

Phenolic compounds and antioxidant activity of the *Douce de Djerba* apple compared to introduced cultivars grown in Tunisia

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Abstract - The total phenolic and flavanol contents and the antioxidant activity of flesh and peel of 5 apple cultivars from Tunisia were investigated. Concentrations of the parameters measured differed significantly among the apple cultivars and were highest in the peel compared to the flesh. Total phenolic content (mg GAE/100 g fresh matter) ranged from 150.58 (Anna) to 288.03 (Douce de Djerba) in the flesh and from 405.48 (Anna) to 1022.11 (Douce de Djerba) in the peel. Total flavanoid content (mg CAE/100 g fm) varied from 12.47 (Anna) to 30.7 (Douce de Djerba) and from 48.77 (Anna) to 146.02 (Douce de Djerba) in the flesh and peel, respectively. The Douce de Djerba flesh and peel had the highest antioxidant activity while Anna flesh and peel had the lowest. The total phenolic content and antioxidant activity were significantly correlated in both flesh ($R^2= 0.95$) and peel ($R^2= 0.99$). The contribution of phenolics to the antioxidant activity in peel suggests that their removal may induce a significant loss of antioxidants.

Keywords: Douce de Djerba, Total phenolic content, Total flavanoid content, Antioxidant activity.

1. Introduction

Apples (*Malus domestica* Borkh) are an important source of phenols and antioxidants (Hertog et al. 1995; Vinson et al. 2001) and their consumption has been attributed to the prevention and protection from several degenerative diseases in humans, mainly because of their antioxidants that prevent free-radical damage (Boyer and Liu 2004; Di Pietro et al. 2007; Hyson et al. 2000). The phenolic compounds in apples are responsible for most of the antioxidant activity of the fruit (Lee et al. 2003; Tsao et al. 2005). Less than 0.4% of the antioxidant activity (AOA) of apples is attributed to ascorbic acid content, indicating that other factors, such as phenolics, are the main contributors (Eberhardt et al. 2002). Recent studies have shown that the content of phenolic compounds in apples varies considerably among different cultivars, and also within different apple parts (Drogoudi et al. 2008; Iglesias et al. 2008; Khanizadeh et al. 2008; Lata et al. 2009; Leccese et al. 2009; Petkovsek et al. 2007; Vieira et al. 2009a). Furthermore, the phenolic content of fruits seems to be regulated by environmental and post-harvest factors, including fruit season, fruit maturity, light exposure, storage and processing (Lata, 2007; McGhie et al. 2005). However, it is well established that the cultivar play a major role in controlling the polyphenol composition in apples (Khanizadeh et al. 2008; McGhie et al. 2005; Scalzo et al. 2005). Thus, the apple cultivar may substantially influence the total content of phenolics, flavanols, and anthocyanins and the AOA (Drogoudi et al. 2008; Lata et al. 2009; Vieira et al. 2009a). In addition, the polyphenolic content and AOA is higher in the peel compared with the flesh of apples (Chinnici et al. 2004; D'Abrosca et al. 2007; Drogoudi et al. 2008; Leccese et al. 2009; Vieira et al. 2009b). The AOA of apples has been measured by different assays. The methods based on the scavenging of free radicals such as ABTS [2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] and DPPH [1,1-diphenyl-2-picrylhydrazyl] are some of the most employed techniques, however, there is no agreement regarding the standard methodology and the consensus is that different methods should be employed to assess the antioxidant capacity of the fruits (Roginsky and Lissi 2005).

