Mol. Nutr. Food Res. 2005, *49*, 239 – 246 DOI 10.1002/mnfr.200400083

Comparison of the 2,2′-azinobis-3-ethylbenzotiazoline-6-sulfonic acid (ABTS) and ferric reducing antioxidant power (FRAP) methods to asses the total antioxidant capacity in extracts of fruit and vegetables

Jessica Nilsson[1], Deepa Pillai[1], Gunilla Önning[1], Christine Persson[1], Åke Nilsson[2] and Björn Åkesson[1,2]

[1]Biomedical Nutrition, Lund Institute of Technology, Lund University
[2]Department of Clinical Nutrition, University Hospital of Lund, Lund, Sweden

A comparison was made on the use of two spectrophotometric methods, the ferric reducing antioxidant power (FRAP) method and the 2,2′-azinobis-3-ethylbenzotiazoline-6-sulfonic acid (ABTS) method, for the measurement of the total antioxidant capacity (TAC) of plant foods. The correlations of TAC measured by the two methods were highly significant in both water-soluble (r^2 = 0.90) and water-insoluble extracts (r^2 = 0.98) from 13 strawberry samples. Also a corresponding comparison of TAC in extracts from 14 plant species showed high correlation coefficients, r^2 = 0.98 for water-soluble extracts and r^2 = 0.88 for water-insoluble extracts. The ratio of TAC values obtained with the two methods (ABTS/FRAP) varied between 0.7 and 3.3 for different plant extracts indicating that they contained antioxidants with varying reactivity in the two methods. TACs in six pure antioxidant substances were ranked in the following order by both methods: quercetin > ferulic acid > catechin > rutin > caffeic acid > Trolox = chlorogenic acid. The two methods showed similar TAC values for quercetin, rutin, caffeic acid and chlorogenic acid while ferulic acid and catechin gave higher results with the ABTS method than with the FRAP method, and such differences probably explain the varying ratios of ABTS/FRAP obtained in foods. Regarding storage TAC in water-soluble strawberry extracts stored at –20 or –80°C was stable for at least five months while storage at 4°C decreased the TAC value with 40% during five weeks of storage. The study showed that both the ABTS and FRAP methods can be used for convenient monitoring of the antioxidant capacities in fruit and vegetables, and that different antioxidants had varying reactivity in the two methods..

Keywords: Antioxidant capacity / Antioxidant solubility / 2,2′-Azinobis-3-ethylbenzotiazoline-6-sulfonic acid / Ferric reducing antioxidant power / Molar reactivity

Received: July 14, 2004; revised: November 8, 2004; accepted: November 22, 2004

1 Introduction

Foods contain a number of antioxidants that have important functions both in the foods and as sources of bioactive compounds for the consumer. Since analysis of many antioxidants, especially so-called natural antioxidants in food, is a formidable task, a need has emerged to find simpler methods to assess the total antioxidant potential of different foods [1]. This would be a valuable tool to compare different cultivars of plant foods and food subjected to storage and processing [2, 3]. Recently, several methods for the measurement of antioxidant capacity in food extracts have been developed [4–8], but only few comparisons between them have been made. Some studies have indicated that different methods do not always give the same responses [7] pointing to the need for further development and characterization of the methods. The objectives of the present study was (i) to compare two methods for the assay of total antioxidant capacity (TAC), the 2,2′-azinobis-3-ethylbenzotiazoline-6-sulfonic acid (ABTS) method measuring the

Correspondence: Dr. Björn Åkesson, Biomedical Nutrition, Lund Institute of Technology, Lund University, P.O. Box 124, SE-22100 Lund, Sweden
E-mail: bjorn.akesson@kc.lu.se
Fax: +46-46-222-3853

Abbreviations: ABTS, 2,2′-azinobis-3-ethylbenzotiazoline-6-sulfonic acid); **FRAP**, ferric reducing antioxidant power; **fw**, fresh weight; **TAC**, total antioxidant capacity; **TPTZ**, 2,4,6-tris(2-pyridyl)-*s*-triazine; **Trolox**, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

scavenging of a free radical, and the ferric reducing antioxidant power (FRAP) method, measuring the potential to reduce ferric to ferrous ion. (ii) Since both hydrophilic and hydrophobic antioxidants are common, the two methods were evaluated both for the assay of water-soluble and to water-insoluble extracts from fruit and vegetables. (iii) The objective was to gain insight in the role played by different antioxidants in foods by also studying selected pure reference compounds.

