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The effect of solvent extraction on antioxidant properties of apricot fruit

Research Article

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Abstract: Two solvent extraction procedures were used to investigate the extraction efficiency in terms of total antioxidant capacity and total phenols in apricot fruit. Samples were either sequentially extracted with aqueous ethanol (ethanol/water 80% v/v) and tetrahydrofuran or directly extracted with tetrahydrofuran. Each extract was analyzed for total antioxidant capacity by the Trolox Equivalent Antioxidant Capacity (TEAC) assay and total phenols by the Folin-Ciocalteu assay. The results showed that using sequential solvent extraction, the majority (85%) of the total antioxidant capacity and total phenols was due to hydrophilic compounds. In tetrahydrofuran direct extractions, the total antioxidant capacity and total phenols were higher than values obtained with aqueous ethanol and the sum of results obtained from sequential extracts for either total antioxidant capacity or total phenols was similar to the tetrahydrofuran-extract antioxidant values. A linear correlation between total antioxidant capacity and total phenols was found and was independent of the solvent extraction method. In conclusion, the choice of solvent is related to the antioxidant potential of fruit and depends on the food hydrophilic/lipophilic composition.

Keywords: *Antioxidant capacity • Solvent extraction • Fruit quality • Prunus armeniaca L.*

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1. Introduction

A daily-dose of antioxidants from fruit and vegetable consumption is recommended for a healthy life-style. Fruits and vegetables are rich in antioxidant compounds able to act against free radicals that are generated in human cells. The more plant foods are preferred in a daily diet, the more antioxidant compounds are consumed. Many studies have focused on the effect of bioactive compounds in the prevention of several diseases [1-4].

The antioxidant profile of apricot fruit (*Prunus armeniaca* L.) has recently been analysed with particular reference to the total antioxidant capacity, polyphenols and carotenoids [5-9]. Given that a diverse germplasm exists for this species, the effect of the genotype on antioxidant properties has been considered, and the nutraceutical profiles of many cultivars have been studied in order to find outstanding genotypes rich in antioxidant compounds [10-12].

Overall, apricot fruit has great nutritional value because of fibre, minerals (potassium, calcium, iron, magnesium, zinc, phosphorus and selenium) [13,14], and low energy intake (50 Kcal/100 g fresh weight) that combined with the nutraceutical plus-value (vitamin C, A, carotenoids, phenols, thiols, thiamin, riboflavin, niacin and pantothenic acid) make apricots '*healthy & easy-to-eat*'. The total antioxidant capacity of this fruit has been measured, especially in the hydrophilic fruit extracts, which mainly contain polyphenols and vitamins. Many phenols have been identified in apricots [8,15,16] that are also known to be a rich source of carotenoids, which are important compounds for the eyes and skin. Recent investigations of the antioxidative effect of carotenes such as phytoene and phytofluene have suggested that they may be part of the oxidative stress defence system in human tissues [17,18]. The influence of solvent extraction on phenolic content and total antioxidant capacity has been reported in fruit and vegetable

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species [19-23]; however, the antioxidant power of lipophilic extracts from apricots and their contribution to the total antioxidant capacity has not been widely investigated and in many cases the reported total antioxidant capacity has only referred to the hydrophilic antioxidant level. Given that apricot fruit is an important source of liposoluble compounds such as carotenes, it could be useful to evaluate the contribution of lipophilic extracts to the total antioxidant properties in mature fruit. We have investigated the total antioxidant capacity and total phenols either in sequential aqueous ethanol and tetrahydrofuran solvent extractions or directly in tetrahydrofuran extracts in mature fruits of some apricot cultivars.

2. Experimental Procedures

2.1 Cultivars and fruit sampling

Seven apricot cultivars were used: six Italian germplasms ('Bona', 'Pisana', 'Boccuccia spinosa', 'Silvana', 'Ammiraglia' and 'Sillari') and one North American cultivar ('Harcot'). Fruits were harvested at physiological maturity (ready-to-eat) from the experimental fields of Department of Trees Science, Entomology and Plant Patology 'G. Scaramuzzi', University of Pisa located in Venturina-Livorno, Italy (altitude 6 m, lat. 43.02N, long. 10.36E).

For each cultivar, 30 fruits were randomly collected and samples of 3g (for three independent replicates) of fresh material (pulp and peel) were immediately frozen in liquid nitrogen and stored at –80°C until extraction.

2.2 Extraction procedure

Two procedures were tested (see below). The absorbance of each extract was measured at two wavelengths (280 and 450 nm) against a blank (extraction solvent) using a UV-Vis spectrophotometer in order to measure the absorbance ratios between the extracts obtained with aqueous and organic solvents.

