

JOURNAL OF OASIS AGRICULTURE AND SUSTAINABLE DEVELOPMENT

www.joasdjournals.org



p-ISSN: 2724 – 699X
e-ISSN: 2724-7007

Pomological evaluation of some genotypes of figs (*Ficus carica* L.)

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Article info

Article history:

Received 08/02/2022

Accepted 25/04/2022

Keywords: *Ficus Carica*, total polyphenols, total anthocyanins, antioxidant activity.



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Conflict of Interest : The authors declare no conflict of interest.

Abstract

In order to enhance some cultivars of fig widespread in the Oasis of Gafsa located in the south of Tunisia (arid bioclimatic stage). Four cultivars of Fig (*Ficus Carica* L.) were evaluated 'Sawoudi', 'Bayoudhi', 'Mlouki', 'Assal'. The phenolic composition and antioxidant activities of skin, pulp and whole fruit were determinate. In this study the results showed that the total polyphenol contents of Assal cultivar were the highest in the various tissues (190.98-697.94 mg/100g FW). In all cultivars and whatever the tissue examined (whole fruit, peel, and flesh), the flavonoid content varied from 14.71 to 63.92 mg/100gFW). Also, Assal cultivar was the richest with flavonoids in comparison to other cultivars. The ortho-diphenol content varied from 8.21 to 118.62 mg/100g FW. Indeed, the flesh of Sawoudi and Bayoudhi cultivars had the higher ortho-diphenol content. On the other hand, Bayoudhi (flesh) was the more concentrated in anthocyanins which was around 2.8 mg/100g FW. However, the highest anthocyanin content in the peel was registered in Sawoudi cultivar (0.934mg/100g FW). This cultivar had the most important antioxidant activity ($p < 0.05$) evaluated by the four test DPPH, ABTS, reducing power and phosphomolybdenum. The comparison between the different cultivars has shown that Assal had the highest levels of total polyphenols and flavonoids as well as potential antioxidant activity. The results of the present study suggest that fig skin may be useful as a viable source of natural antioxidants for agri-food applications.

1. INTRODUCTION

The fig tree (*Ficus carica* L.) is one of the very old fruit species in Tunisia (Mars, 1995). This culture has a very important economic interest. In Tunisia, the fig tree is present in all regions, in diversified environmental conditions. However, its cultivation remains sporadic, despite the richness of this phylogenetic heritage and its age (Mars et al. 1994 and Mars 1995). The cultivation of the fig tree occupies an area of 37,774 ha and 72% of which is dry-farmed (Mars, 2003). The national production is 25,000 T/year. Many researches had been carried out with the aim of identifying the morphological and pomological characteristics of *Ficus carica* L. cultivars (Gozlekci, 2010). The quality of the fig depends on several factors such as the amount of total

sugars, the content of soluble solid sugars, the weight, the shape, the size and even the cultivar (Babazadeh Darjazi, 2001). In addition, the fig is a very nutritious food and has an irreproachable nutritional value. The fruit has been consumed by humans for a long time for its high nutritional value. In several producing countries, most of the harvest is dried (85 to 90%), 10% is put in canned and less than 3% is marketed fresh (Bolin and King, 1980). In addition to their consumption in the fresh or dried state, figs are used in preserves (candied figs and figs in syrup), for the extraction of aromas, natural food colorings, food preparations (cakes, pasta, chocolate, etc.) (Loizzo et al., 2014). Dried figs are a staple food for many families in rural areas during difficult times (Bourayou et al., 2005). The presence of

phytosterols (433mg/g FW) is also reported, in addition to the presence of high levels of polyphenols and flavonoids (Vinson, 1999). Figs have a laxative effect and contain many antioxidants. The concentrations of phenolic compounds are strongly dependent on fig cultivars, which can significantly influence total antioxidant activity and other properties such as total flavonoids, and total anthocyanins (Caliskan and Polat, 2011; Ercisli et al., 2012). These compounds are not evenly distributed in the fruit; generally they are more concentrated in the fig peel than in the flesh. Despite its nutritional importance and its richness in natural antioxidants, fig peel is not appreciated by consumers. In this context, the objective of our work consists in determining the biochemical composition of the peel and the flesh and the whole fruit of some fig cultivars as well as their antioxidant properties. In addition, the most suitable cultivars will be selected and will be the most valued as a source of natural antioxidants for the use in agro-food.

