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Evaluation of the antioxidant properties of fruits

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Abstract

Twenty-eight different fruits were analysed for antioxidant activities using two different methods, one that determines the inhibition of ascorbate/iron-induced peroxidation of phosphatidylcholine, by means of thiobarbituric acid-reactive substances (TBARS) measurement, and another that evaluates the scavenging of the radical cation of 2,2′-azinobis (3-ethyl-benzothiazoline-6-sulphonate) (ABTS) relative to Trolox C, a water-soluble vitamin E analogue. The values of the antioxidant capacities of the analysed fruits were in a wide range from 50 g to more than 5 mg for the IW_{50} (dry weight causing 50% inhibition of lipidic peroxidation) and from it 406 μmol/g for the TEAC (μmol Trolox equivalents g^{-1}). There was no significant correlation between antioxidant activity (IW_{50} and TEAC) and the contents of flavanol of the samples. The antioxidant activities of the analysed fruits cannot only be attributed to their flavanol contents, but to the result of the action of different antioxidant compounds present in the fruits and to possible synergic and antagonist effects still unknown.

Keywords: Antioxidant activity; TEAC; TBARS; Fruits; Flavanols

1. Introduction

The association between a diet rich in fruit and vegetables and a decreased risk of cardiovascular disease and certain forms of cancer is supported by considerable epidemiological evidence (Hertog, Feskens, Hollman, Katan, & Kromhout, 1993, 1994; Hertog et al., 1995).

Different studies have shown that free radicals present in the human organism cause oxidative damage to different molecules, such as lipids, proteins and nucleic acids and thus are involved in the initiation phase of some degenerative illnesses. As a consequence, those antioxidant compounds that are capable of neutralizing free radicals, may play a major role in the prevention of certain diseases, such as cancer, cataracts, cerebral pathologies and rheumatoid arthritis (Clifford, 1995). Fruits and vegetables contain different antioxidant compounds, such as vitamin C, vitamin E and carotenoids, whose activities have been established in recent years. However, these compounds are not the only ones contributing to the antioxidant activity of fruit and vegetables. Recent work shows that the presence of polyphenol compounds, such as flavonoids (in fruits and vegetables) also contribute to beneficial effects of this group of foods (Bors, Heller, Michael, & Saran, 1990; Clifford, 1995; Hertog et al., 1993, 1994, 1995). Apart from their biological properties, flavonoids are also of interest in the food, cosmetic, and pharmaceutical industries, as they can be used as substitutes for synthetic antioxidants (Moure et al., 2001).

Flavonoids are a family of compounds with a C6-C3-C6 skeleton structure. Flavanols, flavonols and anthocyanins are included in this group. All of them are found ubiquitously in the plant kingdom and have been shown to possess antioxidant activity, which depends mainly on the number and position of hydroxyl groups within their structure (Rice-Evans, Miller, & Paganga, 1996). Between flavanols, the most common in fruits are of the catechin and gallocatechin types (Fig. 1) and they can exist in the monomer form or can polymerise, giving rise to condensed tannins or proanthocyanidins.

There are different methods to evaluate the in vitro antioxidant capacities of isolated compounds, mixtures of compounds, biological fluids and tissues. The results

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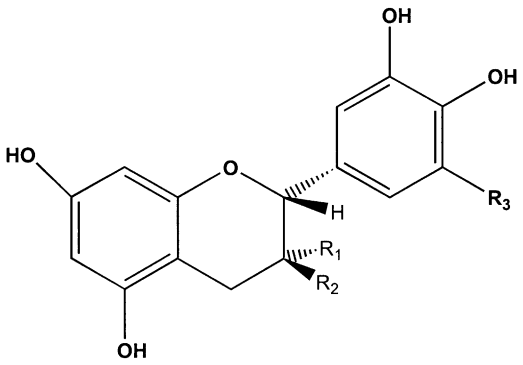

Catechin: R_1 =H, R_2 =OH, R_3= H

Gallocatechin: R_1 =H, R_2 =OH, R_3= OH

Fig. 1. Structures of catechin and gallocatechin.

obtained depend on the method used (Sánchez-Moreno & Larrauri, 1998). For this reason, in the present work, two different methods were selected for the evaluation of the antioxidant activity, one that determines the inhibition of ascorbate/iron-induced peroxidation of phosphatidylcholine in lipid media (thiobarbituric acid-reactive substances, TBARS) and Trolox equivalent antioxidant capacity (TEAC), which evaluates the scavenging of the radical cation of 2,2′-azinobis (3-ethyl-benzothiazoline-6-sulphonate; ABTS) relative to Trolox C, a water-soluble vitamin E analogue. Both assays have already been widely applied for the analysis of the antioxidant capacity in different samples (Chambers, Lambert, Plumb, & Williamson, 1996; Fogliano, Verde, Randazzo, & Ritieni, 1999; Plumb et al., 1996; Plumb, de Pascual-Teresa, Santos-Buelga, Cheynier, & Williamson, 1998; Rice-Evans & Miller, 1994).