2 Materials and methods

2.1 Chemicals

Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) 97%, TPTZ (2,4,6-tris (2-pyridyl)-*s*-triazine) >98%, ABTS, potassium persulfate (Sigma-Aldrich, St. Louis, MA, USA), ferric chloride (ICN Biomedicals, Costa Mesa, CA, USA), acetic acid (glacial, p.a), acetone (p.a) (Merck, Darmstadt, Germany), sodium acetate (BDH Chemicals, Poole, UK), and ethanol >95% (Kemetyl, Haninge, Sweden) were used for the analysis of TAC. The antioxidant substances (chlorogenic acid, quercetin, (+)-catechin, rutin, caffeic acid, ferulic acid) were purchased from Sigma-Aldrich or Extrasynthèse (Genay, France). Solutions of these compounds were prepared in ethanol at the concentrations of 20, 50, 100, 150, and 200 μmol/L for the ABTS assay and of 100–1000 μmol/L for the FRAP assay.

2.2 Plant materials

Vegetables, berries, and concentrates prepared from some plant species were provided by the following companies in Sweden: Findus R&D AB, Bjuv; Orkla Foods R&D, Eslöv; Kiviks Musteri AB, Kivik; Svalöf Weibull AB, Svalöv, and Skånemejerier, Malmö.

2.3 Sample preparation

Thawed or fresh vegetables, berries, and plant concentrates (5–100 g) were homogenized in a rotating blade mixer after the addition of buffer (sample : liquid ratio 1 : 1–1 : 6) or without any addition for approximately 2 min. The samples were then centrifuged in a Beckman centrifuge (JA21 rotor) at 26 000 × *g* for 30 min at 4°C. The supernatant was recovered and stored in aliquots at –80°C. One g of the remaining pulp was extracted with 8 mL acetone with occasional shaking for 30 min and then the mixture was centrifuged in a Beckman GPR Centrifuge at 1200 × *g* for 10 min at room temperature. The acetone supernatant was stored at –80°C until analyzed.

2.4 Test of different extraction procedures

To test the efficiency of the extraction technique, peas were chosen for an experiment with multiple extractions. One purpose was to study the possibility that water-soluble antioxidants contaminated the water-insoluble extracts. Thawed peas were homogenized in an equal weight of 0.1 mol/L sodium acetate buffer (pH 5.0) and the homogenate was centrifuged as described above. The supernatant was recovered, and 1 g of the pulp was taken away and from it the water-insoluble antioxidants were extracted twice with 8 mL acetone. Both acetone supernatants were saved. The remainder of the first pulp not extracted with acetone was homogenized a second time with buffer and after centrifugation, 1 g of this second pulp was extracted twice with acetone. The remainder of the second pulp was homogenized with acetate buffer a third time. In total, the water-soluble antioxidants were extracted three times and the water-insoluble ones were extracted twice. Strawberries, raspberries, puree made of strawberries or raspberries, and intermediate fractions from the processing of the purées were homogenized with sodium acetate buffer (pH 5.0) in normal air atmosphere and in the presence of nitrogen. To exclude the oxygen from the extraction process, both the buffer and the samples were flushed with nitrogen before homogenization. Nitrogen was also flushed over the sample during the homogenization. The homogenate was centrifuged as described above and TAC in the water-soluble fractions was analyzed with the FRAP method.

2.5 Effect of storage

To determine a suitable storage temperature for the sample supernatants, strawberry supernatants were stored at room temperature, in a refrigerator (4°C) or in freezers (–20 or –80 °C). The samples were analyzed with the FRAP method every second day during the first week, then once a week for five weeks, and after that once a month to a total storage time of five months.

2.6 Measurement of TAC

The methods used to assess TAC were the ABTS method [9] and a modification of the FRAP method [10]. Both are spectrophotometric methods and the absorbance readings were performed on the spectrophotometer model Ultrospec 3000 (Pharmacia Biotech, Uppsala, Sweden) at 25°C. As a standard compound Trolox was used, which is a water-soluble analogue of α-tocopherol. The stock solution contained 5 mmol/L of Trolox in ethanol, and it was stored at –20°C. A new standard curve was made for each assay and the data were expressed as Trolox equivalent antioxidant capacity, μmol/g fresh weight (fw).

