

Original article

A comparison of antioxidant properties between artisan-made and factory-produced chocolate

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Summary The antioxidant capacities and the total phenolic content in cocoa liquor directly manufactured chocolate from an artisan manufacturer were measured using different *in vitro* methods (BR, TEAC, and Folin–Ciocalteu Reagent). These parameters were then compared with those of a chocolate made by a leading manufacturing company producing chocolate and cocoa-containing products. A statistical analysis of the collected data showed that the antioxidant properties of the artisan-made chocolate are significantly better than those of the factory-produced one. These results were ascribed to the fact that all the bioactive components in the cocoa beans are better preserved in the artisan-made chocolate.

Keywords Antioxidant properties, Briggs–Rauscher reaction, chocolate, TEAC method, total phenolics.

Introduction

In recent years cocoa and dark chocolate have attracted continuous interest not only for their palatable treat and nutritional properties, but especially for its potential health benefits. These beneficial effects on cardiovascular diseases (Kris-Etherton & Keen, 2002; Heiss *et al.*, 2003), blood pressure (Taubert *et al.*, 2007a,b), plasma LDL and HDL cholesterol (Baba *et al.*, 2007), diabetes (Makoto Tomaru *et al.*, 2007), platelet function (Rein *et al.*, 2000; Pearson *et al.*, 2002), breast cancer (Ramljak *et al.*, 2005), antioxidant status and oxidative stress (Heiss *et al.*, 2003), are mainly ascribed to the high flavanoid content of cocoa and chocolate.

Many papers have been published about the antioxidant capacity and phenolic content of cocoa and cocoa related products (Waterhouse *et al.*, 1996; Adamson *et al.*, 1999; Arts *et al.*, 1999; Vinson *et al.*, 1999; Won Lee *et al.*, 2003; Gu *et al.*, 2006; Miller *et al.*, 2006).

A more recent study investigated the effect of some steps of manufacturing chocolate on single components and antioxidant activity of cocoa beans (Arlorio *et al.*, 2008). The authors found that the roasting step caused a dramatic reduction in clovamide, parallel to an overall decrease of the antioxidant capacity.

Before becoming cocoa powder or chocolate, cocoa beans undergo the following treatments: fermentation,

drying, deshelling, sterilisation, roasting and grinding. The first three steps are usually done at the site of cultivation, while the others are factory-based. From the grinding a cocoa liquor (CoL) is obtained that will be transformed into cocoa powder or chocolate products (Cooper *et al.*, 2007). The transformation process of CoL into dark chocolate commonly used by the multinational companies implies pressing, mixing, conching and tempering. Cocoa liquor is heated up to 95–105 °C and is then pressed. As a result, a great part of the fat (cocoa butter) is separated from cocoa paste. Cocoa paste can be alkalisated to favour aggregation, but it is known that this step considerably reduces the polyphenol content (Gu *et al.*, 2006).

Cocoa paste, part of the cocoa butter, sugar and possible natural flavours are then mixed in specified proportions. After the mixing, the mass is ground. This chocolate mass is then subjected to conching which is intensive mixing at a high temperature. Conching is a very long process (up to 24 h) and, as a result, the superfluous moisture is evaporated from the chocolate mass. Finally this mass is tempered. In the tempering process, which is long and complex, the chocolate mass is gradually cooled to 27 °C, heated again to 37 °C and then slowly re-cooled into a solid state thus leading to the desired uniform crystallisation of the chocolate.

In many countries, some artisan manufacturers make dark or baking chocolate directly conching cocoa liquor (maybe added with sugar and natural flavours). In this case the conching treatment requires much longer duration, more than 5 days.

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The aim of the present work is to compare the antioxidant capacity and the total phenolic content of a chocolate produced by a worldwide known company with those of an artisan manufacturer.

The antioxidant capacity was evaluated using two tests: the Briggs-Rauscher (BR) oscillating reaction method that works at pH \sim 2 and the trolox equivalent antioxidant capacity (TEAC) assay working at pH 7.4. Total phenolic content was determined using the Folin-Ciocalteu reagent.

