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Influence of Tunisian *Ficus carica* fruit variability in phenolic profiles and *in vitro* radical scavenging potential

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Abstract: *Ficus carica* L., Moraceae, is one of the first plants that were cultivated by humans, being the fruit an important crop worldwide for dry and fresh consumption. In this work, phenolics and antioxidant potential of dried fruits of seventeen Tunisian *F. carica* varieties, from green, red and black phenotypes, were assessed for the first time. HPLC-DAD analysis was performed. All samples presented a similar qualitative profile. The phenolics content ranged between 29.18 and 55.56 mg/kg (in black and red phenotypes, respectively) and quercetin-3-*O*-rutinoside was always the major compound. The antioxidant potential against DPPH•, superoxide and nitric oxide radicals of three varieties representing each phenotype was checked. All samples exhibited activity against the first two radicals in a concentration-dependent way, "Bayoudi" variety being the most effective one (IC25 values of 10.32 and 2.89 µg/mL, respectively). Nevertheless, only "Hammouri" variety presented some capacity to scavenge nitric oxide radical. Our results reveal nice perspectives for these typical fruits, as they present an interesting phenolic composition and good antiradical activity and may encourage their consumption for health protection.

Introduction

The common edible fig (*Ficus carica* L.), belonging to the Moraceae family, has been cultivated since ancient times. In Tunisia, fig plantations are widespread over the country and cover different climates and soils (Mars, 1995). The crop has an essential role in the development of many areas, especially those with arid or semi-arid climate (Oukabli, 2003). The fruit can be consumed fresh, dried and canned, being an interesting source of carbohydrates, essential amino acids, vitamins A, B1, B2 and C and minerals (Solomon et al., 2006). Additionally, figs contain relatively high amounts of crude fibres and polyphenols, which represent 1230 and 360 mg/100 g in the dried form, respectively (Vinson et al., 2005; Solomon et al., 2006).

The drying processing of some fruits and vegetables is one of the oldest forms of preservation. The main purpose in drying foods is the reduction of moisture/water activity to a level which allows safe storage over an extended period. On the other hand, it enables a substantial reduction in weight and volume, minimizing packaging, storage and transportation costs (Okos et al., 1992). Sun-drying is a traditional method used to obtain dried figs, requiring low capital and simple equipment. This method produces figs with good flavour and consistency (Piga et al., 2004).

Phenolic compounds are secondary metabolites quite widespread in nature. Despite this almost ubiquity, experimental evidence has demonstrated that some of them can work as useful markers of the botanical origin of several plant food products (wine, coffee beans, and jams) (Spanos & Wrolstad, 1992). Additionally, within each species, the nature of these compounds can vary from organ to organ and several other factors can contribute to the variability in the phenolic composition, such as cultivar and genetics, geographical origin, maturity, climate, position on tree, agricultural practices, industrial processing and storage conditions (Spanos & Wrolstad, 1992). These compounds have demonstrated a wide range of important biological effects, including antioxidant, anti-carcinogenic, anti-atherogenic, anti-inflammatory, and antimicrobial activities (García-Alonso et al., 2004; Yildirim, 2006).

Some studies have been performed in dried figs to characterize the presence of mycotoxins (Karaca & Nas, 2008), to describe several morphological, chemical and sensorial alterations occurring during the drying process (Piga et al., 2004; Aljane & Ferchichi, 2007; Aljane et al., 2008) and to determine their total phenolic content and antioxidant potential (Vinson et al., 2005). However, to our knowledge there is no report concerning phenolics profile and antioxidant capacity of the most abundant fig varieties in southern Tunisia, where several phenotypes are found: "Zidi", "Sawoudi", "Magouli-ekchine", "Hami" and "Kahli" correspond to black varieties, "Bayoudi", "Besbassi", "Bither", "Makbech", "Chaâri", "Magouli" and "Naasan" are green ones, while "Minouri", "Hamouri", "Ragoubi", "Weldeni" and "Croussi" are red.