2.6.1 The ABTS method

The ABTS method is a decolorization assay applicable to both lipophilic and hydrophilic antioxidants [9]. To oxidize the colorless ABTS to the blue-green $ABTS^{\bullet+}$ radical cation ABTS (7 mmol/L) was mixed with potassium persulfate (final concentration: 2.42 mmol/L) and kept for 12 – 16 h at room temperature in the dark. This reagent was stable for 2 – 3 days when stored in the dark. On the day of analysis the $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.70 (±0.02) at 734 nm. After the addition of 1.0 mL $ABTS^{\bullet+}$ solution to 100 µL of sample the mixture was stirred for 30 s and the absorbance reading was started after another 30 s and finished after 6 min. The readings were performed at 734 nm and 25°C. The % inhibition of the sample was then compared with a standard curve made from the corresponding readings of Trolox (20 – 200 µmol/L).

2.6.2 FRAP method

In the FRAP method the yellow Fe^{3+}-TPTZ complex is reduced to the blue Fe^{2+}-TPTZ complex by electron-donating substances under acidic conditions. Any electron donating substances with a half reaction of lower redox potential than Fe^{3+}/Fe^{2+}-TPTZ will drive the reaction and the formation of the blue complex forward. To prepare the FRAP reagent, a mixture of 0.1 mol/L acetate buffer (pH 3.6), 10 mmol/L TPTZ, and 20 mmol/L ferric chloride (10 : 1 : 1 v : v : v) was made. To 900 µL reagent 90 µL water and 30 µL sample were added. The absorbance readings were started immediately after the addition of sample, and they were performed at 593 nm with readings every 20 s for 10 min. The blank consisted of 120 µL water and 900 µL reagent. The final absorbance of each sample was compared with those obtained from the standard curve made from Trolox (100 – 1000 µmol/L).

2.7 Statistical calculations

Linear correlation coefficients were calculated using the SPSS program (Version 11.0).

3 Results

3.1 Reproducibility of the TAC assays

A low between-day imprecision was observed for both the ABTS method and the FRAP method when five concentrations of Trolox were analyzed in duplicates on ten days. The ABTS method showed coefficients of variation (CVs) of 4.8 – 7.8% for the five concentrations of Trolox (20 – 200 µmol/L) while the values for the FRAP method were 2.6 – 9.2% (100 – 1000 µmol/L). When five different concentrations of a water-soluble strawberry extract were analyzed in duplicates at three occasions with the ABTS assay, the results showed a good reproducibility (CV < 4%), and there was a good linearity ($r^2 = 0.99$) between sample concentration and % inhibition (Fig. 1). For concentrations giving high percentage inhibitions (approx. 80%) the curve became nonlinear and results obtained under such conditions were not included.

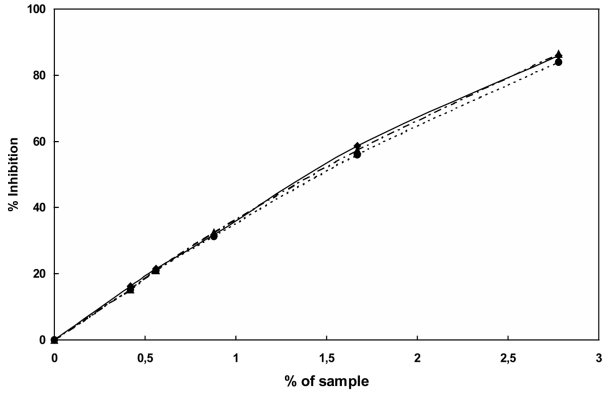

Figure 1. Reproducibility of the ABTS assay at analysis of five concentrations in duplicate of a strawberry extract on three occasions. (♦) Day 1 (r^2 = 0.99), (●) day 2 (r^2 = 0.99), (▲) day 3 (r^2 = 0.99)

3.2 Test of different extraction procedures

When peas were extracted three times with acetate buffer and twice with acetone, the TAC in the extract decreased with each extraction (Fig. 2). The first extraction with buffer recovered about 76%, the second 18%, and the last one 5% of the recovered water-soluble TAC (µmol/g peas). After the first and the second buffer extraction the pulp was extracted twice with acetone and in both cases about 80% of the recovered water-insoluble TAC was found in the first acetone extraction. The acetone-extractable TAC was similar in the first and second pellet. In another experiment homogenization of strawberries, raspberries, strawberry and raspberry purées, and intermediate fractions from the

