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Characterization of commercial Spanish non-citrus juices: Antioxidant and physicochemical aspects

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ABSTRACT

The presence of many different antioxidant species makes fruit juices to be perceived by populations as a very healthy beverage easy to include in the daily diet. These antioxidant actions have been reported in a large number of papers, however the information correlating the antioxidant profile with the physicochemical characteristics derived from the industrial processing of fruit juices is limited. In a previous paper, our research group demonstrated that the antioxidant properties of citrus juices were underestimated when measuring by traditional methods and that our improved methodology, so-called GAR, is a better approach to analyze the global antioxidant response of juices. In this paper, we confirm that statement, establishing that the overall antioxidant capacity of non-citrus juices is 10-times higher with the GAR method (including an *in vitro* gastrointestinal digestion) that with the other methodologies. In some cases, such as pineapple juice, the antioxidant action was distributed between the soluble and non-soluble fractions almost at 50%. But, surprisingly, in some other (like tomato juice) the non-soluble fraction accounted for the higher antioxidant capacity. This fact definitively underlines the importance of the non-soluble fraction and shows the suitability of the GAR method to consider it. Physicochemical parameters, such as color, fluorescence, 5-hydroxymethylfurfural and furfural contents were correlated with antioxidant characteristics in some samples. Lastly, we unravel a mathematical model to classify non-citrus juices depending on their nature or storage conditions.

1. Introduction

Fruit juices provide energy in the form of simple sugars, vitamins, minerals and a small amount of fiber, hydrating the body and contributing a good part of the nutritious qualities of the fruit. These beverages are fundamental in a healthy diet with the potential to positively impact metabolic outcomes mediated by the antioxidant activity (Crowe-White et al., 2017). Carotenoids, vitamin C and phenolic compounds are among the antioxidant species naturally occurring in fruit juices (Quitao-Texeira, Odriozola-Serrano, Soliva-Fortuny, Mota-Ramos, & Martín-Belloso, 2009). The analysis of antioxidant capacity is usually performed with the soluble fraction resulting from *in vitro* digestion (Ryan & Prescot, 2010) or with juice extracts (Stella, Ferrarezi, dos Santos, & Monteiro, 2011). However, these approaches do not measure the overall antioxidant capacity of juices, like protocols such as the GAR method (Pastoriza, Delgado-Andrade, Haro, & Rufián-Henares, 2011), which has been successfully applied for citrus juices (Álvarez, Pastoriza, Alonso-Olalla, Delgado-Andrade, & Rufián-Henares, 2014).

Thermal treatment is usually applied to fruit products aimed to ensure safety and extend their shelf life (Rattanathanalerk, Chiewchan, & Srichumpoung, 2005). However, heating processes can affect the nutritional and organoleptic quality of product leading to consumer dissatisfaction. In the case of the nutritional quality it is also affected by industrial manufacture since nutrients like vitamin C, sugars and proteins take part in nonenzymatic reactions (Tulek & Yilmaz, 2006). During the Maillard reaction and caramelization, 5-hydroxymethylfurfural (HMF) and furfural are intermediate undesirable compounds which production reduces the juices' acceptance by consumers (Buedo, Elustondo, & Urbicain, 2001). HMF is an indicator of the decrease on quality since it is related with an excessive processing temperature of juices or inadequate storage conditions (Rodrigo et al., 2003). Furthermore, it is necessary to include the analysis of HMF in the nutritional assessment of juices, since recent finding have established that the compound is metabolized by human beings to 5-sulfoxymethylfurfural (Pastoriza et al., 2017), a derivate with demonstrated nephrotoxicity (Nadiya, Bernhard, Heinz, Albrecht, & Hansruedi, 2009) and mutagenic activity (Glatt & Sommer,

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2006; Surh, Liem, Miller, & Tannenbaum, 1994).

In Spain, the consumption of juices along 2016 reached 457.6 million L (Mercasa, 2017), 30% corresponding to orange juices and 70% to non-citrus juices: 19% to pineapple juices, 18.2% to peach juices, 16.8 to multifruit juices and 3.5% to apple juices. In a previous study (Álvarez et al., 2014) we investigated the antioxidant and physicochemical characteristics of citrus juices. However, due to the economic importance of non-citrus juices in Spain (spending of 428.1 million euros in 2016; Mercasa, 2017) the aim of the present study was to analyze the antioxidant and physicochemical characteristic of commercial Spanish juices with non-citric origin. In the case of the antioxidant characteristics we studied the amount of total phenols as well as the global antioxidant capacity of juices through the GAR method, specially paying attention to the insoluble fraction. For the physiochemical profile color, fluorescence and furanic compounds like HMF and furfural were measured. Then we estimated the contribution of juices consumption to the overall antioxidant capacity intake in Spain. Finally, the relationship among all the evaluated parameters was studied in order to look for an algorithm, which enables us distinguishing the kind of fruit used to manufacture these juices.