2.2.1 Sequential extraction (extraction 1)

This procedure was made on apricot samples from seven cultivars 'Bona', 'Pisana', 'Boccuccia spinosa', 'Silvana', 'Ammiraglia', 'Harcot' and 'Sillari'. For each cultivar, three independent samples of 3 g were homogenized for 3 min at 4°C using an ultra-Turrax T-25 (Ika, Staufen, Germany) to avoid oxidation and extracted first with 9 ml of aqueous ethanol (ethanol and water, 80% v/v) (Et_{extr1}) for 30 min in a shaker in the dark. The supernatant was collected after centrifugation at 2600 x g for 10 min at 2-4°C. The pellet was re-extracted with 9 ml of tetrahydrofuran (THF_{extr1}) following the above procedure and the supernatant was collected. The supernatants were immediately analysed for the total antioxidant capacity and total phenols.

2.2.2 Extraction by tetrahydrofuran (extraction 2)

This procedure was made on apricot samples from three cultivars, 'Bona', 'Pisana' and 'Boccuccia Spinosa'. Three independent samples of 3 g were directly extracted as indicated above with 9 ml of THF (THF_{extr2}). The supernatant was collected and immediately analysed for the total antioxidant capacity, total phenols and total carotenoid content.

2.3 Total Antioxidant Capacity (TAC), Total Phenols (TP) and total carotenoids analysis

2.3.1 TAC assay

Total antioxidant capacity was evaluated using the improved Trolox Equivalent Antioxidant Capacity (TEAC) method [24]. The TEAC value was calculated in relation to the reactivity of Trolox, a water-soluble vitamin E analogue which was used as an antioxidant standard (calibration curve y=0.030x, R^2=0.997). In the assay, 40 µl of the diluted samples, controls or blanks were added to 1960 µl $ABTS^{\bullet+}$ solution, which resulted in a 20-80% inhibition of the absorbance. The decrease in absorbance at 734 nm was recorded at 6 min after an initial mixing, and plotted against a dose-response curve calculated for Trolox (0-30 µM). Antioxidant activity was expressed as micromoles of Trolox equivalents per gram of fresh fruit weight (µmol TE g FW^{-1}).

2.3.2 TP assay

Total phenolic content was determined according to the improved Folin-Ciocalteu (F-C) method [25]. This assay provides a rapid and useful indication of the antioxidant status of the studied material, and has been widely applied to different food samples. Gallic acid (GA) was used as a standard compound (0-500 mg/L) for the calibration curve (y=0.098x, R^2=0.998). Total phenol content was calculated as milligrams of GA equivalent (GAE) per gram of fresh fruit weight (mg GAE g FW^{-1}). The absorbance of the blue coloured solutions was read at 765 nm after incubation for 2 h at room temperature.

2.3.3 Total carotenoid content (expressed as β-carotene content)

Total carotenoid content was determined from extraction 2 and measured by the simple spectrophotometric method as described by the Nagata and Yamashita equation [26].

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2.4 Statistical analyses

Data were reported as means of three independent determinations. Student's t-test was used to compare data from different extraction procedures. Analysis of variance (ANOVA) and Pearson's correlation were performed using statistical package Statistica (StatSoft, Turla, USA). Differences between means from ANOVA were considered statistically significant at $P \leq 0.05$ according to Newman-Keuls test.

3. Results and Discussion

The total antioxidant capacity in the aqueous ethanol extracts ranged from 1.49 to 11.72 µmol TE g FW^{-1} and the subsequent THF extracts showed a TAC value from 0.5 to 1.53 µmol TE gFW^{-1} (Table 1). Total phenols in aqueous ethanol ranged from 0.35 to 1.81 mg GAE g FW^{-1} and in THF from 0.077 to 0.257 mg GAE g FW^{-1} (Table 1). The range of variation for both variables was much higher in aqueous ethanol extracts compared with sequential THF. ANOVA results for TAC and TP from aqueous ethanol extractions differentiated cultivars better than the subsequent THF extracts. Overall, the 'Ammiraglia' cultivar achieved the highest TAC and TP values while 'Boccuccia Spinosa' achieved the lowest values independently of the solvent used (Table 1).

TAC and TP values were much higher in the aqueous ethanol extracts than the less polar solvent (THF) and nearly 85% of the total antioxidant capacity was due to the hydrophilic TAC. Scalzo *et al.* [9] performed sequential extractions with methanol/water (80% v/v) and acetone on fruit samples of the 'San Castrese' apricot cultivar and found that the lipophilic extracts contributed 7% of the total antioxidant capacity. Pellegrini *et al*. [27] reported that tomato lipid-soluble antioxidants in the acetone extracts for TAC determination were lower than those obtained from THF extraction, which is more efficient than acetone at extracting carotenoids. In view of these results, the higher percentage of the lipophilic TAC contribution, nearly 15%, to the total TAC obtained in our study, compared to published results, is likely related to the specific solvent efficiency for less polar compounds such as carotenes in apricot fruit.