Interest in fruits as source of natural antioxidants prompted us to investigate the phenolic constituents of *F. carica* dried fruits. So, the work herein represents a contribution to the knowledge of the seventeen Tunisian fig varieties from the distinct phenotypes above referred. Thus, their phenolic profile was determined by HPLC coupled to diode array detector (HPLC-DAD). Their antioxidant potential was also studied, by evaluating their 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), nitric oxide and superoxide scavenging properties.

Materials and Methods

Plant material

Fig trees *Ficus carica* L., Moraceae were grown in the implementation area of Institute of Arid Regions (IRA), located in Medenine, southern Tunisia. Fruits from seventeen varieties, grouped according to the phenotype (black, green and red), were collected in August 2008 (Table 1). Identification was performed by Ali Ferchichi, Ph.D. (IRA). The drying process took place at IRA. The fruits were arranged in a single layer on a plate, in a greenhouse made with tulle that allowed receiving direct sunlight and protected against insects. The ambient temperature under the tulle greenhouse, during one typical day of the drying period, fluctuated between 29 and 40° C and the maximum values were reached between 10 am and 4 pm. During the night the fruits were kept in the laboratory, at room temperature, to avoid receiving the night humidity. The figs were considered dried when the moisture content was lower than 25 % (from 6 to 15 days), which was determined by the loss in mass measured following AOAC official method 934.06 (AOAC, 1990). Dried samples were then kept in a desiccator, in the dark.

Standards and reagents

Quercetin-3-*O*-rutinoside, quercetin and ferulic acid were from Sigma-Aldrich (St. Louis, MO, USA) and 5-*O*-caffeoylquinic acid was from Extrasynthése (Genay, France). *N*-(1-Naphthyl)ethylene-diamine dihydrochloride, phosphoric acid, methanol and formic acid were from Merck (Darmstadt, Germany). Sodium nitroprusside dehydrate, sulphanilamide, β-nicotinamide adenine dinucleotide (NADH), nitroblue tetrazolium chloride (NBT), phenazine methosulphate (PMS) and DPPH• were obtained from Sigma-Aldrich (St. Louis, MO, USA). The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA, USA). The Chromabond C18 SPE columns (70 mL / 10000 mg) were purchased from Macherey-Nagel (Duren, Germany).

Extracts preparation

Dried figs were triturated in a commercial mill (Moulinex®), homogenised and approximately *ca*. 5 g of each sample was mixed with 100 mL of acid water (pH 2 with HCl) with magnetic stirring (300 rpm), for 15 min. The obtained solution was filtered through a Büchner funnel, under vacuum, and then passed through an SPE C18 column, previously conditioned with 30 mL of methanol and 70 mL of acid water. The phenolic compounds retained in the column were eluted with 40 mL of methanol. Afterwards, this solution was evaporated to dryness under reduced pressure (40 ºC) and the dried extract was kept at -20 °C, in the dark, until analysis.

HPLC-DAD for phenolic profile determination

Each extract was redissolved in methanol, filtered and 20 µL were analysed on an analytical HPLC unit (Gilson), using a Spherisorb ODS2 (25.0 x 0.46 cm; 5 µm, particle size) column, applying an identification methodology already validated and applied for other matrices, including *F. carica* materials (Silva et al., 2001, 2002; Oliveira et al., 2012). Detection was achieved with a Gilson Diode Array Detector (DAD). Spectral data from all peaks were accumulated in the range 200-400 nm. The identity of 3-*O*-caffeoylquinic acid was confirmed by comparing its chromatographic behavior and spectral characteristics with those of 3-*O*-caffeoylquinic acid obtained before by our group by transesterification of 5-*O*-caffeoylquinic acid using tetramethylammonium hydroxide (Silva et al., 2002) and those observed by us for the same compound in *Cydonia oblonga* Miller and *F. carica* fresh fruits using the same analytical conditions (Silva et al., 2002; Oliveira et al., 2009). The data were processed on Unipoint System software (Gilson Medical Electronics, Villiers le Bel, France). Phenolic compounds quantification was achieved by the absorbance recorded in the chromatograms relative to external standards. Phenolic acids were determined at 320 nm and flavonoids at 350 nm. As no 3-*O*-caffeoylquinic acid standard was available,

and as it is an isomer of 5-*O*-caffeoylquinic acid, with the same molecular weight and UV spectrum, it was quantified as 5-*O*-caffeoylquinic acid. The other compounds were determined as themselves.