A number of studies have been carried out on the phenolic compounds and AOA of different apple cultivars grown in several countries (Drogoudi et al. 2008; Khanizadeh et al. 2008; Lata et al. 2009; Leccese et al. 2009; Petkovsek et al. 2007; Wojdylo et al. 2008), however, a fewer of researches were done in Tunisia and the fewer of them were interested to the introduced varieties grown in the region of Sbiba and particularly no researchers were found about local varieties. «Douce de Djerba» or

«Meski Djerba» is a variety of apple tree very known in the Tunisian arid areas and especially in the island of Djerba (South-East of Tunisia). It is very adapted to the conditions of aridity (drought); it has good organoleptic qualities and it is very productive (Boudabous 2008). Recently, statistics show that «Douce de Djerba» has started to rarefy and we can say that it is seriously threatened by extinction (Mehrez 2005). The objective of this study was to compare the total content of phenolics, flavanols and the antioxidant activity (AOA) measured by different methods, in the flesh and peel of «Douce de Djerba» variety in comparison with the introduced varieties. The relationship between AOA and polyphenolic contents was also examined.

2. Materials and methods

2.1. Apple cultivars and chemicals

Five apple cultivars (Douce de Djerba, Golden, Startcrimson, Anna and Richared), the first one was collected from the island of Djerba (South-east of Tunisia) and the other were from the region of Sbiba (North-ouest of Tunisia), were used for this study. Samples were collected at maturity stage at June-July 2010. The «Douce de Djerba» variety, the most threatened in south Tunisia, was taken as reference for all analysis.

2.2. Sample preparation and extraction procedure

Apple flesh and peel samples were tested. The flesh or peel was obtained from five randomly selected apples in each trial to minimize variation. The flesh was the edible portion of the apple without the peel. The peel was the part of the apple removed with a legume knife, and as a thin layer of apple flesh remained adhered to the peel it can be considered as the epidermic zone of the apple. Samples of 5 and 10 g of peel and fresh pulp, respectively, were extracted at room temperature and in darkness with methanol containing 1% 2,6-di-*tert*.-butyl-4-methylphenol (BHT) using an ultrasonic bath. The extraction was carried out according to a method previously optimized to obtain a quantitative extraction. The sample was extracted with 10 ml of solvent for 1 h, 10 ml for 30 min, and then 5 ml for 30 min. The three extracts were combined to a final volume of 25 ml (Escarpa and Gonzalez 1998).

2.3. Total phenolic (TP) content

The TP content was measured using a modified Folin–Ciocalteu method (Budini et al. 1980). Sample extract (100 μ L) was mixed with 2.5 mL of water in a 10 mL volumetric flask. Folin–Ciocalteu reagent (0.5 mL) was added and allowed to react for 5 min. Then, 1.5 mL Na_2CO_3 solution (20 g/100 mL) was added and the mixture was made up to 10 mL with water. After 120 min of incubation at room temperature, the absorbance at 765 nm was read using a Hewlett–Packard spectrophotometer model HP 8452A (Cheadle Heath, Stockport, Cheshire, UK). TP was expressed as mg gallic acid equivalent/100 g fresh matter (mg GAE/100 g fm).

2.4. Total flavonoid (TF) content

Total flavonoid content was measured according to a colorimetric assay (Zhishen et al. 1999). A 1 mL aliquot of standard solution of catechin at different concentrations (10–1000 mg/mL, external calibration with $n = 6$ concentrations) or sample (was added to 10 mL volumetric flasks containing 4 mL water. At the onset of the experiment, 0.3 mL of 5% NaNO_2 was added to the flask. After 5 min, 0.3 mL of 10% AlCl_3 was added. At 6 min, 2 mL of 1 M NaOH was added to the mixture. Immediately, the solution was diluted to a final volume of 10 mL with water and mixed thoroughly. Absorbance of the mixture was determined at 510 nm versus the prepared blanks. Total flavonoid content in apples was expressed as mg catechin equivalents (CE) per 100 g fresh matter (mg CAE/100 g fm).

2.5. Antioxidant activity (AOA)

Several methods have been proposed to evaluate the antioxidant activity of plant extracts and pure compounds and it is widely accepted that their effectiveness depends on the environmental conditions and procedures used. Each method relates to the generation or use of a different radical that is directly involved in the oxidative process, acting through a variety of mechanisms. Antioxidants act by scavenging these radicals and by reducing their oxidative power. Among the commonly used in vitro

assays, we selected the DPPH assay, based on the inactivation of stable synthetic radicals, the DPPH, first envisaged by Blois (1958) and the ABTS to determine the antioxidant activity of apple extracts.