Pearson's correlation coefficients among hydrophilic/lipophilic TAC and TP and total carotenoids were statistically significant. A strong correlation between TAC and TP values was found and was independent of the solvent extraction (r=0.99 between TAC and TP in aqueous ethanol and r=0.98 between TAC and TP in tetrahydrofuran). Lipophilic TAC was more strongly correlated with total less polar phenols (r=0.98) than total carotenoids (r=0.44).

For 'Bona', 'Pisana' and 'Boccuccia spinosa' cultivars, TAC and TP were measured in fruit samples extracted directly with THF (see Extraction 2 in Experimental Procedures) which was used in parallel with extraction 1 (sequence of solvents aqueous ethanol and tetrahydrofuran). In Table 2, Student's t-test results between Total TAC/TP values (which are the sum of aqueous ethanol TAC/TP and subsequent tetrahydrofuran TAC/TP) and TAC/TP values from

Cultivar	$TAC_{Et\ extr1}$ µmol TE g FW^{-1}	$TAC_{THF\ extr1}$ µmol TE g FW^{-1}	$TP_{Et\ extr1}$ mg GAE g FW^{-1}	$TP_{THF\ extr1}$ mg GAE g FW^{-1}	Tot car* mg kg^{-1}
B. Spinosa	1.49 (74.9) f	0.5 (25.1) d	0.35 (82) f	0.077 (18) d	9.79 b
Pisana	3.02 (83.9) e	0.58 (16.1) d	0.59 (86.3) ef	0.094 (13.7) d	7.55 c
Bona	3.58 (80.1) e	0.89 (19.9) c	0.665 (83.2) de	0.134 (16.8) c	14.90 a
Sillari	4.69 (83.8) d	0.91 (16.2) c	0.78 (81.7) d	0.175 (18.3) b	12.90 a
Harcot	6.86 (84.7) c	1.24 (15.3) b	1.15 (84.3) c	0.214 (15.7) ab	14.30 a
Silvana	8.26 (86.2) b	1.32 (13.7) b	1.31 (86) b	0.213 (14) ab	13.65 a
Ammiraglia	11.72 (88.5) a	1.53 (11.5) a	1.81 (87.6) a	0.257 (12.4) a	10.31 b
Mean	5.66 (85.1)	0.99 (14.9)	0.95 (84.8)	0.17 (15.2)	11.91

Table 1. Total antioxidant capacity (TAC, µmol TE g FW^{-1}) and total phenols (TP, mg GAE g FW^{-1}) in both aqueous ethanol (EtOH 80%) and tetrahydrofuran (THF) fractions from sequential extractions in seven cultivars of apricot fruit at ready-to-eat stage. Total carotenoid content, (Tot car, mg kg^{-1}) from extraction 2 by tetrahydrofuran. The % contribution of aqueous ethanol and tetrahydrofuran to the total value of TAC and TP are given in parentheses. Values are expressed as micromoles of Trolox Equivalent per gram of fresh weight (µmol TE g FW^{-1}) and milligrams of Gallic Acid Equivalent per gram of Fresh Weight (mg GAE g FW^{-1}).

One-way ANOVA. Letters indicate different means by Newman-Keuls test, P<0.05
**Total carotenoid content was determined from extraction 2 by tetrahydrofuran*

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independent THF extracts were reported. Small differences in total antioxidant capacity compared with independent THF were shown and they were not observed for TP content. In the lower part of Table 2, the absorbance readings at 280 nm and 450 nm wavelengths were reported as ratios between different solvents in order to highlight the effect of solvent at extracting phenolics and carotenoids. In particular, in the sequential extraction procedure, the antioxidant compounds were mostly extracted with the first aqueous ethanol solvent, as shown from TAC and TP values, while the 8-13 fold higher reading-values obtained at 450 nm in THF than aqueous ethanol did not consistently increase the respective TAC level.

In order to find possible relationships between TAC and TP, a regression analysis was performed for each solvent. A good linear relationship between TAC and TP was found and was independent from the solvent extraction (Figure 1). Phenolic compounds either more or less polar seem to mainly contribute to the total antioxidant capacity when measured by the TEAC assay.