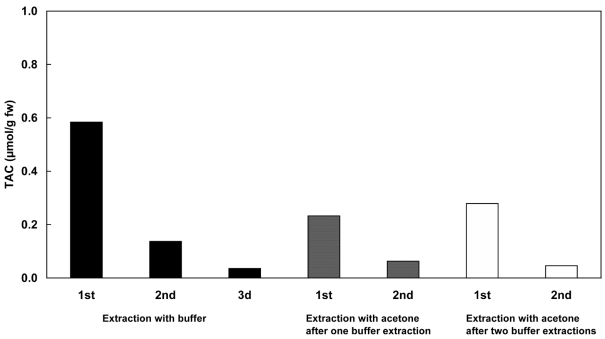

Figure 2. TAC in extracts from green peas extracted one, two, or three times with buffer and extracted once or twice with acetone.

processing of the berry purées was performed in either air or nitrogen but the use of nitrogen did not result in any differences in TAC values.

3.3 Effect of storage

Water-soluble extracts from one batch of strawberries were stored at different temperatures. There was no difference in TAC between the extracts stored at –20 or –80°C, and the TAC values were unchanged during five months of storage. For the extracts stored at 4°C the TAC value was constant during the first week and then decreased rapidly by 40% during the following two weeks. The TAC value in the extract stored at room temperature decreased by 30% during the first four days and the analyses were then discontinued. When five fruit- and berry-based products (three purées, one soup, and one beverage) where stored for eleven months at room temperature in their normal paper packages the TAC in all samples decreased. The percentage decreases of TAC in the products were between 15 and 42% during eleven months.

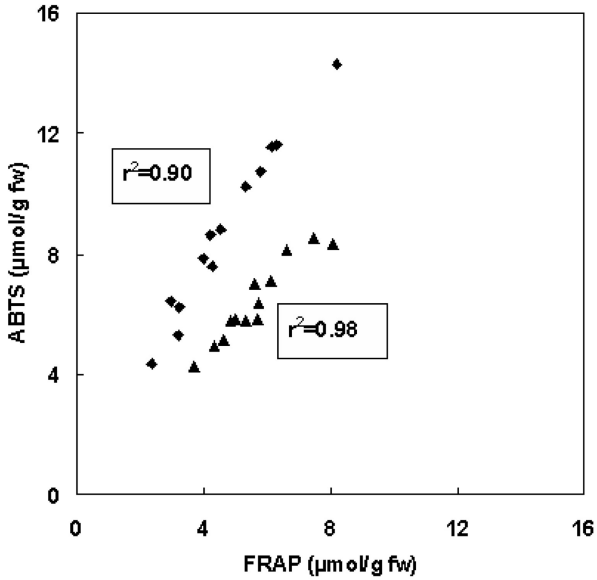

Figure 3. Relation between TAC measurements of 13 samples of strawberries using the FRAP method and the ABTS method. (▲) Water-soluble TAC, (♦) water-insoluble TAC.

3.4 Comparison of TAC assays

Several experiments were performed to compare the ABTS and FRAP assays. The water-soluble and water-insoluble extracts from 13 samples of strawberries were analyzed with both assays. All samples were analyzed on at least two occasions and the mean for each sample was used for calculating the correlation coefficient between results obtained by the two methods. The TAC results were highly correlated to each other in both the water-soluble and water-insoluble extracts (Fig. 3). For the water-soluble extracts r^2 was 0.905 ($p < 0.001$) and the ABTS/FRAP ratio ranged between 1.0 and 1.3. For the water-insoluble extracts r^2 was 0.98 ($p < 0.001$) and the ABTS/FRAP ratio ranged between 1.7 and 2.2.

Table 1. Comparison of TAC (µmol/g fw) in different extracts from plant species as measured by the ABTS and FRAP methods in water-soluble and water-insoluble extracts