Materials and methods

Chemicals and apparatus

Malonic acid, manganese (II) sulphate monohydrate, NaIO₃, Na₂CO₃ anhydrous, (all reagent grade \geq 99%) were purchased from Merck (Darmstadt, Germany). Gallic acid (3,4,5-trihydroxy benzoic acid; Seelze, Riedel-de Haën, Germany), 2,6-DHBA (2,6-dihydroxy benzoic acid; Aldrich, USA), K₂S₂O₈, ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid), Folin-Ciocalteu reagent (FC), HClO₄ and H₂O₂ were purchased from Fluka (St. Louis, MO, USA) and Trolox (6-hydroxy-2,5,8-tetramethylchroman-2-carboxylic acid) from Aldrich (St. Louis, MO, USA). HClO₄ was analysed by titration against a standard 0.1 M NaOH solution (from Merck). H₂O₂ was standardised daily by manganometric analysis. All stock solutions were prepared with double distilled (dd), deionised water.

Potentiometric measurements were performed by recording the potential of an iodide ion selective electrode (model 9453; Orion, Beverly, MA, USA) using a multimeter (WTW model pH 540; GLP, Weilheim, Germany) controlled by an IBM-compatible PC. As a reference a double junction Ag/AgCl electrode (model 373-90-WTE-ISE-S7; Ingold, Urdorf, Switzerland) was used. Spectrophotometric measurements were made on a Shimadzu 1601 PC UV-vis spectrophotometer (Kyoto, Japan).

Chocolate samples

Multinational manufactured (MM) 99% cocoa. Ingredients: cocoa paste + cocoa powder + cocoa butter 99%, sugar cane. Artisan manufactured (AM) classic 100% cocoa. Ingredients: cocoa liquor 99.98%, sweeteners (0.02%: sodium cyclamate, sodium saccharinate, acesulfame k). AM red pepper flavour 100% cocoa. Ingredients: cocoa liquor 99.96%, red pepper powder, sweeteners (0.02%: sodium cyclamate, sodium saccharinate, acesulfame k). AM rosemary flavour 100% cocoa. Ingredients: cocoa liquor 99.95%, rosemary powder, sweeteners (0.02%: sodium cyclamate, sodium saccharinate, acesulfame k). All samples were produced from cocoa beans Criollo variety, Ecuador origin.

Sample preparation

Bioactive compounds from chocolate were extracted using the procedure suggested by Serafini *et al.* (2003). A sample of 2 g were ground in a mortar. Exactly one gram of this powder was defatted by adding 10 mL of *n*-hexane, the mixture was ultrasonicated for 10 min at 30 °C and then centrifuged at 3000 rpm for 10 min. This operation was repeated twice. The residue was extracted with 5 mL of a mixture of acetone, water and acetic acid (70.0:29.8:0.2 by volume). The mixture was put in an ultrasound bath for 15 min at 30 °C, then centrifuged for 4 min at 875 g. The supernatant liquid was separated and the operation was repeated with 2 mL of the acetone-water-acetic acid solution. The collected supernatant liquids were filtered with a PTFE 0.45 μ m filter, then the organic solvent was removed in a water-bath at 45 °C. After filtration doubly distilled water was added to a final volume of exactly 10.0 mL.

Antioxidant activity assay based on the Briggs-Rauscher reaction

The chemical *in vitro* method reported by Cervellati *et al.* (2001), is based on the inhibitory effects by free radical scavengers on the oscillations of the BR reaction. In brief, when antioxidant scavengers of free radicals are added to an active oscillating BR mixture there is an immediate quenching of the oscillations, an inhibition time (t_{inhib}) that linearly depends on the concentration of the antioxidant added, and a subsequent regeneration of the oscillations. Relative antioxidant activity (r.a.c.) with respect to a substance chosen as standard (2,6-DHBA; Höner & Cervellati, 2005) is determined on the basis of concentrations of sample and 2,6-DHBA that give the same t_{inhib} . r.a.c. is expressed as mg 2,6-DHBA equivalents/g chocolate.

Antioxidant activity based on the trolox equivalent antioxidant capacity assay

The protocol suggested by Re *et al.* (1999) was used. Antioxidant activity is expressed as mg Trolox equivalents/g chocolate.

