants used for infusion

Ana Jiménez-Zamora [a], Cristina Delgado-Andrade [b], José A. Rufián-Henares [a,*]

[a] Departamento de Nutrición y Bromatología, Facultad de Farmacia, Universidad de Granada, 18071 Granada, Spain
[b] Dpto. Fisiología y Bioquímica de la Nutrición Animal, Estación Experimental del Zaidin (EEZ-CSIC), Armilla, Granada, Spain

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ABSTRACT

Many plants, like tea, are widely used for preparing herbal infusions. These plants have an interesting antioxidant capacity that may change after harvesting depending on the technological processing and the storage conditions. We determined the antioxidant capacity (ABTS, DPPH and FRAP methods), total phenolic content and color analysis (reflectance) of 36 plants traditionally consumed in Spain as infusion. Green tea was the most antioxidant herb, although oregano and lemon balm showed also a very high antioxidant capacity, as well as phenolic content. The antioxidant study after 3-month storage at different temperatures showed that up to a 50% of the total antioxidant capacity could be lost. Color analysis correlated with antioxidant capacity evolution, being a quick tool to control the storage conditions. Finally, our data confirm that the intake of one serving of plant infusion could release the equivalent of up to 1500 μmol trolox, being a good source of antioxidants for the human diet.

1. Introduction

In the last years, many studies have shown the relation between oxidative stress, cellular senescence and some diseases (Finkel & Holbrook, 2000). In addition, current lifestyle is causing the overproduction of free radicals and reactive oxygen species (ROS) in our organism, increasing the physiological level of oxidative stress while decreasing the antioxidant activity (Ward et al., 2004). Free radicals and other ROS, such as singlet oxygen, hydroxyl radical, superoxide anion, and peroxyl radical, can be generated from normal metabolism in the human body, and can cause oxidative damage to functional macromolecules such as DNA, proteins and lipids (Apel & Hirt, 2004). This increases the chance of occurrence of neurodegenerative diseases, inflammation, atherosclerosis, cancer and age-related disorders, among others. However, antioxidants protect the human body from free radicals, preventing oxidative stress and associated diseases (Halliwell, 1996). Several studies demonstrated that medicinal herbs are a rich source of antioxidant compounds such as phenolics, vitamins, and alkaloids, which may be used as pharmacologically active products to balance the physiological antioxidant/prooxidant status (Katalinic, Milos, Kulisic, & Jukic, 2006; Yoo, Lee, Lee, Moon, & Lee, 2008).

Herbal plants are generally defined as one year gramineous herbs with not any strict contexture (Farzaneh & Carvalho, 2015). The infusion, for example, of tea or peppermint leaves, is a popular beverage known for its refreshing taste and peculiar aroma. Additionally, the nutritional application of natural phytochemicals from rich sources such as leaves, branches, flowers as well as roots of different plants is quite general in human beings, because of existence of compounds with bioactive potentials and well-being advantages (Chivian, 2002). Among them, the most reputed groups of compounds with antioxidant efficacy in plants are vitamins, such as vitamins E and C, polyphenols, flavonoids as well as pigments amongst carotenoids and anthocyanins. In Spain, some of the most popular plants have been widely used to prepare beverages that are drunk after meals as herbal infusions (Pardo de Santayana, Blanco, & Morales, 2005). This is the case for the species studied herein (Supplemental Table 1). However, the antioxidant capacity of plants may change after harvesting depending on the technological processing and the storage conditions (Wahid, Gelani, Ashraf, & Foolad, 2007). In this sense, the development of color is an extremely important and obvious sign of the technological process and storage, mainly to dehydration and the development of the advanced Maillard reaction (Nursten, 1986). The quantitative measurement of the browning rate is used as an indicator of heat treatment severity or storage conditions in foods (Rufian-Henares, Guerra-Hernandez, & Garcia-Villanova, 2006). However, to the best of our knowledge, color analysis has not been

* Corresponding author at: Departamento de Nutrición y Bromatología, Universidad de Granada, Facultad de Farmacia, Campus de Cartuja, 18071 Granada, Spain. Tel.: +34 958 241000x20463; fax: +34 958 24 95 77.
E-mail address: jarufian@ugr.es (J.A. Rufián-Henares).

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used previously in herbs in order to control the evolution of their antioxidant capacity.

As far as we know, the studies regarding the antioxidant stability of plants used to obtain infusions during their storage and shelf-life are rare (Gião, Pereira, Pintado, & Malcata, 2013; Guimarães et al., 2011) and less is known about the evolution of color and the antioxidant capacity. Therefore, the main objective of this research was to investigate the stability along storage of the antioxidant capacity and total phenolic content of those plants traditionally consumed in Spain and other locations as infusions, as well as the evolution of color and the relationship among them. In this sense, we also aimed to model the storage evolution of antioxidant capacity, phenol content and color by means of multivariate methods. Finally, we proposed to determine the contribution of the intake of plant infusions to the daily antioxidant capacity of the Spanish diet.