Sample	Water-soluble			Water-insoluble		
	ABTS	FRAP	Ratio ABTS/FRAP	ABTS	FRAP	Ratio ABTS/FRAP
Rosehip	1000 (52)	632 (48)	1.6	–[b]	–[b]	–
Acerola	494 (11)	434 (35)	1.1	–[b]	–[b]	–
Spinach	92.4 (4.7)	30.0 (0.8)	3.1	0.59 (0.09)	0.48 (0.05)	1.2
Red cabbage	54.2 (2.6)	30.6 (1.5)	1.8	0.83 (0.01)	0.87 (0.02)	1.0
Orange	37.4 (1.9)	18.8 (3.2)	2.0	0.97 (0.15)	0.85 (0.01)	1.1
Lemon	29.6 (7.0)	16.2 (2.8)	1.8	0.77 (0.05)	0.93 (0.02)	0.8
Guava	16.9 (0.3)	10.2 (1.0)	1.7	2.82 (0.19)	1.99 (0.02)	1.4
Carrot	17.2 (0.5)	9.3 (0.6)	1.9	0.70 (0.05)	0.29 (0.00)	2.4
Wheat germs	10.6 (0.3)	6.7 (0.7)	1.6	12.90 (0.1)	3.89 (0.01)	3.3
Banana	7.6 (0.1)	3.0 (0.3)	2.5	1.38 (0.06)	0.88 (0.03)	1.6
Mango	4.0 (0.1)	1.67 (0.2)	2.4	1.35 (0.06)	0.61 (0.01)	2.2
Spinach[a]	2.0	1.89	2.3	1.63	1.35	1.2
Tomato[a]	1.1	0.87	1.2	0.11	0.16	0.7
Carrot[a]	0.34	0.31	1.1	0.05	0.05	1.0

a) Fresh sample
b) No pulp was recovered.
Data are expressed as mean (SD), for fresh vegetables as means of two observations.
All extracts were analyzed in duplicates on three occasions.

In another comparison of the TAC methods, concentrates from 11 plant species (guava, rosehip, acerola, spinach, carrot, mango, orange, lemon, red cabbage, wheat germs, and banana) together with three fresh vegetables (carrot, tomato, spinach) were assayed for their water-soluble and water-insoluble TAC (Table 1). There was a high correlation between the results from the two methods both for the water-soluble TAC, $r^2 = 0.88$ ($p < 0.001$) and the water-insoluble TAC, $r^2 = 0.86$ ($p < 0.001$). To avoid the possibility that single high values had a large effect on the correlation, the values for water-soluble TAC in rosehip and acerola and the water-insoluble TAC in wheat germs were excluded from these calculations. The ABTS/FRAP ratio between the water-soluble TAC values obtained with the two methods ranged between 1.1 and 3.1 and for the water-insoluble TAC between 0.7 and 3.3. There were no obvious similarity for each sample between the ratios from the water-soluble and the water-insoluble TAC measurements. This indicates that the water-insoluble and water-soluble extracts from plant materials contained varying proportions of antioxidants with different activities in the FRAP and ABTS methods.

3.5 TAC in six antioxidant reference substances

Six commercially available antioxidant substances (chlorogenic acid, quercetin, catechin, rutin, caffeic acid, and ferulic acid) were analyzed with the ABTS and FRAP assays (Table 2). Since the ABTS method lacked linearity for the higher concentrations of test compounds, the TAC values, expressed as µmol/µmol, from the lowest concentrations were used for the comparison. The two methods gave similar TAC values for chlorogenic acid, caffeic acid, rutin, and quercetin and the ratios between results from the two methods were in most cases close to 1 (Table 2). Ferulic acid and catechin gave higher results with the ABTS method than with the FRAP method.

Table 2. TAC in antioxidant substances as measured by the ABTS and the FRAP methods

Substance	Molar TAC (µmol/µmol) ABTS	Molar TAC (µmol/µmol) FRAP	ABTS/FRAP ratio
Quercetin	3.74	3.73	1.0
Ferulic acid	3.51	1.40	2.5
Catechin	3.30	1.26	2.6
Rutin	1.45	1.17	1.2
Caffeic acid	1.18	1.13	1.0
Chlorogenic acid	1.00	0.99	1.0

All solutions were analysed in duplicates on three occasions.

4 Discussion

4.1 Comparison of the ABTS and FRAP assays

Most pure antioxidant substances analyzed with the FRAP and ABTS methods in the present study had a higher molar activity than Trolox in the following rank order: quercetin > ferulic acid > catechin > rutin > caffeic acid > Trolox = chlorogenic acid (Table 3). The results from the two methods were well correlated except that for ferulic acid and catechin a higher TAC value was obtained with the ABTS method than with the FRAP method (Table 2). Although no previous study of these compounds by both the ABTS and FRAP methods seems to have been performed, several investigations using one of the methods have been done (Table 3). Most studies showed that quercetin had a higher activity than the other compounds with any of the methods. There were some variations in the TAC values between methods and among studies which can have several explanations. In one study using the FRAP method prolongation of the reading time from 4 to 30 min resulted in increased TAC values for all the antioxidants studied but the increases were of different magnitudes [11]. The same tendency was shown [12] when comparing TAC of various antioxidants

