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Nutritional and physicochemical characteristic of commercial Spanish citrus juices

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ABSTRACT

Citrus juices are perceived as healthy foods by consumers due to their richness in antioxidant compounds. Despite the large number of papers about the antioxidant activity of citrus juices, less is known about the relationship with physicochemical properties. This paper shows that the overall antioxidant activity of citrus juices is underestimated with the standard methodologies, being up to 10-times higher with the GAR method (including an *in vitro* gastrointestinal digestion). 70% of the antioxidant activity was found in the soluble fraction and citrus juices contributed up to 12% of the overall antioxidant intake within the Spanish diet. Physicochemical parameters, such as colour, fluorescence, 5-hydroxymethylfurfural and furfural contents, were correlated with nutritional parameters in some samples. The intake of HMF was negligible from commercial citrus juices and was absent in freshly squeezed ones. Finally, a mathematical model is developed to classify juices depending on their nature or storage conditions.

1. Introduction

Citrus juices have received much attention in recent years. Evidence from a large number of *in vitro* and *in vivo* studies as well as epidemiological investigations have shown that human health is promoted by the consumption of citrus juices. They have been proposed as antimicrobial (Su, Sangster, & D'Souza, 2010), as antitumoral (Dahlawi, Jordan-Mahy, Clench, & Le maître, 2012) as anti-inflammatory agents (Hollebeeck et al., 2012) and as anti-atherosclerotic (Aviram et al., 2008). Therefore, citrus juices contribute to the prevention of degenerative processes, particularly lowering the incidence and mortality rate of cardio and cerebrovascular diseases and cancer (Poulose, Harris, & Patil, 2005). These beneficial properties of citrus juices have been attributed to their content of fibre, vitamins, minerals and specially to antioxidant compounds, such as carotenoids, ascorbic acid (vitamin C), flavonoids and phenolic compounds (Quitao-Texeira, Odriozola-Serrano, Soliva-Fortuny, Mota-Ramos, & Martín-Belloso, 2009). Thus, researchers have been attracted to measure the total antioxidant capacity of citrus juices in order to rank them based on their total protective capacity against reactive oxygen species (Ryan & Prescot, 2010). The measurement of antioxidant activity has been performed usually with juice extracts (Stella, Ferrarezi, dos Santos, & Monteiro, 2011) or with the soluble fraction obtained after *in vitro* digestion (Ryan & Prescot, 2010). However, the main drawback of these approaches is that the overall antioxidant activity is not measured in the same way as more recent methods, like the GAR method (Pastoriza, Delgado-Andrade, Haro, & Rufián-Henares, 2011).

The colour of citrus juices is the first quality factor appreciated by the consumer and has a remarkable influence on its acceptance. Conventional thermal processing ensures safety and extends the shelf life of citrus juices, but it often leads to detrimental changes in sensory quality through caramelisation and non-enzymatic browning. The last one includes the Maillard reaction and vitamin C degradation in citrus juices, both affecting sensorial properties, like texture, colour, taste and flavour (Bull et al., 2004). Nutritional quality is also affected by industrial manufacture because many nutrients, such as vitamins, sugars and proteins take part in nonenzymatic reactions. In addition, the formation of intermediate undesirable compounds, such us furfural and 5-hydroxymethylfurfural (HMF), (Buedo, Elustondo, & Urbicain, 2001) reduce the product's acceptance by consumers. The presence of HMF in citrus juices is an indicator of their quality loss, because furfural and HMF are related with an excess of processing temperature and storage time

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(Nagy & Randall, 1973; Rodrigo et al., 2003). In addition, HMF is metabolised by human beings to 5-sulphoxymethylfurfural (SMF), which has mutagenic (Glatt & Sommer, 2006; Surh, Liem, Miller, & Tannenbaum, 1994) and nephrotoxic activities (Nadiya, Bernhard, Heinz, Albrecht, & Hansruedi, 2009). There is evidence that a high intake of HMF in rats promotes the formation of premalignant lesions in various organs such as hepatocellular adenomas (Zhang et al., 1993). It is therefore necessary to include the analysis of HMF in the nutritional assessment of citrus juices.

In Spain, 26% of the total amounts of juices sold are orange juices and another 5% comes from mandarin juices, which is steadily expanding (Mercasa, 2012). Therefore, due to the economic importance of these juices, the objective of the present study was to investigate both the nutritional and physicochemical characteristics of commercial Spanish citrus juices. To assess the nutritional characteristics we measured the antioxidant activity by conventional methods and by the GAR method, in order to establish the antioxidant capacity of the whole juices. In addition we measured the amount of phenolic compounds of these juices. To characterise the physiochemical profile we measured colour and fluorescence as well as the presence of HMF and furfural. We next aimed to unravel the contribution of the intake of citrus juices to the overall antioxidant activity intake in Spain as well as their contribution to the daily intake of HMF. Finally we studied the relationship amongst the different parameters studied according to the kind of fruit used to manufacture the juices.