The main objective of this work was to evaluate the antioxidant activity of extracts from a total of 28 different fruits, in which the flavanol composition had been analysed, and to establish whether there was any correlation between the antioxidant activity and the content of flavanols of low degrees of polymerization, i.e. those more likely to be bioavailable and thus to have biological significance (Deprez, Mila, Huneau, Tome, & Scalbert, 2001; Plumb et al., 1998).

2. Materials and methods

2.1. Chemicals and reagents

Butylated hydroxytoluene (BHT) was purchased from Aldrich (Milwaukee, USA), 2,2′-azinobis (3-ethyl-benzothiazoline-6-sulphonate) (ABTS), (±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox C) and trichloroacetic acid (TCA) were obtained from Fluka Chemie (Buchs, Switzerland), thiobarbituric acid (TBA), phosphatidylcholine and myoglobin were obtained from Sigma (St. Louis, USA), and $FeCl_3$ was obtained from Panreac Química SA (Barcelona, Spain). All other chemicals used were of analytical grade.

2.2. Fruits material

Twenty-eight different fruits collected from local markets were studied. They included: apple (four different varieties), apricot, strawberry-tree fruit, avocado, banana, blackberry, blueberry, cherry, custard apple, fig, grape (red and white), kiwi, medlar, peach, pear (two different varieties), persimmon, pineapple, plum (*var*. Claudia), pomegranate, quince, raspberry, redcurrant and strawberry. The fruits were immediately washed, peeled (in the cases of banana, custard apple, fig, kiwi, medlar, persimmon, pineapple, pomegranate, and quince) and frozen to be further freeze-dried. Freeze-dried samples were maintained in a helium atmosphere at −30 °C until their analysis.

2.3. Sample preparation

For their analysis, freeze-dried samples (3 g) were homogenized in cold methanol (3×25 ml) and centrifuged at 12 000*g* for 10 min; water was added to the supernatants, the methanol evaporated under vacuum and the aqueous extract obtained made up to 10 ml with water.

2.4. HPLC and analysis

The analysis of flavanols was carried out by HPLC under conditions previously described (de Pascual-Teresa, Rivas-Gonzalo, & Santos-Buelga, 2000; de Pascual-Teresa, Treutter, Rivas-Gonzalo, & Santos-Buelga, 1998). Briefly, the quantifications of individual flavanols were achieved from the areas of their peaks recorded at 640 nm, after post-column reaction with *p*-dimethylaminocinnamaldehyde (DMACA), by comparison with calibration curves obtained using standard solutions of the monomers: (+)-catechin, (−)-epicatechin, (+)-gallocatechin, (−)-epigallocatechin, (−)-epicatechin gallate, the dimers: B1, B2, B3, B4, B5, B7, gallocatechin-(4→8)-catechin, and the trimers: C1 and epicatechin-(4→8)-epicatechin-(4→8)-catechin, previously isolated in our laboratory. The concentrations of flavan-3-ols in the fruits analysed were expressed in mg/100 g of dry weight.

2.5. Lipid phase antioxidant activity: TBARS method (Buege & Aust, 1978)

Phosphatidylcholine, at a final concentration of 1 mg/ml, and assayed sample, at different concentrations, were added to 150 mM KCl containing 0.2 mM $FeCl_3$.

Peroxidation was started by adding ascorbate at a final concentration of 0.05 mM to complete a volume of 0.4 ml. Samples were incubated at 37 °C for 40 min and the reactions terminated by the addition of 0.8 ml of 20% (w/v) trichloroacetic acid (TCA)/0.4% (w/v) thiobarbituric acid (TBA)/0.25 N HCl, and 0.01 ml of BHT in ethanol. The production of TBARS was measured spectrophotometrically at 535 nm after incubation at 80 °C for 20 min and expressed as dry weight (μg) causing 50% inhibition (IW_{50}).

2.6. Aqueous phase antioxidant activity: TEAC method (Rice-Evans & Miller, 1994)

The assay was based on the relative ability of antioxidants to scavenge the radical cation 2,2′-azinobis (3-ethyl-benzothiazoline-6-sulphonate) ($ABTS^{\bullet+}$). The radical was generated by the interaction of ABTS with the ferrylmyoglobin radical species, generated by the activation of metmyoglobin with H_2O_2. The extent of quenching of the ABTS radical was measured spectrophotometrically at 734 nm and compared with Trolox C, a water-soluble vitamin E analogue. Results were expressed as μmol Trolox equivalents g^{-1} (TEAC).