Table 3. TAC in flavonoids and phenolic acids as analyzed with the ABTS and the FRAP methods in different studies

Method / Substance	FRAP (µmol/µmol)				ABTS (µmol/µmol)		
	(Pulido *et al.* 2000)		(Hunter *et al.* 2002)	Present study	(Miller *et al.* 1995)	(Re *et al.* 1999)	Present study
Quercetin	2.55[a)]	3.50[b)]	1.51	3.73	4.72	3.10	3.74
Ferulic acid	0.83[a)]	1.01[b)]	1.22	1.40	1.90	1.90	3.51
Catechin	0.93[a)]	1.68[b)]	1.75	1.26	2.40	–	3.30
Rutin	1.02[a)]	1.92[b)]	0.87	1.17	2.42	–	1.45
Caffeic acid	1.64[a)]	2.32[b)]	1.65	1.13	1.26	0.98	1.18
Trolox	1.00[a)]	1.00[b)]	1.00[c)]	1.00	1.00	1.00	1.00
Chlorogenic acid	–	–	–	0.99	1.24	–	1.00

a) 4 min reading time
b) 30 min reading time
c) α-Tocopherol was studied instead of Trolox.
Results were recalculated to allow a comparison so that Trolox had a TAC-value of 1.00 µmol/µmol.

using 10 s and 6 min as reaction times. In the present study 10 min was chosen as reading time since only little increase in absorbance occurred after this time point. Another explanation for the variation between methods and studies can be the solvent used. Several studies have shown that the TAC values are dependent on the solvent used but the dependence varies among antioxidants [11 – 13].

The molar reactivity of different antioxidants in TAC methods may also reflect the variation in molecular structure. The ABTS method measures the ability of hydrogen-donating antioxidants to scavenge the $ABTS^{\bullet+}$ radical cation. A hierarchy of antioxidant potential among flavonoids and phenolic acids related to molecule structure has been documented previously [14]. Quercetin and catechin have the same number of hydroxyl groups located at the same positions but the C-ring in the catechin is saturated while that in quercetin has one double bond in the 2,3-position. The double bond structure allows the electrons to delocalise across the molecule giving quercetin a higher antioxidant potential than catechin. Glycosylation of the 3-hydroxyl group in the C-ring of quercetin resulting in rutin, which has a lower antioxidant potential in agreement with previous data [14]. Also elimination of the 3-hydroxyl group (as in luteolin) decreases TAC substantially [14]. The antioxidant capacity of the hydroxycinnamic acids (caffeic acid, ferulic acid, and chlorogenic acid) depends generally on the number of hydroxyl groups and the influence of the carboxylate group on the hydrogen donating abilities [14]. Substitution of the 3-hydroxyl group of caffeic acid by a methoxy group to ferulic acid enhanced TAC, confirming previous results [14]. These considerations on the activity of individual antioxidants with the ABTS method were thus confirmed in the present study and moreover the same order in TAC among the substances was noticed using the FRAP method.

4.2 Role of different antioxidants for TAC

In the present study, data from the analysis of strawberry extracts with the two methods correlated well and for the water-insoluble TAC the results from the ABTS method were somewhat higher than with the FRAP method. Strawberries contain a wide range of antioxidants, *e.g.*, ascorbic acid, ellagic acid, and phenolic acids. The most probable reason for the higher activity in the water-insoluble extract using the ABTS method was that these extracts contained a high proportion of antioxidants with a higher activity in the ABTS method than in the FRAP method. Among the water-insoluble antioxidants, strawberries are rich in ellagic acid (1.3 – 1.9 μmol/g fw) [15] but also contain *p*-coumaric acid (0.04 – 1.04 μmol/g fw) [16]. No TAC values on ellagic acid measured with the FRAP or ABTS methods have been found in the literature but in a parallel study of strawberries the ellagic acid content correlated well with the water-insoluble TAC [17]. The molar TAC values for *p*-coumaric acid was shown earlier to be 2.00 with the ABTS method [9] but only 0.16 with the FRAP method [13] and thus *p*-coumaric acid could account for a sizeable part of the TAC value obtained by the ABTS method if its content in strawberries is in the higher range. Regarding the water-soluble TAC strawberries contain a high amount of ascorbic acid, 2.4 – 2.7 μmol/g fw [18], and it probably contributes appreciably to TAC values obtained by both methods since it has a similar molar TAC value in both methods, 1.05 in the ABTS method [9] and 0.98 in the FRAP method [13]. Further support for an important role of ascorbic acid for water-soluble TAC was found in another study where the TAC values in the water-soluble extracts of strawberries correlated well with the amount of ascorbic acid in the same samples [17]. Another possibility is that synergy effects or other interactions among antioxidants may affect the results and give rise to different activities in the ABTS and FRAP methods. Further studies are necessary to verify the importance of this hypothesis.