2. MATERIAL AND METHODS

2.1. Plant material

In order to enhance some widespread fig cultivars in the oasis of Gafsa (Fig. 1), we were interested in this work to study four fig cultivars "Sawoudi", "Baoudhi", "Mlouki", "Assal" grown in this oasis chosen as 'pilot oasis'. Geographically, the oasis of Gafsa is located at the northern limit of the southwestern plain which is considered to be the beginning of the arid expanses before the great desert of southwestern Tunisia. The plant material studied is the figs harvested at the end of August 2019. The study was made on the whole fruits (WF), peel and flesh.

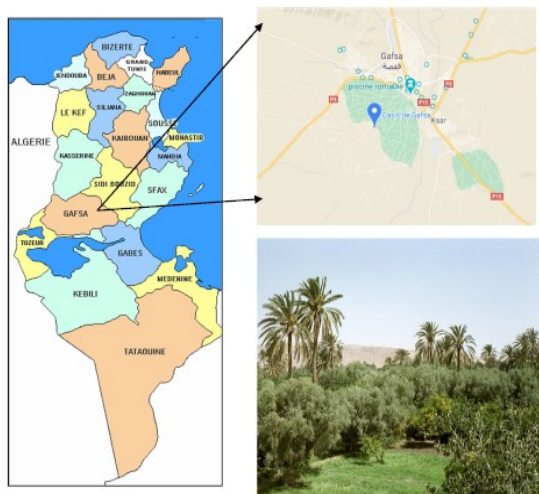


Fig. 1. Geographical location of the oasis of Gafsa.

2.2. Dosage of phenolic compounds

Methanolic extracts were prepared as described previously (Dabbou et al. 2016) using [methanol/water (80:20, v/v)] solution. The obtained methanolic extracts were stored at -20°C for further analysis. The concentration of total phenols was measured by the method of (Montedoro et al. 1992). Briefly, after the dilution of the methanolic extract, Folin-Ciocalteu reagent (1/10; v/v) and sodium bicarbonate Na_2CO_3 ($75\text{g}\cdot\text{l}^{-1}$) were added. The obtained solution was mixed and incubated for 2h at room temperature. Finally, the absorbance was measured at 765 nm and total phenols contents were expressed as mg hydroxytyrosol 100g^{-1} fresh weight (FW). The *o*-diphenols contents were determined colorimetrically (Montedoro et al. 1992). In fact 1 ml of HCl (0.5N) was added with 1 ml NaOH (1N) and 1 ml of mixed solution (NaNO_2 (1.45N) and $\text{Na}_2\text{Mo}_4\cdot 2\text{H}_2\text{O}$ (0.4N)) to diluted samples and incubated for 30 min. Finally, the absorbance was measured at 500 nm and the results were expressed as mg hydroxytyrosol equivalents 100g^{-1} FW. Total flavonoids were measured by a colorimetric assay according to the method of Zhishen et al. (1999). A volume of $75\mu\text{l}$ of NaNO_2 (5%) was added to a diluted sample and the mixture was allowed to stand for 6min. Then, $150\mu\text{l}$ of AlCl_3 (10%) were added. After 5 minutes, 0.5 ml of NaOH (1M) was added and the final volume adjusted to 2.5 ml. The obtained solution was mixed well and the absorbance was measured at 510 nm. Total flavonoids were expressed on a FW basis as mg catechin equivalents (CE) 100g^{-1} . The flavonols content was evaluated from diluted methanolic extracts (Romani et al. 1996) with 10% ethanol, 0.1% HCl in 95% ethanol and 2% HCl. The obtained solution was mixed and incubated at room temperature for approximately 15 min. The absorbance was read at 360 nm and the total flavonols content was expressed as mg quercetin equivalents (QE) 100g^{-1} FW. For the total anthocyanin content we had followed the method described by Padmavati et al. (1997) and Chung et al. (2005). Anthocyanins were measured in the skin and the pulp of the fruits. Before the preparation of the extracts, the fruits were peeled by hand and the skin was separated from the pulp. For each tissue 5 g of fresh weight was mixed with 10 ml of a 0.5 N HCl solution in 80% MeOH, and homogenized by a Polytron homogenizer then incubated in the dark for 16 h at 4°C. Then, they were centrifuged for 20 min at 4000 rpm at 4°C. Subsequently, the absorbance is

