Food Chemistry 115 (2009) 119–123

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Antioxidant capacity of some caramel-containing soft drinks

Oreste V. Brenna *, Elena L.M. Ceppi, Gabriella Giovanelli

Department of Food Science and Microbiology, University of Milan, Via Celoria 2, 20133 Milan, Italy

ARTICLE INFO

Article history:
Received 29 July 2008
Received in revised form 13 October 2008
Accepted 20 November 2008

Keywords:
Antioxidant capacity
Soft drink
Caramel colours
Carbonated beverages
Chinotto

ABSTRACT

Antioxidant properties of foods and beverages have been widely studied; however, few data have been reported on the antioxidant capacity of soft drinks. Apart from fruit juice-based drinks, some of the most common soft drinks contain as a colouring agent one of the four caramel colours allowed in foods (E150 d). Caramels contain melanoidin compounds, which have been reported to contribute to the antioxidant powers of some foodstuff. This study aimed to ascertain the contribution to the antioxidant activity of some caramel-containing soft drinks, such as cola drinks, and chinotto, an original Italian soft drink. Some commercial caramel colours were analysed for main parameters, i.e. HMF (5-(hydroxymethyl)-2-furfural), residual glucose and fructose content, total reducing compounds by the Folin–Ciocalteau reagent, and the antioxidant activity by the FRAP and DPPH® methods. Similar analyses were performed on various soft drinks coloured with E150 d. The results showed that even if soft drinks have a lower antioxidant activity than other beverages such as tea, coffee or chocolate, they may contribute to the antioxidant pool assumed with the diet, since the antioxidant activity ranged from 0.2 for cola-like soft drinks to 1.0 mmoles Trolox equivalent/l for chinotto drinks.

1. Introduction

Recent studies have widely demonstrated that a diet rich in antioxidants can protect from a variety of degenerative pathologies (Block, Patterson, & Subar, 1992; Kinsella, Frankel, German, & Kanner, 1993; Renaud & de Lorgeril, 1992). In particular, polyphenols have anti-inflammatory (Subbaramaiah et al., 1998), anti-carcinogenic (Kuroda & Hara, 1999), anti-atherogenic and cardioprotective (Dell'Agli, Buscialà, & Bosisio, 2004) effects, and also play a protective role towards brain degenerative processes (Conte, Pellegrini, & Tagliazucchi, 2003). Polyphenols are present in drinks derived from fruits such as wine and fruit juices, from malted seeds such as beer, from roasted seeds such as coffee and cocoa or from leaves such as tea. For example, in wines the amount of phenolic compounds (expressed as GAE, measured by the Folin–Ciocalteau reagent) can range from less than 500 mg/l in white wines to some g/l in red wines (Lee, Kim, Lee, & Lee, 2003). A lower level of phenolics, derived from cereal grains and added hops, was observed in dark and lager beers (Lugasi & Hóvári, 2003). A cup of espresso coffee (30 ml) contains 130–160 mg GAE [unpublished data]. Much higher levels of total phenolics (611 mg of GAE) are present in cocoa beverages (200 ml per serving); whilst the GAE content of tea varies from about 120 GAE for black tea to 165 for green tea (200 ml per serving) (Lee et al., 2003). Recently, the antioxidant power of some ready-to-drink polyphenol-rich beverages available in the United States has been reported (Seeram et al., 2008). With regard to cola-like drinks, recipes are generally covered; only two papers (Atawodi et al., 2007; Rababah, Hettiarachchy, & Horax, 2004) report a partial characterisation of African cola, and the reported phenolic content is very poor. Therefore, phenolics cannot be assumed to contribute to the antioxidant power of these drinks.

Most of the above-mentioned beverages suffer from some drawbacks as the alcoholic content in wine and beer or the presence of stimulant compounds, such as caffeine in coffee and tea and theobromine in chocolate drinks. Therefore, due to medical (children and pregnant women), practical (i.e. driving a car), or ethical–religious reasons, many consumers turn their attention to different beverages, such as the so-called "soft drinks". Concerning these kinds of beverages, most of them contain a rather high content of sugars, that contribute to energy intake and this fact must be taken into account too.