2. Materials and methods

2.1. Chemicals

Folin–Ciocalteu reagent, gallic acid and sodium carbonate were from Panreac (Madrid, Spain). 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (trolox), was from Sigma–Aldrich (St. Louis, USA). 2,2′-azobis-(3-ethylbenzothiazoline-6-sulfonic acid), 2,2′-diphenyl-1-picrylhydrazyl and 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) were from Fluka Chemicals (Madrid, Spain). Bidistilled deionized water was obtained from a Milli-Q system (Millipore, Milford, MA).

2.2. Plant materials and storage conditions

Thirty-six plants, traditionally consumed in Spain in infusion and marketed in different herbalists from the metropolitan area of Granada, were selected (Supplemental Table 1). For time zero, the samples were used directly without undergoing a storage process. Parallel, aliquots of the samples were maintained for 3 and 6 months at room temperature (RT, simulating the conservation under usual conditions) or at 50 °C (50, simulating accelerated conservation in extreme conditions). The codes used to identify each sample were respectively 0, RT/3, RT/6, 50/3 and 50/6. Each sample was obtained from three different places and was analyzed in triplicate.

2.3. Sample preparation

Plant beverages were prepared by infusion of 150 ml of boiling mineral water with 2.00 g of each sample and left to stand at room temperature for 7 min, being finally filtered. The obtained infusions were stored at −80 °C until analysis.

2.4. Antioxidant capacity analysis

The trolox equivalent antioxidant capacity (TEAC) of plant infusions was analyzed with the ABTS, DPPH and FRAP methods. The ABTS assay was conducted as described by Pastoriza, Delgado-Andrade, Haro, and Rufián-Henares (2011) with slight modifications (Re et al., 1999). The ferric reducing ability of the infusions was estimated following the procedure described by Pastoriza et al. (2011) slightly modified (Benzie & Strain, 1996). The antiradical capacity of samples in a methanolic medium was estimated according to the procedure reported by Rufián-Henares, Guerra-Hernández, and Garcia-Villanova (2013) slightly modified (Brand-Williams, Cuvelier, & Berset, 1995). The analyses were performed by using a Fluostar Omega microplate reader (BMG Labtech, Germany) with temperature control set at 37 °C. Trolox solutions were used to perform the calibration curve. The results are expressed as mmol equivalents of trolox per litre of sample.

2.5. Total phenolic content

Total phenolics were determined according to the Folin–Ciocalteu method as described by Marfil et al. (2011) with slight modifications (Singleton, Orthofer, & Lamuela-Raventos, 1999). Measures were performed on a Fluostar Omega microplate reader (BMG Labtech, Germany). Quantification was carried out on the basis of the standard curve of gallic acid, and results were expressed as mg gallic acid equivalent per litre of sample.

2.6. Color analysis

The color of samples was determined using a Chroma Meter CR-400 optical sensor (Konica Minolta Sensing, Inc., Osaka, Japan) according to the CIE Lab scale (CIE, 1974). The system provides the values of three color components; L^* (black-white component, lightness), and the chromaticity coordinates, a^* (+red to −green component, redness) and b^* (+yellow to −blue component, yellowness) (Hunter, 1942). The samples were placed in a 34 mm optical glass cell and illuminated with D65-artificial daylight (10° standard angle) in accordance with the manufacturer's instructions. Each color value reported was the mean of six determinations at 22–24 °C.

2.7. Statistical treatment

Homogeneity of variance was first assessed using the Levene test and a significance level of 5% ($P < 0.05$). Statistical significance of data was then tested using one-way analysis of variance (ANOVA). The evaluation of the relationship between different assays was carried out by computing the relevant correlation coefficient (Pearson linear correlation) at the $P < 0.05$ confidence level. Multivariate analysis was performed by cluster analysis. After study the samples clustering, those samples farther from their group that had any outlier in the parameters included in the analysis, were removed. Finally, the cleaned groups were further analyses by principal component analysis. All the statistical analyses were performed using the Statgraphics Centurion XVI statistical software (2009).

3. Results and discussion

3.1. Antioxidant capacity of plants

Table 1 shows the antioxidant capacity measured by the ABTS method. In the case of non-stored samples, their TEAC (values ranged from 22.89 to 0.11 mmol trolox/l of green tea and carduus marianus, respectively. The antioxidant values of other teas were lower than that found for green tea, which is in line with other authors (Horžić et al., 2009; Kim, Goodner, Park, Choi, & Talcott, 2011). The results obtained for other samples, such as olive, thyme, lavender, lemon balm or nettle, fennel or mint, and hibiscus were also similar to those published by other research groups (Gil, Rebelo, Serralheiro, & Falé, 2011; Komes et al., 2010; and Surveswaran, Cai, Corke, & Sun, 2007; respectively). When plants were grouped by means of botanical families at time 0, we found statistically significant differences ($P < 0.05$) between theaceae and asteaceae, fabaceae, lamiaceae, malvaceae, oleaceae or umbiliferae families. The tea group was the most antioxidant (mean value 8.73 mmol trolox/l), followed by lamiaceae (2.44 mmol trolox/l). In the case of the lamiaceae family, we removed the values obtained for oregano and lemon balm, due to a very high