measured at different wavelengths. Anthocyanins are calculated according to the formula described by Gould et al. (2000) and expressed mg Cyanidin-3-glucoside equivalent $100 \text{ g}^{-1} \text{FW}$. $A = A535 - A700$

Or : A535 was the absorbance at 535 nm and A700 was absorbance at 700 nm.

The total anthocyanin contents are calculated by the following formula (Giusti and Wrolstad, 2001):

$$\text{TAC} = A \times \text{MW} \times V \times \text{DF} \times 100 / \epsilon \times 100$$

Or : A = absorbance; MW = molecular mass (449.2 g/mol); DF = dilution factor; ϵ = molar absorbance coefficient (25965 L/mol/cm).

2.3. Antioxidant activities

The antioxidant activities were tested using four assays: The ABTS \bullet^+ radical scavenging activity of sample was determined according to the method of Re et al. (1999) with slight modifications. ABTS \bullet^+ cation was prepared by reacting 39.2 mg of ABTS \bullet^+ solution with 6.7 mg of potassium persulfate and allowing the mixture to stand in the dark at 4°C for 12 h before use. Prior to assay, the ABTS \bullet^+ solution was diluted with ethanol to an absorbance at 734 nm of 0.700 ± 0.02 . Five methanolic extracts (50 μl) were reacted with 1.95 mL of the ABTS \bullet^+ solution in the dark for 6 min. Then, the absorbance at 734 nm was measured for each sample relative to a blank. The phenolic concentration of the sample required to scavenge 50% of the ABTS free radical, was determined from the plot between % inhibition and concentration of tested samples and labeled as effective concentration EC_{50} expressed as $\text{mg}100 \text{ g}^{-1}$.

The effect of the methanolic extracts on DPPH \bullet (1,1-diphenyl- 2-picrylhydrazyl radical) was estimated according to the method described by Brand-Williams et al. (1995). Extracts can scavenge the DPPH \bullet and the reduction of DPPH is monitored by the decrease of the absorbance at 515 nm at 25 °C. An aliquot of methanolic extracts (0.1 ml) was added to 3.9 ml of DPPH solution ($6 \times 10^{-5} \text{ M}$ in methanol), mixed well and the absorbance was measured after 30 min. The absorbance of the DPPH without any antioxidant in methanol extracts was measured daily and kept in the dark. The phenolic concentration of the sample required to scavenge 50% of the DPPH free radical, was expressed as $\text{EC}_{50} / \text{mg}100 \text{ g}^{-1} \text{FW}$.

The capacity of methanolic extracts to reduce Fe^{3+} (reducing power assay) was assessed by the method of Oyaizu (1986). In brief, 250 μL of each sample was mixed with 250 μL of sodium

phosphate buffer (0.2 M, pH 6.6) and 250 μL of 1% $\text{K}_3\text{Fe}(\text{CN})_6$ and the obtained solution was incubated at 50 °C for 20 min. Then, 250 μL of 10% trichloroacetic acid were added and the mixture was centrifuged (3750 g for 10 min). Further, 100 μL of the supernatant was mixed with 100 μL of methanol and 25 μL of 0.1% ferric chloride and the solution was incubated for 10 min. The absorbance was finally determined against blank at 700 nm. The extract concentrations providing 0.5 of absorbance (EC_{50}) was calculated by the graph of absorbance at 700 nm against phenolic concentration and it was expressed as $\text{mg}100 \text{ g}^{-1} \text{FW}$.