The aim of this study was to evaluate the antioxidant capacity of soft drinks coloured with caramel, such as the cola-type carbonated beverages, which are largely consumed around the world and do not have the main drawbacks of most of the above-mentioned beverages. Owing to their manufacture procedures, some caramels contain melanoidins that were reported to act as antioxidant (Borrelli, Visconti, Mennella, Anese, & Fogliano, 2002; Wang et al., 2007).

* Corresponding author. Tel.: +39 02 50319169; fax: +39 02 50319190.
E-mail address: Oreste.Brenna@unimi.it (O.V. Brenna).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.11.059

2. Materials and methods

2.1. Materials

2.1.1. Samples

Four commercial caramel colours (E150 a, b, c and d) were kindly provided by SICNA S.r.l. (Cassina Nuova di Bollate, Milan, Italy). Eleven samples of caramel coloured soft drinks, three classic colas (A, B and C), three colas light (D, E and F), two colas without caffeine (G and H), one cola with lemon juice (I) and two soft drinks flavoured with chinotto (*Citrus myrtifolia*) (Italian law: DPR 19/5/1958, art. 5), were also analysed (chinottos A and B).

2.1.2. Chemicals

HPLC-grade methanol, glucose, fructose, sucrose, Folin–Ciocalteau reagent, Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), TPTZ (2,4,6-tripyridyl-striazine), $FeCl_3 \cdot 6H_2O$, DPPH* (1,1-diphenyl-2-picrylhydrazyl radical) and HMF (5-(hydroxymethyl)-2-furfural) were purchased from Sigma–Aldrich Italia (Milano – Italy). All solutions were prepared using deionised water.

2.2. Methods

2.2.1. Dry weight for caramel colours

The analysis was carried out according to the AOAC 925,45 D method (AOAC, 1995, chap. 44).

2.2.2. Colour intensity EBC units

This parameter was determined according to the EBC procedure no. 6.8: colour of caramel (European Brewery Convention, Anaytica-EBC, 2004). The absorbance of all samples was read at 430 nm, after a suitable dilution, in 10 mm pathlength cells, using a Beckman DU 650 spectrophotometer.

2.2.3. Spectrophotometric parameters

The absorbance of caramel colours (0.1% w/v, dry matter) and undiluted soft drinks using deionised water as reference was determined in a 10 mm pathlength cell at 610, 560 and 280 nm; the ratio $R_{280/560}$ values were also calculated this being an important parameter to distinguish between caramel colours II and IV (Licht et al., 1992).

2.2.4. HPLC determination of HMF

This analysis was performed according to the method of Porretta and Sandei (1991). The HPLC system consisted of a Merck Hitachi L-7100 pump, a Merck Hitachi UV detector L-7400 (set at 280 nm) and Merck Hitachi integrator D-7500. A Spherisorb ODS2 column was used, equipped with a C18 pre-column. The flow rate was 1.2 ml/min and injection volume was 20 μl. Isocratic elution was carried out with 10% methanol in water. A calibration curve was obtained with different amounts of HMF standard. All samples were filtered through Waters HA 0.45 μm filters before injection. Results are expressed as g/kg dry matter (d.m.) for caramels and as mg/l for the analysed soft drinks.

2.2.5. HPLC determination of simple sugars

Glucose and fructose contents were determined essentially as described by Calull, Marsé, and Borrull (1992). The HPLC system used consisted of a Waters 600 E Multisolvent Delivery System, a RI detector (1037 A, Hewlett Packard), an Aminex HPX-87 H column (Bio–Rad, Richmond, USA) equipped with a Microguard Cation H^+ pre-column (Bio–Rad). Samples were eluted with 0.01 N sulphuric acid at a flow rate of 0.7 ml/min at 60 °C. Before injection (20 μl), samples were filtered through a 0.45 μm membrane (Waters HA). The amounts of glucose and fructose were calculated from the respective calibration curves and were expressed as g of sugar per litre of sample.