2.5.1. ABTS [2, 2-azino bis (3-ethylbenzothiazoline-6-sulfonic acid)] assay

The ABTS assay described by Re et al. (1999) was used. The ABTS radical cation (ABTS^{•+}) was produced by a reaction of 7 mM ABTS with 2.45 mM K₂S₂O₈. The reaction mixture was kept in the dark at room temperature for 16 h before use. The ABTS^{•+} solution was diluted with methanol to an absorbance at 734 nm of 0.700 ±0.020 in time t =0min (t₀). After addition of 1.0 mL of diluted ABTS^{•+} solution to 0.01 mL of sample the absorbance was measured after 7 min of reaction (t) using a Hewlett–Packard spectrophotometer model HP 8452A (Cheadle Heath, Stockport Cheshire, UK). A standard curve of Trolox was prepared and the percentage reduction of ABTS^{•+} was calculated as: (100-[Absorbance t₇/Absorbance t₀] ×100).

2.5.2. DPPH [1, 1-diphenyl-2-picrylhydrazyl] assay

For the DPPH assay the modified method of Brand-Williams et al. (1995) was used. Methanol DPPH• solution (0.1 mM) was prepared fresh daily. The DPPH• solution (2.9 mL) was placed in a glass cuvette and absorbance at 515 nm in time t =0 min (t) was measured. Then 0.1 mL of sample was added and the mixture was shaken vigorously and kept in the dark at room temperature for 30 min (t₃₀). The absorbance at 515 nm was then measured using a Hewlett–Packard spectrophotometer model HP 8452A (Cheadle Heath, Stockport, Cheshire, UK). A standard curve of Trolox was prepared and the percentage reduction of DPPH• was calculated as: (100 - [Absorbance t₃₀/Absorbance t₀] ×100).

2.6. Statistical analysis:

Data were evaluated by mean analysis. Significance testing was performed using SAS 9.0. Data were analyzed using analysis of variance (ANOVA) for a completely randomized design (CRD). Duncan multiple range test was used to separate the means for significant effects. The correlation between the parameters studied was done by STATBOX 6.5.

3. Results and discussion:

3.1. Total phenolic and total flavanol content

The total phenolic (TP) and total flavanol (TF) contents in all cultivars were significantly higher in the peel than in the flesh (Table 1). In addition, a great variability in terms of TP and TF content was observed among the apple cultivars analyzed and the differences were significant (p < 0.05). In the flesh, the TP content ranged from 150.58 (Anna) to 288.03 mg GAE/100 g fm. The flesh of «Douce de Djerba» followed by «Golden» had the highest TP content whereas the lowest value was found in «Richared» followed by «Startcrimson» and «Anna». The TP content of the peel samples ranged from 405.48 to 998.78 mg GAE /100 g fm. The peel of the «Douce de Djerba» cultivar followed by «Golden» had the highest phenolic content, whereas the lowest value, as in the flesh, was found in «Anna».

Table 1. Total phenolic (TP) and total flavanol (TF) content in the flesh and peel of 5 apple cultivars

Cultivar	TP (mg GAE/100g fm)		TF (mg CAE/100g fm)	
	Flesh	Peel	Flesh	Peel
Douce de Djerba	288.03±6.71 ^A	998.78±3.19 ^A	30.70±2.49 ^A	146.02±1.03 ^A
Golden Sbiba	171.39±6.73 ^B	826.84±11.79 ^{AB}	20.93±0.52 ^B	74.48±1.75 ^C
Startcrimson Sbiba	173.61 ±5.2 ^{BC}	588.40±6.82 ^{BC}	13.12±1.61 ^D	60.23±0.25 ^D
Richared Sbiba	167.49±4.39 ^{BC}	667.31±32.34 ^{BC}	17.36±1.06 ^C	82.19±0.97 ^B
Anna Sbiba	150.58±5.36 ^C	405.48±7.15 ^C	12.47 ±0.45 ^D	48.77±0.19 ^E

Results as mean ±standard deviation from three replicates.