The procedure of Prieto et al. (1999) was followed in order to estimate the total antioxidant activity of methanolic extracts of peach fruit by the phosphomolybdenum assay. 200 μL of each extract was mixed with 2 mL of the phosphomolybdenum reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). All were incubated for 90 min at 95°C, then cooled to room temperature and the absorbance was measured at 695 nm. Results were expressed as μg ascorbic acid/mL extract. EC_{50} expressed as $\text{mg}100 \text{ g}^{-1}$ was calculated by the graph of the content in μg ascorbic acid/ml extract against phenolic concentration. EC_{50} is the effective concentration at which the total antioxidant activity (TAA) was 50% and was obtained by interpolation from linear regression analysis.

2.4. Statistical analysis

Statistical analyzes were performed using SPSS software (version 17.0 for Windows, SPSS, Chicago, IL, USA). Analysis of variance (ANOVA) was performed. Duncan's test was used to compare means between different fig cultivars. Values were represented by mean \pm standard deviation.

3. RESULTS AND DISCUSSION

The results exposed in table 1 showed that the total polyphenol contents of the whole fruit, peel and flesh of *Ficus Carica* varied from 116.59 to 697.94 mg /100g FW. In addition, we had found that fruit of Assal cultivar was the richest in total polyphenols compared to other cultivars. Furthermore, the content of total polyphenols in flesh (697.94 mg of EAG/g of FW) was significantly higher than that measured in peel (350.31 mg /100g FW) and in the whole fruit (190.98 mg /100g FW). For Sawoudi, the total polyphenol contents in the peel was higher (319.04 mg /100g FW) than that measured in

flesh part. For Bayoudhi and Mlouki cultivars, the total polyphenol contents of various tissues (WF, peel, flesh) varied between 116.59 and 204.49 mg of EAG/g FW. Our results had also, illustrated that the fruit of Bayoudhi presented the lowest concentration of total polyphenols in the different parts.

The results confirmed that the cultivars studied in this work showed higher levels of phenolic compounds than that presented in previous study. In fact, the work of Vallejo et al. (2011), carried out in other fig cultivars, reported that the contents of total polyphenol varied between 19.1 and 140 mg /100g FW in the peel and between 0 and 11.3 mg/100g FW in the flesh. The contents of phenolic compounds varied hugely from one cultivar to another (depending-cultivar). Moreover, Oguzhan et al. (2011) mentioned that the total polyphenol contents of fresh figs varied from 28.6 to 211.9 11.3 mg/100g FW. Our results are in agreement with those of Louaileche et al (2015), Ivanov et al (2018) and Hoxha et al. (2015) who showed that the dark varieties are more concentrated in phenolic compounds than the varieties whose colored green and white. Indeed, the content of total polyphenols is influenced not only by the cultivar but also by the part of the fruit (Vederic et al. 2008). Similarly, rainfall and the harvest season also vary the concentration of phenolic compounds in the fig. Previous work has shown that the polyphenol contents was higher in the

second harvest season (September) compared to the first (June) (Vederic et al. 2008).

In addition, the results exposed in table 1, showed that the highest values of flavonols were measured in the peel of Sawoudi and Bayoudhi cultivars (60.1 and 35.67mg/100g FW, respectively). However, the lowest flavonol contents were recorded in Assal cultivar, especially in the whole fruit (15 mg/100g FW). Similarly, in Assal fruit, the flavonol contents in the peel and flesh were very weak and did not exceed 25mg/100g FW. The results had also confirmed that, for the most of the cultivars studied, flavonol contents were much higher in the peel than in the flesh parts. Del Caro et al (2004) recorded higher flavonol contents (105 and 178 mg /100g FW) than that found in this study.