2.2.6. Total reducing substances content

These were determined using the Folin–Ciocalteau reagent (Singleton & Rossi, 1965). Briefly, 1.0 ml of appropriately diluted sample was mixed with a small amount of water and 1.0 ml of the Folin–Ciocalteau reagent; after ca. 4 min, 4 ml of 10% w/v sodium carbonate solution was added; the flask was then filled to the mark with distilled water. The mixture was incubated in the dark at room temperature for 90 min before reading the A_{750} nm using distilled water as the reference. Results were expressed as mg/l of gallic acid equivalents (GAE), a standard curve being prepared using pure gallic acid.

2.2.7. Ferric reducing antioxidant power (FRAP)

The procedure of Benzie and Strain (1996) was followed with some modifications. Briefly, 10 ml of freshly prepared reagent containing 10 mM TPTZ in 40 mM HCl, 20 mM $FeCl_3 \cdot 6H_2O$ and 300 mM acetate buffer, pH 3.6, in the ratio of 1:1:10 (v:v:v) were mixed with 330 μl of appropriately diluted sample. The mixture was allowed to stand for 60 min at room temperature before absorption was measured at 593 nm. Aqueous solutions of $FeSO_4 \cdot 6H_2O$ in the concentration range of 0.125–1.0 mM were used for calibration of the FRAP assay, and Trolox was used as a standard (in the range from 0.1 to 0.5 mM). FRAP values were expressed as mmol Trolox equivalents (TE) per kg dry matter for caramel colours and as mmol TE/l of sample for soft drinks.

2.2.8. 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH*) assay

The antioxidant activity was measured by the DPPH* assay (Sanchez-Moreno, Larrauri, & Saura-Calixto, 1998), which measures the free radical scavenging capacity**.** Briefly, 30 μl of diluted sample was added to 5 ml of methanolic DPPH* solution (30 mg/l). After 30 min at 40 °C ($A_{t=30}$), the absorbance at 515 nm was measured using methanol as the reference. At least four different dilutions were assayed for each sample, in order to obtain a percentage inhibition between 20% and 80% (in our case, dilutions were in the range 1–5 fold). The antioxidant activity was expressed as TE by using Trolox as a standard for calibration.

2.3. Statistical analysis

All samples were analysed at least in triplicate, and data were reported as mean value ± standard deviation (SD). One-way ANOVA and the multiple range test were carried out using Statgraphics plus 5.1 (Graphics Software Systems, Rockville, USA). Multivariate analysis was performed applying the PCA (Principal Components Analysis), in order to discriminate samples on the basis of their analytical characteristics. Afterwards analytical data were correlated with the antioxidant capacity using the PLS algorithm (Partial Least Squares). PCA and PLS processing were performed with The Unscramble v. 9.7 software (Camo, Inondhaim, Norway).

Table 1
Spectrophotometric parameters of the four caramel samples.

Sample	EBC units	A_{560}	A_{280}	$R_{280/560}$	A_{610}
E150 a	7.040 ± 28	0.072 ± 0.001	7.04 ± 0.21	97.78	0.032 ± 0.001
E150 b	18.300 ± 460	0.242 ± 0.001	10.58 ± 0.07	43.72	0.121 ± 0.000
E150 c	34.850 ± 141	0.496 ± 0.001	15.41 ± 0.08	31.07	0.256 ± 0.001
E150 d	40.425 ± 247	0.892 ± 0.004	12.05 ± 0.16	13.51	0.513 ± 0.001

The absorbance values were recorded on a 0.1% (w/v, dry matter) solutions. The EBC units are expressed according to the European Brewery Convention, Anaytica-EBC (2004), as A 430 * dilution factor * 25.