2.7. Statistical analysis

The possible correlation between the antioxidant activities and contents of flavanols of the extracts of the fruits was analysed by analysis of variance (ANOVA) using Statview 4.1. Differences at $P<0.05$ were considered significant.

3. Results and discussion

3.1. Flavanol content

For determination, an extraction of the fruits with methanol was carried out. This solvent had been previously shown to provide a good extraction of low polymerized flavanols (Kallithraka, Garcia-Viguera, Bridle, & Bakker, 1995). The global content of flavanols with a low degree of polymerization (monomers + dimers + trimers; presented in Table 1) was calculated from the sum of the concentrations of the individual compounds determined. It can be seen that the highest contents are those of plum (*var*. Claudia), apples, custard apple, peach, strawberry tree fruit and cherry, with concentrations of total flavanols between 70 and 370 mg/100 g of dry weight. On the other hand, the lowest contents are in the samples of avocado, banana and pear (*var*. blanquilla), with values lower than 4 mg/100 g of dry weight. Catechins are present in all the flavanol-containing fruits, the distribution of gallocatechins being much more limited. The presence of these latter is noteworthy in the cases of pomegranate and grapes and also in the berries. In general, among the compounds quantified, the greater proportion corresponds to the monomers. The most abundant monomer is normally (−)-epicatechin, followed by (+)-catechin, which is predominant in the berries. Although in most cases the monomers represent more than half of the total concentration of flavanols of low degree of polymerization, in some cases the dimers and trimers predominate, e.g. in blueberry, custard apple, plum (*var*. Claudia), apples, peach, quince, banana and grapes. The galloyl flavanols are less widespread and are only important in strawberry, medlar and grapes.

Table 1
Global content of flavanols with a low degree of polymerization

Sample	(mg/100 g dry weight)
Avocado	0.24
Apricot	4.65
Blueberry	44.46
Fig	ND
Cherry	70.03
Custard apple	129
Plum, Claudia	366
Raspberry	69.1
Strawberry	55.3
Pomegranate	4.48
Redcurrant	27.3
Persimmon	10.2
Kiwi	1.68
Strawberry-tree	91.0
Apple, Golden	39.0
Apple, Granny Smith	153
Apple, Renette	211
Apple, Red Delicious	251
Peach	98.3
Quince	22.6
Blackberry	31.9
Medlar	20.6
Pear, blanquilla	3.54
Pear, conferencia	14.1
Pineapple	ND
Banana	0.26
Grape, white	4.99
Grape, red	9.79

3.2. Antioxidant activity

The results obtained for antioxidant activity by the TBARS method are presented in Fig. 2. The antioxidant activity of the samples varies within a wide range of values, the greatest antioxidant effect, lowest IW_{50}, being found in strawberry (50 μg), raspberry (55 μg), cherry (74 μg), blackberry (76 μg) and blueberry (78 μg) and the lowest in banana (1281 μg), white grape (2216 μg), kiwi (4487 μg) and avocado (>5000 μg). It is striking that the fruits which demonstrate greater antioxidant activity are all rich in anthocyanins, suggesting that these pigments could be contributing to this activity.