Determination of total phenolics (antioxidant reducing capacity quantification)

This test is based on the oxidation of phenolic groups by phosphomolybdic and phosphotungstic acids (FC reagent). After oxidation the absorbance of a green-blue complex can be measured at 765 nm. The procedure for 20 mL total volume of the reacting mixture (Singleton & Rossi, 1965) was used. Total phenolic content is expressed as mg gallic acid equivalents (GAE)/g chocolate.

Results and discussion

In Table 1, the first two columns show the antioxidant data for the examined chocolate samples. The third column shows the values of the total phenolic content (total reducing power).

The statistical significance among the mean values of the different samples was evaluated using the Student's *t*-test, taking the MM sample as reference because its Factory is considered one of the leaders in manufactur-

Table 1 Relative antioxidant capacity and total phenolic content of the chocolate extracts

Sample	BR (r.a.c.) _{m±σ} mg/g 2,6-DHBA	TEAC mg/g Trolox	TPC GAE mg/g GA
MM	2.9 ± 0.3	20 ± 2	13.2 ± 0.8
AM classic	5.8 ± 0.4	33 ± 3	15 ± 1
AM red pepper	6.2 ± 0.4	35 ± 7	15 ± 1
AM rosemary	6.4 ± 0.5	40 ± 5	16 ± 1

Data in the table are average values of five measurements at different extract concentrations ± SD.

ing chocolate and cocoa-containing products. The results are reported in Fig. 1.

From the data in Fig. 1a,b it can be seen that the artisan-produced chocolate has significant higher antioxidant activity (with both methods) than the reference sample. As far as the total phenolic content is concerned only the two flavoured handicraft chocolates show significant higher GAE than the factory-based one (Fig. 1c). It can be excluded that these significant differences can be due to the 1% difference in the total cocoa content between MM and AM chocolates.

There are no great differences among the three AM samples; this is not surprising because the main ingredient, cocoa liquor, is the same. Only the parameters of the AM rosemary flavoured chocolate seem a little higher than those of the other two chocolates. This can be due to some very high antioxidant capacity compounds contained in the rosemary: rosmarinic acid, carnosic acid and carnosol (Cervellati *et al.*, 2002; Costa *et al.*, 2007).

As pointed out in the Introduction several authors have investigated antioxidant properties of cocoa and related products, but it is difficult to compare their results with those presented here. In fact the