4.3 Assay performance

In different studies on TAC in food samples a wide range of extraction techniques have been used. In the present study, the water-soluble antioxidants were extracted with acetate buffer (pH 5.0) and the water-insoluble antioxidants remaining in the pellet after the centrifugation of the buffer homogenate were extracted with acetone for 30 min. It was shown that extraction of strawberries, white grapes and oranges with acetone for 30 min resulted in much higher TAC-values than extraction for 2 min, determined with the oxygen radical absorbance capacity (ORAC) method [5]. Prolonging the extraction time to 1 and 4 h only increased the TAC-values in the white grape extracts and even decreased the values in the strawberry and orange extracts. In another study, combined extraction of vegetable soups with sodium phosphate buffer and ethyl acetate was performed and the contribution of hydrophilic and lipophilic compounds to the TAC was evaluated [19]. Usually the hydrophilic antioxidants dominated over the lipophilic ones, and ascorbic acid and carotenoids were important compounds in the respective phases. A large screening study of TAC in dietary plants [20] used methanol for the sample extractions but no comparison with other extraction procedures was made. More comparative studies of different extraction procedures are necessary.

Even if the ABTS and FRAP methods showed good performance with the samples analyzed in the present study, the use of one-dimensional methods may be misleading using samples with more complex structures, such as emulsions [21]. Application of these methods on different fractions of milk showed that proteins often interfered with the

FRAP method and that many factors affected the reactivity of milk using the ABTS method [22]. Also when applied on human plasma, different results were obtained by the ABTS, ORAC, and FRAP methods although there was a weak correlation between data obtained using the two latter methods [23]. In contrast, widely varying correlation coefficients between data obtained with the FRAP and ORAC methods were found in extracts from several vegetables [24]. Another factor which can influence the TAC of flavonoids is the interaction with proteins [25]. It is evident that the choice of the TAC method to be used must be done carefully taking into account the composition of the sample matrix and related factors.

4.4 Conclusions

Both the FRAP and the ABTS method evaluated in the present study are reliable, fast, and easy-to-handle methods. They offer great advantages in monitoring total antioxidant capacity compared to analysis of all the individual antioxidant components in food samples. The results were very reproducible in comparisons of samples containing the same types of antioxidants, *e.g.*, different cultivars of fruit and vegetables or in following TAC in foods during storage. As shown for the first time in the present study, the relative reactivity in the ABTS and FRAP methods may also give some guidance on which antioxidants that are the main contributors to TAC.

The study was supported by the Swedish Research Council for Environment, Agricultural Science, and Spatial Planning (project No. 50.0331/97), the Swedish Agency for Innovation Systems (project No. P13652-1), Lund University Hospital and the Physiographic Society at Lund University. We thank representatives from different companies for providing us with food samples, especially Annika Nilsson, Lena Nyberg, Rolf Stegmark, and Ulla Svensson. We also thank Kerstin Svensson, Eva Sjöström, and Margareta Johnsson for skilful laboratory work.

5 References

[1] Halliwell, B., Gutteridge, J. M. C., The definition and measurement of antioxidants in biological systems. *Free Radic. Biol. Med.* 1995, *18*, 125 – 126.

[2] Ewald, C., Fjelkner-Modig, S., Johansson, K., Sjöholm, I., Åkesson, B., Effect of processing on flavonoids in processed onions, green beans, and peas. *Food Chem.* 1991, *64*, 231 – 235.

[3] Viberg, U., Alklint, C., Åkesson, B., Önning, G., Sjöholm, I., The effect of processing on total antioxidative capacity in strawberries, in: Kumpulainen, J. T., Salonen, J. T. (Eds.), *Natural antioxidants and anticarcinogens in nutrition, health and disease*, The Royal Society of Chemistry, Cambridge 1999, pp. 338 – 340.

[4] Miller, N. J., Diplock, A. T., Rice-Evans, C. A., Evaluation of total antioxidant activity as a marker of the deterioration of apple juice on storage. *J. Agric. Food Chem.* 1995, *43*, 1794 – 1801.