A-E Different superscript letters between cultivars denote significant differences (Duncan's test, p < 0.05).

The TF content in the flesh ranged from 12.47 to 30.7mg CAE/100 g fm. The «Douce de Djerba» flesh contained the highest amount of TF, while the lowest was found in Anna. The flavanol content in the

peel was highest in «Douce de Djerba» (146.02 mg CAE/100 g fm), whereas the lowest value, as in the flesh, was found in Anna (48.77 mg CAE/100 g f m) (Table 1). This study showed that apple peel, in general, has a 73.58-78.01 % higher TP content and 79.63-82.62 % higher TF content compared with the flesh and the values varied between apple cultivars, suggesting that peel removal may lead to a more significant nutrient loss in some cultivars than others. Significant quantitative differences between the apple varieties were also found, the «Anna» variety showing the lowest content of phenolic compounds and «Douce de Djerba» variety the highest (Table 1). Comparison of the total phenolic content values obtained in this study with those of other studies suggests similar results, although differences in the units reported and spectrophotometric standards employed make a direct comparison difficult. Vieira et al. 2009a, were analysed the total phenolic content in the flesh and the peel of three cultivars of apple, grown in southern of Brazil, they were found that in the flesh the TP content ranged from 128.3 to 212.0 mg GAE/100g fm whereas, the TP content in the peel ranged from 304.6 to 712.6 mg GAE/100g fm, which is agreement with our results. While, Eberhardt et al. (2002) and Wolfe et al. (2003) reported values of total phenolics for apple cultivars grown in the United States between 119 and 290 mg GAE/100g fm. McGhie et al. (2005) demonstrated that the growing region influences the phenolic compounds composition in apples, but the amount of the difference was highly cultivar-dependent. In general, total polyphenols are more concentrated in apple peel than in pulp for all advanced lines and cultivars studied, as was observed by other authors in dessert or cider apple varieties (Awad et al. 2000; Alonso-Salces et al. 2004). Apple phenolic content is characterized by some major classes of phenolic compounds present in the peel and in the flesh of this fruit: flavan-3-ols monomers (catechin and epicatechin), flavan-3-ols polymers (procyranidins), dihydrochalcones (phloretin glyco- sides), flavonols (quercetin glycosides), hydroxycinnamic acids (chlorogenic and caffeic acid) and, in red skin cultivar, anthocyanins (Alonso-Salces et al. 2001). A great variation in terms of phenolic contents have been reported among cultivars of different fruit species, such as apple (Vieira et al. 2011, Drogoudi et al. 2008; Petkovsek et al. 2007; Vieira et al. 2009a), mulberry (Özgen et al. 2009), red grape (Orak, 2007), sea buckthorn (Ercisli et al. 2007) and plum (Rupasinghe et al. 2006). Our results are in agreement with those of previous studies on several apple cultivars showing that the peel is the fruit portion with the highest bioactivity (Escarpa and Gonzalez 1998, Khanizadeh et al. 2008; Leccese et al. 2009; McGhie et al. 2005; Petkovsek et al. 2007; Vieira et al. 2009b). Petkovsek et al. (2007) reported that these quantitative differences are mainly due to the flavanol glycosides, as well as the high levels of catechins and chlorogenic acid in the peel. Comparison of the phenolic and flavanol content values obtained here with those of other studies suggests similar results (Khanizadeh et al. 2008; Petkovsek et al. 2007; Wolfe et al. 2003; Vieira et al. 2009b).

3.2. Antioxidant activity (AOA)

The AOA values found for the apple extracts are reported in Table 2. Similarly to the results for phenolics and flavanols, the peel showed significantly higher AOA values than the flesh for all cultivars and a great variability in AOA, measured by all methods, between the cultivars was also observed ($p < 0.05$).