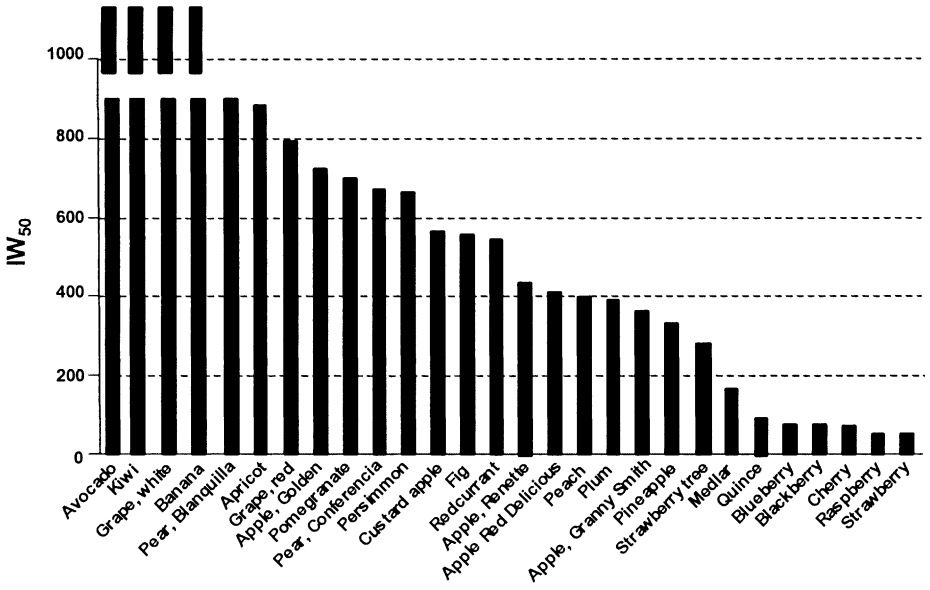

Fig. 2. Antioxidant capacity obtained by TBARS method.

Nevertheless, other fruits, such as the pomegranate, red currant and red grape, which also have anthocyanins, do not present such a high antioxidant activity. This indicates that other compounds might affect the antioxidant activity measured, and also flavanol composition of the samples, as different compounds within this family could have different activities.

The antioxidant activity of some fruits (plum, apple, pear and peach) had previously been determined by Plumb et al. (1996) using the same method. Their results are similar to our observations, except for plum. These authors found a greater antioxidant activity in the case of this fruit, that could be due to the fact that a red variety was used, therefore containing anthocyanins while, in our case, Greengage plums (*var*. Claudia) were used.

The results of the TEAC analysis can be seen in Fig. 3. As in the previous assay, the range of antioxidant activity obtained was very wide, although it must be emphasized that, in all the cases, with the exception of avocado, the TEAC values obtained were positive, which is to say their activity was equal to or greater than that of a solution 1 mM Trolox C. As can be appreciated, the sample with the lowest antioxidant activity was avocado (1 μmol/g), followed by green fig and pear (c.v. blanquilla), which had TEAC values of 4 and 3 μmol/g, respectively. The samples with greatest antioxidant capacities in this assay were persimmon (406 μmol/g), blackberry (192 μmol/g), blueberry (187 μmol/g) and strawberry-tree fruit (163 μmol/g). The fact that all the samples, with the exception of avocado, give

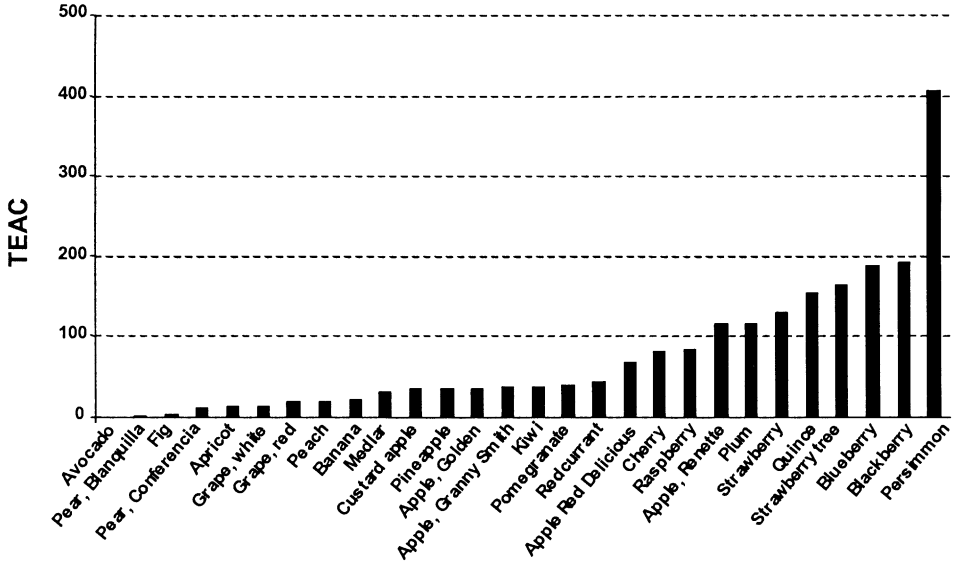

Fig. 3. Antioxidant capacity obtained by TEAC method (μmol Trolox equivalent g^{-1}).

TEAC values greater than 1 confirms what has frequently been indicated in nutritional, biochemical and epidemiological studies; in general, fruits are a very good source of antioxidants.

The antioxidant activities of some of these fruits have been previously published by other authors, also using methods for assessing activity in the aqueous phase. Miller, Rigelhof, Marquart, Prakash, and Kanter (2000), using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as substrate, for the samples of plum, red apple, red grape, kiwi, white grape, banana and pear, always obtained absolute values of antioxidant activity higher than those obtained in this study, which could be explained by differences between the methods employed. Nonetheless, the relative orders, from greater to lesser antioxidant activity of the mentioned fruits, are the same, with the exceptions of red and white grapes which, in our ranking, occupy a position corresponding to less activity.