2. Materials and methods

2.1. Chemicals

The enzymes used for *in vitro* digestion (α-amylase, pepsin, pancreatin and bile salts) were from Sigma-Aldrich (St. Louis, MO, USA). For HMF-furfural assays, zinc acetate, potassium ferrocyanide and HMF-furfural standards were from Sigma-Aldrich (St. Louis, MO, USA). In the case of the antioxidant capacity methods 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (trolox), 2,2′-azobis-(3-ethylbenzo-thiazoline-6-sulfonic acid) and 2,4,6-tri(2-pyridyl)-*s*-triazine (TPTZ) were obtained from Fluka Chemicals (Madrid, Spain). Folin-Ciocalteu reagent and gallic acid were from Panreac (Madrid, Spain).

2.2. Samples

A total amount of fifty-six juices were selected to cover the main brands consumed in Spain. They were as follows: 9 antioxidant juices (declared as “antiox” on their labels), 5 apple juices, 4 banana juices, 3 mango juices, 10 multifruit juices (declared as “multifruit” on their labels and composed of a mixture of different types of juices), 9 peach juices, 13 pineapple juices and 3 tomato juices. Samples were obtained from three different retail stores and stored under refrigeration or at room temperature (according to manufacturer's instructions) for a maximum of 3 days before analysis. Freshly squeezed juices were obtained from apple, mango and pineapple fruits. Fruits, from two different retailers, were cut and squeezed with an orange squeezing machine (Taurus TC600, Spain) and immediately frozen and stored at − 80 °C until analysis. At least three determinations for each procedure were carried out in different samples.

2.3. Antioxidant capacity

The antioxidant capacity was assayed either in the whole juice or in the soluble and insoluble fractions obtained after *in vitro* gastrointestinal digestion by the GAR method (Pastoriza et al., 2011). The methods used to evaluate the antioxidant activity were the standard ABTS and FRAP methods.

2.3.1. In vitro digestion

The enzymatic digestion was performed as stated in Pastoriza et al. (2011). Juice samples were digested with α-amylase, pepsin, pancreatin and bile salts simulating the oral, gastric and intestinal phases. Then, the soluble bioaccessible and the insoluble non-accessible fractions were separated and stored at − 80 °C until analysis.

2.3.2. Antioxidant capacity of the soluble fraction

The ABTS and FRAP assays were conducted as stated previously by Álvarez et al. (2014) in order to measure the antiradical and reducing capacity of the soluble fraction obtained after *in vitro* digestion of juices. Both spectrophotometric methods were performed on a Fluostar Omega microplate reader (BMG Labtech, Germany). Aqueous solutions of trolox were used for calibration and the results were expressed as mmol equivalents of trolox per litre of sample.

2.3.3. Antioxidant capacity of the insoluble fraction

The antioxidant activity of the remaining insoluble solid fraction obtained after proper digestion of each juice was conducted as described by Álvarez et al. (2014). In brief, the lyophilized solid was mixed and vortexed with the ABTS or FRAP reagents. After a propped period of time, the samples were centrifuged and the absorbance of the optically clear supernatant was measured by using a Fluostar Omega microplate reader (BMG Labtech, Germany). Trolox solutions were used to perform the calibration curve, being the results expressed as mmol equivalents of trolox per litre of sample.

2.3.4. Calculations of global antioxidant response (GAR) of juices

The total antioxidant capacity of juices was calculated as the sum of the antioxidant capacity of each soluble fraction + the antioxidant capacity of the corresponding insoluble fractions (Pastoriza et al., 2011).

2.3.5. 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.

2.4. Chromatographic analysis of HMF and furfural

The levels of HMF and furfural in juices were analyzed with the HPLC method described on Rufián-Henares, Delgado-Andrade, and Morales (2006). In short, each juice was clarified with potassium ferrocyanide and zinc acetate solutions. After vortexing, the mixture was centrifuged and the supernatant filtered through 0.45 μm acetate filters. HMF and furfural were analyzed by reversed-phase HPLC with a Perkin-Elmer series 200 HPLC (PerkinElmer, Spain). HMF and furfural were quantified by the external standard method within the range 0.1–150 μM.

2.5. Color and fluorescence analysis

The color of juices was determined with a Chroma Meter CR-400 optical sensor (Konica Minolta Sensing, Inc., Osaka, Japan) according to the CIE Lab scale (CIE Colorimeter Committee, 1974) and stated previously for citrus juices (Álvarez et al., 2014). The analysis included three values: L* (black-white component, luminosity), a* (+ red to − green component) and b* (+ yellow to − blue component) chromaticity coordinates (Hunter, 1942). The juices were illuminated with D65-artificial daylight (10° standard angle).

The presence of fluorescence in juices associated to Maillard compounds was evaluated using the same extract obtained for HMF and furfural analysis. Fluorescence was measured at 347 nm excitation and 415 nm emission (Delgado-Andrade, Rufián-Henares, & Morales, 2006) on a fluorescence spectrophotometer (LS 55, Perkin-Elmer, Waltham, MA) with quartz glass cuvettes (QS-1.000 Suprasil, Hellma GmbH & Co, Germany). Data were expressed as arbitrary units (AU) × 10^3.