Variables	Cultivar		
	B. Spinosa	Pisana	Bona
Total TAC	1.99	3.60	4.47
$TAC_{THF\ extr2}$	1.86	3.88	4.00
t-test (TotTAC *vs.* $TAC_{THF\ extr2}$)	NS	*	*
Total TP	0.43	0.68	0.79
$TP_{THF\ extr2}$	0.40	0.78	0.76
t-test (TotTP *vs.* $TP_{THF\ extr2}$)	NS	NS	NS
	Absorbance readings ratio at 280 and 450 nm		
$Abs280_{Et\ extr1/THF\ extr1}$	4.16	4.33	3.85
$Abs280_{THF\ extr2/THF\ extr1}$	6.52	6.17	4.86
$Abs280_{THF\ extr2/Et\ extr1}$	1.57	1.42	1.26
$Abs450_{THF\ extr1/Et\ extr1}$	8.62	7.94	12.7
$Abs450_{THF\ extr2/THF\ extr1}$	1.27	1.12	1.12
$Abs450_{THF\ extr2/Et\ extr1}$	9.25	8.94	14.28

Table 2. Fruit total antioxidant capacity (TAC, μmol TE g FW^{-1}), total phenols (TP, mg GAE g FW^{-1}) in the sequential extractions with aqueous ethanol and tetrahydrofuran (Et_{extr1}/THF_{extr1}) and directly in tetrahydrofuran (THF_{extr2}) and absorbance readings ratio between extraction solvents at wavelengths 280 and 450 nm in three apricot cultivars, 'Boccuccia Spinosa', 'Pisana' and 'Bona'. Student's t-test was performed between total TAC and TAC THF_{extr2} as well as total TP and TP THF_{extr2} ($P<0.05$). Values are expressed as micromoles of Trolox Equivalent per gram of fresh weight (μmol TE g FW^{-1}) and milligrams of Gallic Acid Equivalent per gram of Fresh Weight (mg GAE g FW^{-1}).

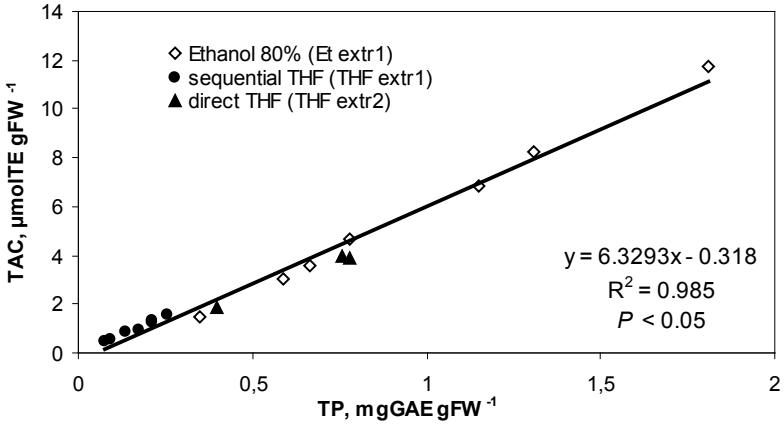

Figure 1. Relationship between total antioxidant capacity (TAC) and total phenols (TP) of apricot fruit in two extraction solvents (aqueous ethanol and tetrahydrofuran).

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4. Conclusions

In apricot fruit, carotenes and polyphenols are important antioxidant compounds which contribute to the total antioxidant properties of mature fruit. Both groups of compounds have important roles either in fruit growth and ripening or for human nutrition. A range of variation of antioxidant attributes related to the genotype was shown and some genotypes studied herein achieved high values of the antioxidant attributes useful for future breeding programs whose main objective is fruit quality improvement.

If a sequence of solvents is used, in particular aqueous ethanol and subsequent tetrahydrofuran as in the present study, the majority (85%) of the total antioxidant capacity measured by the TEAC assay was due to hydrophilic compounds. Polyphenols, more than carotenes, likely contribute to the total antioxidant capacity in mature apricot fruit. However, lipophilic TAC contributes to the total TAC, which might be due either to carotenes or less polar phenolic compounds. Therefore, the characterization of individual compounds that contribute to the observed bioactivity has to be considered as the next step towards the qualitative and quantitative chemical characterization of the individual components responsible for the nutraceutical properties of apricot fruit. In regards to the efficiency of extraction between aqueous ethanol and tetrahydrofuran, when made in parallel on apricot samples, the ability of THF in terms of extracted total phenols and total antioxidant capacity is slightly higher than in aqueous ethanol but much more efficient at extracting carotenes. Despite this solvent effect on the antioxidant properties of apricot fruit, a linear correlation between TAC and TP was shown and was independent of the extraction method. Similarly, Chang and Liu [20] reported a higher antioxidant level in THF than aqueous extracts in four varieties of tomato fruit. The fact that less polar THF extract had higher activity in the TEAC mechanism might be due to a specific additive/synergistic antioxidant effect of the extracted bioactive constituents as suggested by Oboh *et al.* [23]. In conclusion, the total antioxidant capacity measurements from a sequential extraction solvent procedure with aqueous ethanol and tetrahydrofuran is likely appropriate for apricot fruit. Overall, a sequence of extraction solvents with different polarities is indicated to be an efficient procedure to give a consistent estimation of the antioxidant potential of fruit.

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