Table 2. Antioxidant activity measured by DPPH ($\mu\text{g/ml}$) and ABTS ($\mu\text{M ET}/100 \text{ g fm}$) assay in the flesh and peel of 5 apple cultivars

Cultivar	DPPH assay		ABTS assay	
	Flesh	Peel	Flesh	Peel
Douce de Djerba	4.13±0.19 ^A	2.03±0.22 ^A	881.22±1.04 ^A	5188.76±0.19 ^A
Golden Sbiba	3.4±0.34 ^B	1.75±0.13 ^B	608.19±0.71 ^B	3268.71±3.07 ^B
Starcrimson Sbiba	2.82±0.17 ^D	1.01±0.03 ^D	418.14±1.9 ^D	1898.43±10.6 ^D
Richared Sbiba	2.99±0.17 ^C	1.5±0.1 ^C	531.89±2.65 ^C	2384.40±10.49 ^C
Anna Sbiba	1.99±0.12 ^E	0.67±0.08 ^E	399.95±0.1 ^E	1210.81±0.46 ^E

Results as mean ±standard deviation from three replicates.

A-E Different superscript letters between cultivars denote significant differences (Duncan's test, $p < 0.05$).

In the flesh, the AOA values measured by ABTS ranged from 399.95 to 881.22 $\mu\text{M ET}/100 \text{ g fm}$; by DPPH from 1.99 to 4.13 $\mu\text{g}/\text{ml}$. The flesh of the «Douce de Djerba» followed by «Golden Sbiba» had the highest TEAC measured by the two methods, whereas the lowest value was found in «Anna». In the apple peel, the AOA measured by ABTS ranged from 1210.81 to 5188.58 $\mu\text{M ET}/100 \text{ g fm}$; by DPPH from 0.67 to 2.03 $\mu\text{g}/\text{ml}$. «Douce de Djerba» peel followed by «Golden Sbiba» had the highest AOA measured by the two methods, whereas the lowest value, as in the flesh, was found in «Anna». The AOA values for the peel were around 58.4–83% greater than the respective values for the flesh.

Our results are consistent with those of a number of previous studies (Chinnici et al. 2004; Khanizadeh et al. 2008; Tsao et al. 2005; Vieira et al. 2009b; Wojdylo et al. 2008). Chinnici et al. (2004) reported values for AOA measured by the DPPH method in apple peel around 2.5 times higher than the respective values for the flesh. Petkovsek et al. (2007) found values of AOA in apple peel 2–5 times higher than in the apple flesh measured by the DPPH assay. Drogoudi et al. (2008) found that apple peel exhibited from 1.5 to 9.2 times greater AOA compared with flesh, also measured by the DPPH assay. Khanizadeh et al. (2008) found that apples exhibited high AOA, and the values varied between apple cultivars and the parts of the fruit. Similarly to our results, Leccese et al. (2009) reported values of AOA measured by ABTS in apple peels very higher than those of pulps. Wolfe et al. (2003) reported that apple peel is a rich source of antioxidants and has significantly higher amounts of phenolic compounds, antioxidant activity and antiproliferative activity than the flesh of apples. They suggested that regular consumption of apple peels may result in reduced risks of cardiovascular diseases and cancer.

3.3. Correlation analysis

Apples contain several phytochemicals and to establish the extent to which polyphenols contribute to the antioxidant properties of the fruit, the linear regression between AOA and TP contents was analyzed for both the flesh and the peel of the apples (Table 3). There was a significant positive relationship ($p < 0.05$) between TP, TF and AOA measured by ABTS and DPPH methods for both the flesh ($R^2 = 0.95$ and 0.90 , respectively) and the peel ($R^2 = 0.98$ and 0.98 , respectively) samples.