[5] Wang, H., Cao, G., Prior, R. L., Total antioxidant capacity of fruits. *J. Agric. Food Chem.* 1996, *44*, 701 – 705.

[6] Cao, G., Sofic, E., Prior, R. L., Antioxidant capacity of tea and common vegetables. *J. Agric. Food Chem.* 1996, *44*, 3426 – 3431.

[7] Plumb, G. W., Chambers, S. J., Lambert, N., Bartolome, B., *et al.*, Antioxidant actions of fruit, herb and spice extracts. *J. Food Lipids* 1996, *3,* 171 – 188.

[8] Velioglu, Y. S., Mazza, G., Gao, L., Oomah, B. D., Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J. Agric. Food Chem.* 1998, *46*, 4113 – 4117.

[9] Re, R., Pellegrini, N., Proteggente, A., Pannala, A., *et al.*, Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* 1999, *26*, 1231 – 1237.

[10] Benzie, I. F. F., Strain, J. J., The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal. Biochem.* 1996, *239*, 70 – 76.

[11] Pulido, R., Bravo, L., Saura-Calixto, F., Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *J. Agric. Food Chem.* 2000, *48*, 3396 – 3402.

[12] van den Berg, R., Haenen, G. R. M. M., van den Berg, H., Bast, A., Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. *Food Chem.* 1999, *66*, 511 – 517.

[13] Hunter, K. J., Fletcher, J. M., The antioxidant activity and composition of fresh, frozen, jarred and canned vegetables. *Innov. Food Sci. Emerg. Technol.* 2002, *3*, 399 – 406.

[14] Rice-Evans, C. A., Miller, N. J., Paganga, G., Structure-antioxidant activity relationship of flavonoids and phenolic acids. *Free Radic. Biol. Med.* 1996, *20*, 933 – 956.

[15] Häkkinen, S. H., Törrönen, A. R., Content of flavonols and selected phenolic acids in strawberries and *Vaccinium* species: Influence of cultivar, cultivation site and technique. *Food Res. Int.* 2000, *33*, 517 – 524.

[16] Häkkinen, S., Heinonen, M., Kärenlampi, S., Mykkänen, H., *et al.*, Screening of selected flavonoids and phenolic acids in 19 berries. *Food Res. Int.* 1999, *32*, 345 – 353.

[17] Olsson, M. E., Ekvall, J., Gustavsson, K. E., Nilsson, J., *et al.*, Antioxidants, Low-Molecular Weight Carbohydrates and Total Antioxidant Capacity in Strawberries (*Fragaria x ananassa)* – Effects of Cultivar, Ripening and Storage. *J. Agric. Food Chem.* 2004, *52*, 2490 – 2498.

[18] Häkkinen, S. H., Kärenlampi, S. O., Heinonen, I. M., Mykkänen, H. M., Törrönen, A. R., Content of the flavanols quercetin, myricetin and kaempferol in 25 edible berries. *J. Agric. Food Chem.* 1999, *47*, 2274 – 2279.

[19] Arnao, M. B., Cano, A., Acosta, M., The hydrophilic and lipophilic contribution to total antioxidant activity. *Food Chem.* 2001, *73*, 239 – 244.

[20] Halvorsen, B. L., Holte, K., Myhrstad, M. C. W., Barikmo, I., *et al.*, A systematic screening of total antioxidants in dietary plants. *J. Nutr.* 2002, *132*, 461 – 471.

[21] Frankel, E. N., Meyer, A. S., The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *J. Sci. Food Agric.* 2000, *80*, 1925 – 1941.

[22] Chen, J., Lindmark-Månsson, H., Gorton, L., Åkesson, B., Antioxidant capacity of bovine milk as assayed by spectrophotometric and amperometric methods. *Int. Dairy J.* 2003, *13*, 927 – 935.

[23] Prior, R. L., Cao, G., *In vivo* total antioxidant capacity: Comparison of different analytical methods. *Free Radic. Biol. Med.* 1999, *27,* 1173 – 1181.

[24 Ou, B., Huang, D., Hampsch-Woodill, M., Flanagan, J. A., Deemer, E. K., Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: A comparative study. *J. Agric. Food Chem.* 2002, *50*, 3122 – 3128.

[25] Arts, M. J. T. J., Haenen, G. R. M. M., Voss, H.-P., Bast, A., Masking of antioxidant capacity by the interaction of flavoniods with protein. *Food Chem. Toxicol.* 2001, *39*, 787 – 791.