The flavonoid contents in the whole fruit, peel and flesh in the four fig cultivars studied were presented in table 1. The flavonoids varied from 14.71 to 63.92 mg/100g FW. Furthermore, the results showed that Assal cultivar had the highest flavonoid contents in the three tissues (peel, flesh and whole fruit). For Sawoudi, the most elevated concentration of flavonoids was detected in the peel (49.59 mg/100g FW), whereas in the flesh it did not exceed 18.7 mg/100g FW. The study of Del Caro and Piga (2008) mentioned similar results. However, Solomon and al. (2006) found that the flavonoid contents of figs varied between 2.1 and 21.5 mg/100g FW. Also, Vallejo and al.

Table1. Phenolic composition changes (mg 100 /g FW) in the peel, flesh and whole fruit in four fig varieties.

		Sawoudi	Bayoudhi	Mlouki	Assal
Total Polyphenol	Whole fruit	159.89 ^c	146.93 ^c	176.36 ^B	190.98 ^A
	Peel	319.04 ^x	116.59 ^z	204.49 ^y	350.31 ^w
	Flesh	193.75 ^b	143.79 ^c	121.95 ^d	697.94 ^a
Flavonol	Whole fruit	24.44 ^B	25.11 ^B	30.67 ^A	12.11 ^C
	Peel	61.11 ^w	35.67 ^x	28.33 ^y	19.78 ^z
	Flesh	31.89 ^a	26.11 ^b	19.67 ^c	24.11 ^b
Flavonoid	Whole fruit	18.70 ^B	14.71 ^C	18.51 ^B	29.68 ^A
	Peel	49.59 ^x	28.22 ^y	25.85 ^z	53.76 ^w
	Flesh	20.62 ^b	17.38 ^c	18.40 ^c	63.92 ^a
O-diphenol	Whole fruit	27.87 ^C	32.52 ^B	25.86 ^C	59.16 ^A
	Peel	42.90 ^x	8.21 ^z	20.75 ^y	69.07 ^w
	Flesh	119.24 ^a	122.34 ^a	13.94 ^c	71.54 ^b
Anthocyanin	Peel	0.93 ^w	0.44 ^z	0.57 ^y	0.82 ^x
	Flesh	2,22 ^b	2.81 ^a	1.35 ^c	0.45 ^d

Values are the means of three different samples ($n = 3$) \pm standard deviation. Letters (a, b, c, d) indicate significant differences ($p < 0.05$) between the flesh of the four cultivars. Letters (A, B, C, and D) indicate significant differences ($p < 0.05$) between the whole fruits of the four cultivars. . Letters (W, X,Y, Z) indicate significant differences ($p < 0.05$) between the peel of the four cultivars.

(2011) reported that this content was ranging from 2.5 to 16 mg /100g in fresh figs.

Contrariwise to the distribution of Flavonols in the fruit, the ortho-diphenols were more concentrated in the flesh in all varieties. The ortho-diphenol contents varied from 8.21 to 118.62 mg/100g FW (Table 1). In the flesh, Sawoudi and Bayoudhi cultivars had the highest concentration compared to Mlouki and Assal cultivars (from 116.14 to 118.62 mg/g FW, respectively). However, fruits of Assal cultivar showed the higher concentration in the peel and the whole fruit (69.07 and 59.16 mg/g FW, respectively). Moreover, ortho-diphenols represent an important group among polyphenols, and are characterized by the O-dihydroxyl function in the catechol ring. They exert better antioxidant activity than para-hydroxy (tyrosol) or mono-hydroxy compounds. They are also powerful metal chelators (Mc Donald et al., 2001).

Anthocyanins are water-soluble pigments that contribute to the coloring of certain parts of plants (flowers, fruits, leaves) in blue, red, mauve, pink and orange (Wang et al., 1997; Giusti and Wrolstad, 2001). The fruits of Bayoudhi had the highest concentration (2.8 mg /100 g FW) in the flesh (table 1). However it had the lowest anthocyanin contents in the peel (0.445mg /100 g FW). In the other side, the fruit of Sawoudi cultivar showed the highest anthocyanin contents in the peel (0.934 mg/100 g FW). Solomon et al. (2006) recorded that anthocyanin levels were ranging from 4 to 42 mg/100g FW in commercial fig cultivars. In the same context, they showed that the dark peel cultivars contained the highest levels of anthocyanin compared to yellow ones. In general, in the fig the anthocyanin contents are concentrated in the peel. This variability in content between different types of figs can be explained by the sensitivity of anthocyanins to several factors such as temperature and light (Laleh et al, 2006). In addition, the anthocyanin content of figs varied significantly depending on the variety and the part of the fruit considered (mesocarp, epicarp) (Del Caro and Piga, 2008; Dueñas et al, 2008).