2. Experimental

2.1. Chemicals

α-Amylase, pepsin, pancreatin and bile salts were from Sigma–Aldrich (St. Louis, MO). For the ABTS and FRAP methods, the standard antioxidant used was 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), obtained from Sigma–Aldrich. 2,2′-Azobis-(3-ethylbenzothiazoline-6-sulphonic acid) for the ABTS method and 2,4,6-tri(2-pyridyl)-*s*-triazine (TPTZ) for the FRAP method were obtained from Fluka Chemicals (Madrid, Spain). For HMF and furfural analysis, potassium ferrocyanide, zinc acetate and HMF and furfural standards were from Sigma–Aldrich.

2.2. Samples

A total amount of 34 citrus juices were selected to cover the main brands consumed in Spain. They were 23 orange juices, 5 grapefruit juices, 4 mandarin juices and 2 lemon 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 grapefruit, lemon, mandarin and orange 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.

The antioxidant activity 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.

2.2.1. In vitro digestion

The enzymatic digestion was performed as stated in Pastoriza et al. (2011). Briefly, the juice samples were digested with α-amylase, pepsin, pancreatin and bile salts in order to include oral, gastric and intestinal digestions. Once the *in vitro* gastrointestinal digestion was finished, the bioaccessible (soluble) and the non-accessible (or insoluble) fractions were separated and stored at −80 °C until analysis.

2.2.2. ABTS method

The ABTS assay of the juices or the soluble fraction obtained after *in vitro* digestion was evaluated in terms of radical-scavenging activity, conducted as described by Rufián-Henares and Delgado-Andrade (2009) with slight modifications. The $ABTS^{+\cdot}$ was produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 16 h before use. The $ABTS^{+\cdot}$ solution was diluted to an absorbance of 0.70 ± 0.02 at 730 nm with a mixture of ethanol:water (50:50). Twenty microlitres of sample or Trolox standard were added to 280 μl of diluted $ABTS^{+\cdot}$ by using a Fluostar Omega microplate reader (BMG Labtech GmbH, Ortenberg, Germany). Solutions were then set at 37 °C and absorbance readings were taken each minute for 20 min at 730 nm. Aqueous solutions of Trolox were used for calibration and the results were expressed as mmol equivalents of Trolox per litre of sample.

2.2.3. FRAP method

The ferric reducing ability of the citrus juices or the soluble fraction obtained after *in vitro* digestion was estimated following the procedure described by Rufián-Henares and Delgado-Andrade (2009). Briefly, 280 μl of FRAP reagent, warmed at 37 °C and freshly prepared, were mixed with 20 μl of sample. The FRAP reagent was composed of 2.5 ml of a 10 mM TPTZ solution in 40 mM HCl plus 2.5 ml of 20 mM $FeCl3.H_2O$ and 25 ml of 0.3 M acetate buffer, pH 3.6. Readings at 595 nm were taken every minute for 30 min by using a Fluostar Omega microplate reader (BMG Labtech) at 37 °C. Trolox solutions were used to perform the calibration curve. Results are expressed as mmol equivalents of Trolox per litre of sample.

2.2.4. Antioxidant activity of the insoluble fraction

This procedure was applied to the lyophilised insoluble fractions from each juice and conducted as described by Pastoriza et al. (2011). For the ABTS method, 6 ml of ABTS were added to 10 mg of sample and the mixture vortexed for 10 min to facilitate the surface reaction with the ABTS+ reagent. After proper centrifugation at 10500*g* for 3 min, the absorbance of the optically clear supernatant was measured by using a Fluostar Omega microplate reader (BMG Labtech) at exactly 20 min after mixing the sample with the ABTS reagent. In the case of the FRAP assay, the sample–FRAP reagent mixture was shaken for 20 min at 37 °C, centrifuged and the absorbance was measured at 30 min. In both methodologies, when the samples showed a high antioxidant activity, dilutions were made with microcrystalline cellulose, an inert material (Gökmen, Serpen, & Fogliano, 2009). Trolox solutions were used to perform the calibration curve, using microcrystalline cellulose as the blank. Results are expressed as mmol equivalents of Trolox per litre of sample.

2.3. Calculations of global antioxidant response (GAR) of citrus juices

The total antioxidant activity of citrus juices was evaluated by determining their global antioxidant response by the GAR method (Pastoriza et al., 2011). The GAR value was calculated as the sum of the antioxidant activity from their soluble fractions + the antioxidant activity trapped in the insoluble fractions.