Table 3. Relationship between total phenolic (TP) and total flavonol (TF) content and antioxidant activity (AOA) measured by DPPH and ABTS assay in the flesh (A) and peel (B) of 5 apple cultivars

A				
	TP	TF	DPPH	ABTS
TP	1	0.95	0.90	0.95
TF	0.95	1	0.94	1.00
DPPH	0.90	0.94	1	0.94
ABTS	0.95	1.00	0.94	1
B				
	TP	TF	DPPH	ABTS
TP	1	0.90	0.98	0.98
TF	0.90	1	0.87	0.95
DPPH	0.98	0.87	1	0.94
ABTS	0.98	0.95	0.94	1

Our results reported a stronger relation between TP content and the AOA in apple peel compared to the flesh, these results are in collaboration with Chinnici et al. 2004; D'Abrosca et al. 2007; Drogoudi et al. 2008 and Leccese et al. 2009. However, other studies have found that the AOA in apple flesh is higher than in the peel (Khanizadeh et al. 2008; Vieira et al. 2009ab). In contrast, Wolfe et al. (2003) and Eberhardt et al. (2000) did not find any relation between the AOA and TP content in apple tissues. These results are probably due to polyphenolic compounds being present in the peel and not in the flesh, as well as the variation in the relative proportions measured and in the different methods of extraction and analysis used. Concerning the relationship between the AOA measured by ABTS and DPPH methods and TF content, signification is highly positive ($p < 0.05$) for both the flesh ($R^2 = 1$ and 0.94 respectively) and the peel ($R^2 = 0.95$ and 0.87 respectively). In contrast, Vieira et al. (2011a) reported that associations were weak ($p > 0.05$) between TF content and AOA measured by ABTS, DPPH and FRAP methods in the flesh ($R^2 = 0.313$, 0.286 and 0.366 , respectively) and TF content and

AOA measured by FRAP assay in the peel ($R^2= 0.301$). However, there was a significant positive relationship ($p < 0.05$) between TF content and AOA measured by ABTS and DPPH methods in the peel ($R^2= 0.666$ and 0.660 , respectively). In addition, apples with the highest TP content also had a high TF content and this result was confirmed by a positive relationship between TP and TF contents in both the flesh and the peel. A significant positive relationship between TF content and AOA in apple peel samples was also observed by other researchers who reported that flavanols, including monomers, dimers and oligomers, due to their high radical scavenging activity and high concentrations are the most important phenolic compounds in terms of the AOA of both apple peel and flesh (Chinnici et al., 2004; Tsao et al., 2005; Wojdylo et al., 2008). Similarly to our study, Khanizadeh et al. (2008) observed a weak correlation between the TF content in apple flesh and AOA measured by FRAP assay and ascribed this to the other phenolics and/or synergism/antagonism between these compounds and other major phenolics. Although, Tsao et al. (2005) demonstrated that anthocyanins had the highest AOA among all tested phenolic standards, it accountable only for 1% of total polyphenolics which explains the lack of relationship observed in this study.

4. Conclusion

The results of this study indicate that the polyphenolic content contributes significantly to the antioxidant activity of apples, which is strongly dependent on the cultivar and fruit part studied. In both apple flesh and peel, the 'Douce de Djerba' cultivar had the highest total phenolic content and antioxidant activity measured by two methods, whereas the lowest values were found in 'Anna'. The significant differences between apple samples confirmed that the cultivar is the main factor determining the composition of bioactive compounds in apples.

Phenolic compounds tend to accumulate in the dermal tissues of plant bodies because of their potential roles in protection against ultraviolet radiation, to act as attractants in fruit dispersal, and as defense chemicals against pathogens and predators. In this study, significantly higher levels of total phenolics and total flavonoids were detected in the skin of apples. The correlation coefficient between total phenolics and DPPH radical-scavenging activity was found to be 0.90 in the flesh and 0.95 in the peel. Wolfe et al. (2003) reported that apple peel is a rich source of antioxidants and has significantly higher amounts of phenolic compounds, antioxidant activity and antiproliferative activity than the flesh of apples. They suggested that regular consumption of apple peels may result in reduced risks of cardiovascular diseases and cancer. The radical-scavenging and antioxidant properties of methanolic extracts of 'Douce de Djerba' apple reinforce the need for phytochemical study of these fruits.

5. References

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