The antioxidant activities variation in the whole fruit, peel and flesh evaluated by the four tests in the four cultivars was presented in the Fig. 2. We found that Assal and Mlouki cultivars had the highest antioxidant activity (ABTS test), followed by Bayoudh and Sawoudi. The higher ABTS radical scavenger activity founded in the different cultivars studied can be explained by their richness in phenolic compounds, mainly

flavonoids, which are powerful antioxidant capable of inhibiting ABTS+ cation radical in a significant way.

The results of the present study had also showed that the percentage of inhibition (EC50) of the DPPH radical had varied significantly among cultivars (Fig. 2B). For the analysis of the whole fruit, Assal had showed the higher antioxidant activity (23.7 mg/100g). However, Bayoudhi had the lowest antioxidant activity (49.2 mg/100g). The results had also confirmed that flesh had the weakest antioxidant activity (DPPH test), compared to the peel. In addition, Assal and Mlouki exhibited higher antioxidant activity than Bayoudhi and Sawoudi. Although, Solomon and al. (2006) estimated that black figs possessed a higher antioxidant activity than those of green figs. The reducing power measured the ability of an antioxidant to donate an electron (Lim and Tee, 2007), these antioxidants are RLS scavengers and act on certain peroxide precursors and thus prevent the chain reaction of peroxidation (Chang et al, 2006). The results of the reducing power activity of Whole fruit, peel and flesh in the analyzed cultivars are presented in Fig. 2 C.

The most important reducing power activity was registered in Sawoudi and Bayoudhi cultivars.

In addition, the results showed that the whole fruit and the peel part had a higher antioxidant activity compared to the flesh. Previous study had also reported a close relationship between the phenolic compounds and the reducing power in several plants (Zhu et al. 2002; Amarowicz et al. 2004). However, Chung et al. (2006), had mentioned that certain sugars (glucose and fructose) and amino acids can modify the activity of reducing power and act as an antioxidant. According to our results, in fig fruits, the antioxidant activities evaluated by ABTS and DPPH, recorded much higher values than those measured by the reducing power.

The total antioxidant activity expressed as mg/100g FW of molybdate reduction, in the different samples is exposed in Fig. 2D. The highest antioxidant activities were recorded in Mlouki in whole fruit and flesh (64.75 and 63.86 mg/100g, respectively), followed by Assal with an inhibition EC50 around 95.14 mg /100g FW. However, Bayoudhi and Sawoudi had the weakest antioxidant activity.

4. CONCLUSION

The results obtained showed that the fruits of the different fig cultivars studied had high levels of phenolic compounds, as well as considerable