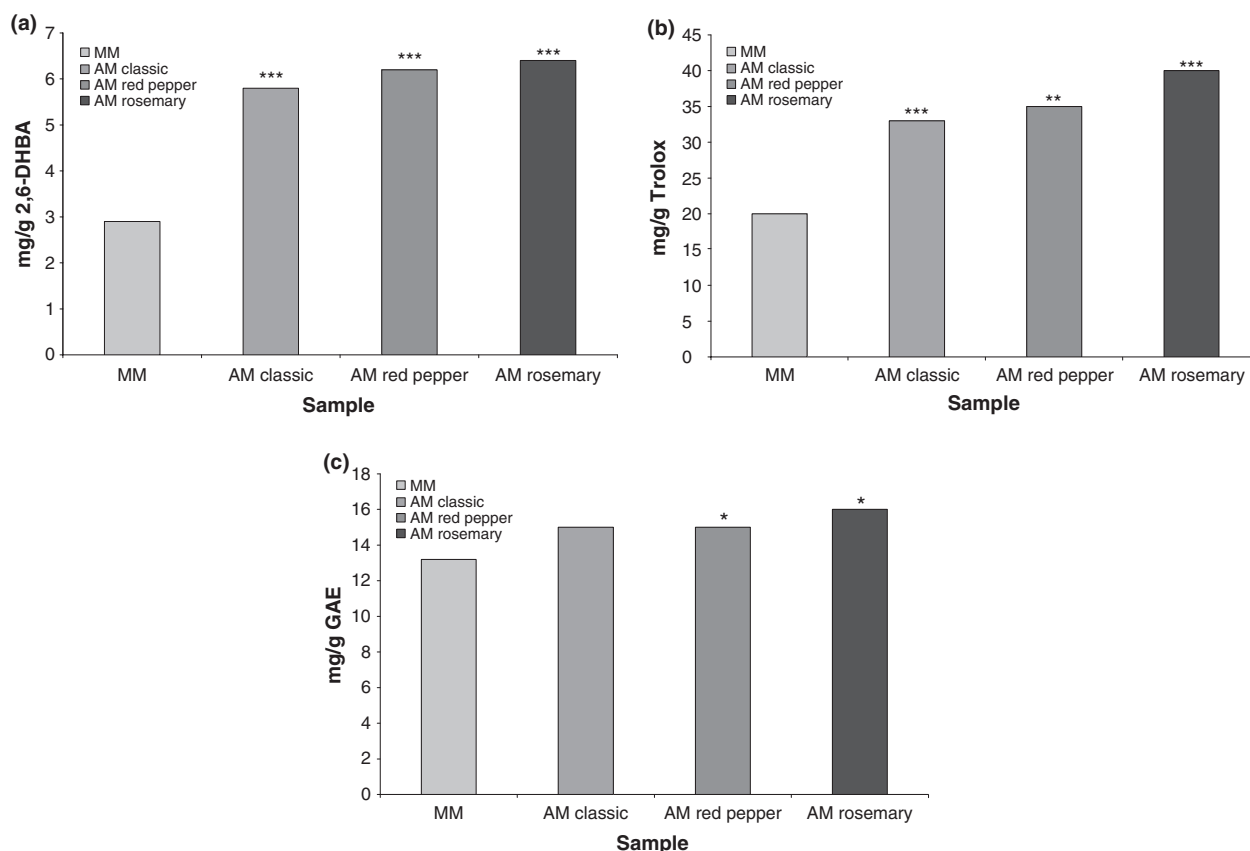


Figure 1 Statistical significances of the antioxidant parameters with respect to the MM sample. (a) BR r.a.c. values, ****P* < 0.0001; (b) TEAC values, ***P* < 0.002, ****P* < 0.0001; (c) GAE values, **P* < 0.01.

concentration of all polyphenols can vary tremendously among cocoa-containing foods, and this can vary depending on the source of the beans, the processing conditions, and how the chocolates are manufactured (Cooper *et al.*, 2007). Moreover, different authors used different extraction procedures and different antioxidant testing methods. Some comparison can be made with the data recently reported by Miller *et al.* (2006) on the total polyphenols (FC method) of chocolate products in the US. For dark chocolate they found GAE values ranging from 11.7 to 14.9 and for baking chocolate from 27.2 to 29.7. These latter values are higher than those reported here. However, Waterhouse *et al.* (1996) reported a value of 8.4 mg GA g⁻¹ baker's chocolate. It must be taken into account that the FC reagent method suffers from a number of interfering substances (Huang *et al.*, 2005) even if it is the recommended method to measure the total reducing capacity (Prior *et al.*, 2005). Since different antioxidant testing methods give different ranking orders of antioxidant capacity due to different experimental conditions, we used two methods in order to obtain a realistic estimate of the antioxidant activity of the examined chocolates, as suggested by Prior *et al.* (2005). Overall, *in vitro* chemical antioxidant assays can only partially mimic physiological conditions. The BR method was adopted because it works at pH ~ 2, similar to that of the fluids in the human stomach. Kanner & Lapidot (2001), observed that some plant-derived antioxidants are able to prevent lipid peroxidation, amplified in the acidic pH of gastric juice. The conception of the stomach as a bioreactor, where reactive oxygen species and food nutrients interact, underlines the importance of determining antioxidant activity of dietary sources at acidic pH.

The pH of TEAC method is 7.4, equal to that of the human plasma. Correlations between the different methods were obtained by Pearson's correlation coefficient in bivariate correlation. Significant correlations were found between GAE and BR and TEAC tests: $R = 0.9505$ ($P < 0.025$), $R = 0.9900$ ($P < 0.01$), respectively. Those data are quite in agreement with the fact that the total reducing capacity is related to the antioxidant activity of the chocolate flavonoids. Good correlation was also observed between the BR and TEAC methods: $R = 0.9753$ ($P < 0.025$).

Our results showed that the antioxidant properties of the artisan-made chocolate are significantly better than those of a factory-produced one. All the bioactive substances in the cocoa beans are better preserved because of the direct process used in artisan-made chocolate which leads to a higher quality product. Further studies are needed to investigate the *in vivo* plasma antioxidant status after the repeated oral administration of these chocolates in animal models in order to verify the *in vitro* chemical results.

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