Wang, Cao, and Prior (1996), using the oxygen radical absorbing capacity (ORAC) method, also found markedly similar results to our observations for strawberry, kiwi and pear. Nonetheless, there is not a good correspondence for the rest of the fruits analysed (plum, red grape, white grape and apple). To explain this discrepancy it should be borne in mind that, in our study, the TEAC values for apple varied with the variety sampled. Moreover, the lower values obtained by Wang et al. (1996) can also be explained by their determination of the antioxidant activity of the peeled fruit, with the consequent loss of antioxidant substances present in the peels.

There was a greater antioxidant activity of both white and red grapes in the results of the ORAC assay of Wang et al. (1996) than by our TEAC. One possible explanation is that, in our case, the extracts were obtained from the grapes after eliminating the seeds, in which there is a high content of flavanols. In the work by Wang et al. (1996) there is no indication of whether the pips were eliminated or not.

The high antioxidant activity in aqueous medium shown by the persimmon cannot be explained by its content in flavanols, since this is low. However, some authors have suggested that the antioxidant capacity of some fruits may be attributed to their gallo-derivatives contents. This is the case with bananas, which have a high content of gallocatechins (Someya, Yoshiki, & Okubo, 2002). It is known that the persimmon is a fruit rich in galloyl derivatives (Nakatsubo et al., 2002), compounds which, with the exception of epicatechin-3-O-gallate, have not been quantified in this study, and may account for the antioxidant effect. On the other hand, the galloyl derivatives have an antioxidant activity, in aqueous medium, superior to those of their non-galloyl homologues (Plumb et al., 1998). Moreover, the results of the TBARS assay for this same sample showed little antioxidant activity, which is also in accordance with this hypothesis since, according to Plumb et al. (1998), the antioxidant activity in the lipid phase is diminished by the presence of galloyl flavanols. Relating the results obtained by the methods of evaluation used, we can conclude that the red fruits, including strawberry, blueberry and blackberry, are those which show the greatest antioxidant capacity. This leads to the consideration of a possible influence of the anthocyanins in this activity. This is also supported by the greater antioxidant power shown by the red grape than the white grape, in both assays. The fruit which follows red fruits regarding antioxidant activity, in both assays, is the plum, which is, among those analysed, that has the greatest total content of flavanols (364 mg/100 g of dry fruit). The avocado is the fruit which has the least antioxidant power.

With the objective of determining the possible correlation between the antioxidant activity of the extracts of the fruits analysed and their contents of flavanols, the coefficients of correlation between the values obtained in this work for TEAC and IW_{50} of the extracts of fruits and the contents of 14 individual flavanols were calculated, and also the total contents of flavanols of these extracts.

In no case was a significant correlation found, all the values of the coefficients of correlation being below 0.3, with the exception of that which was established between the content of catechin and the TEAC value, which was 0.383. The coefficients of correlation between the values of antioxidant activity and the contents in flavanols for the samples of fruits, grouping them by the presence or not of anthocyanins in them, were also calculated. No significant correlation was found in any of the groups. Moreover, in the case of TEAC assay, Arts, Dallinga, Voss, Haenen, and Bast (2003) have recently described the formation of reaction products, which may interfere in the analysis of the antioxidant activity and consequently may introduce an error in its evaluation.

Other compounds which must contribute to the antioxidant activity in the samples analysed should also be considered. For example, besides flavanols and anthocyanins, in most fruits analysed, other flavonoids have been reported, such as quercetin conjugates, flavonols whose antioxidant activity is well known. It should be mentioned that samples such as the avocado or banana, with low antioxidant activities, lack flavonols, whereas others, such as apples, are very rich in this type of flavonoid. Some authors have found positive correlations between the total content of phenolic compounds and the antioxidant activity in fruits (Wang & Lin, 2000). Nevertheless, these results must be interpreted with caution as they have been obtained using the method of Folin-Ciocalteau for the determination of total phenolic compounds, which, in spite of being widely accepted, is not very specific since, not only phenolic compounds,

but also other reducing compounds are simultaneously determined (Santos-Buelga & Scalbert, 2000). Furthermore, in the fruits, variable quantities of diverse antioxidant vitamins can be found.

In conclusion, the fact that the antioxidant activity did not show correlation with the content of flavanols in the samples assayed does not signify that these do not contribute to it, but that this could be the result of the synergies (or antagonisms), still unknown.

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