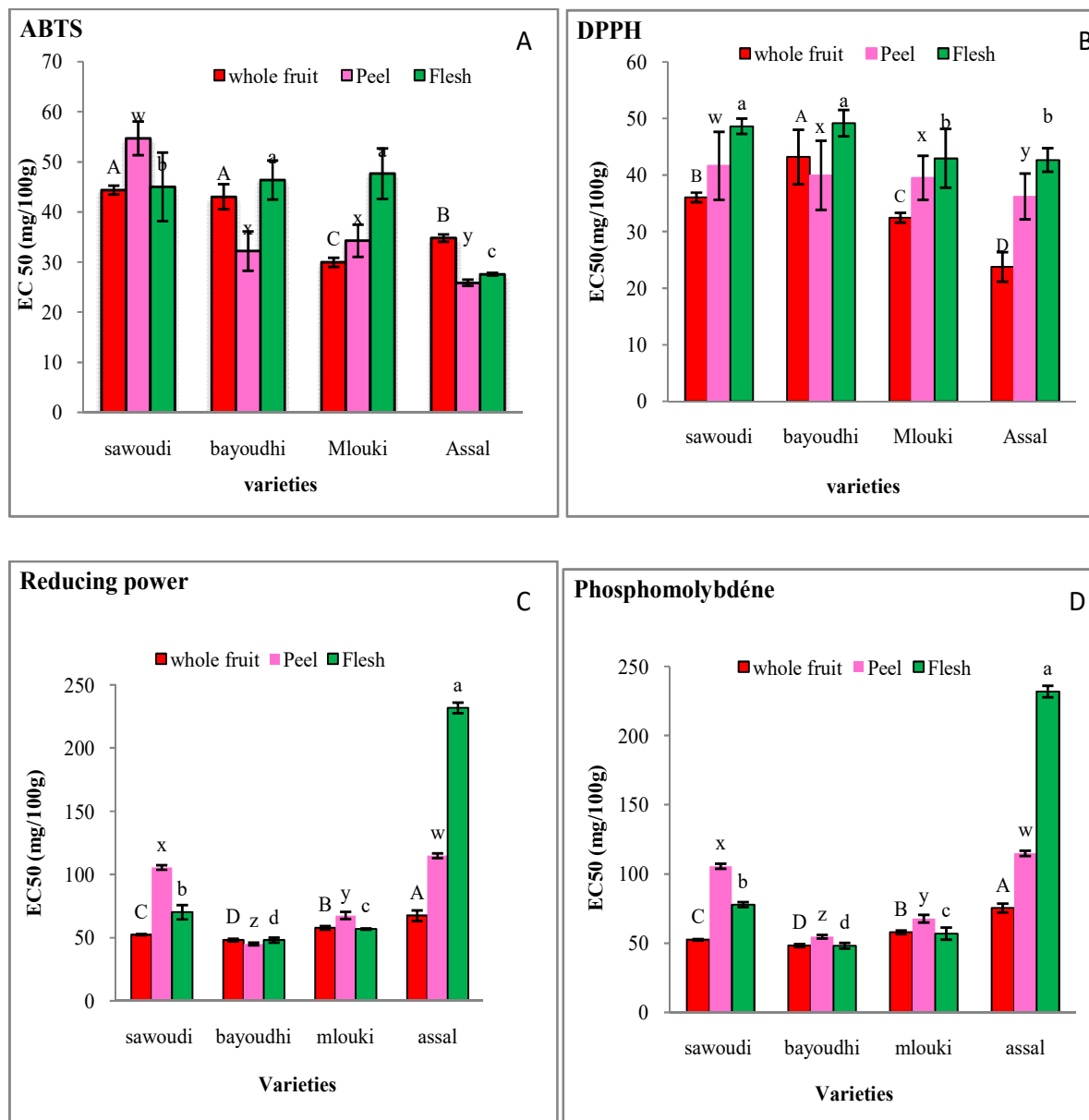


Fig. 2. Effective concentration (EC50) values (mg 100 /g FW) in the peel, flesh and whole fruits in four fig varieties.

Values are the means of three different samples ($n = 3$) \pm standard deviation. Letters (a, b, c, d) indicate significant differences ($p < 0.05$) between the fleshs of the four cultivars. Letters (A, B, C, and D) indicate significant differences ($p < 0.05$) between the whole fruits of the four cultivars. Letters (W, X, Y, Z) indicate significant differences ($p < 0.05$) between the peel of the four cultivars.

antioxidant activities. The cultivars Assal and Mlouki were the richest in total polyphenols and had the powerful antioxidant activity evaluated by the four tests (ABTS, DPPH, reducing power and total antioxidant activity). In general, skin figs fruit can be valued in several sectors, namely food, cosmetics and medical.

ACKNOWLEDGEMENTS

This research was financially supported by the Tunisian Ministry of Higher. We thank the technical staff (Fersi B and Mnafki N) of CRRA for supporting the field survey and providing data.

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