Statistical Analysis

The evaluation of statistical significance was determined by a paired t-test ($p<0.05$). Principal component analysis (PCA) was carried out using XLSTAT 2011.2 software.

Antioxidant activity

The antioxidant activity was assessed with "Zidi" (ZID), "Bayoudi" (BYD) and "Hamouri" (HAM) varieties, which were chosen as representatives of black, green and red phenotypes, respectively (Table 1), because they are the most commonly consumed ones in Tunisia.

DPPH• scavenging activity

Antiradical activity was determined spectrophotometrically in a Multiskan Ascent plate reader (Thermo; electron corporation), by monitoring the disappearance of DPPH• at 515 nm, according to a described procedure (Oliveira et al., 2009).

Nitric oxide scavenging activity

The nitric oxide scavenging capacity was determined in a Multiskan Ascent plate reader, according to a described procedure (Oliveira et al., 2009), based on the reaction with Griess reagent.

Superoxide radical scavenging activity

Superoxide radicals were generated by the NADH/PMS system and the effect of the extracts on radical-induced reduction of NBT was monitored spectrophotometrically in a Multiskan Ascent plate reader, as reported by Valentão et al. (2001).

Results and Discussion

Phenolic compounds

Tunisian figs were characterized by the presence of five phenolic compounds, which were already described in fruits and leaves of other varieties: three hydroxycinnamic acids (3-*O*- and 5-*O*-caffeoylquinic acids and ferulic acid) (Veberic et al., 2008; Oliveira et al., 2009; Vallejo et al., 2012), one flavonol glycoside (quercetin-3-*O*-rutinoside) (Oliveira et al., 2009; Veberic et al., 2008; Vallejo et al., 2012) and one free flavonol (quercetin) (Vaya & Mahmood, 2006) (Figures 1 and 2 and Table 1). Quantitatively, varieties from the red phenotype show a tendency for having the highest amounts of phenolic compounds and those from black the lower ones (Table 1). However, within each phenotype, the several varieties do not exhibit the same quantitative profile, which may be related with their genetic diversity (Harris & Karmas, 1975). For example, there are green varieties, such as NAAS, MAG, MKH and BYD with higher phenolics content than red ones (HAM) (Table 1). The same is observed with some black phenotype varieties (Table 1). These facts seem to suggest that, in a general way, the phenolic composition does not allow distinguishing the three phenotypes.

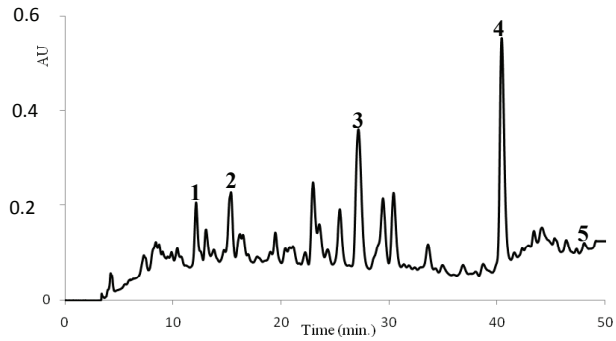

Figure 1. HPLC-DAD phenolic profile of "*Zidi*" variety. Detection at 320 nm. Peaks: 1, 3-*O*-caffeoylquinic acid; 2, 5-*O*-caffeoylquinic acid; 3, ferulic acid; 4, quercetin-3-*O*-rutinoside; 5, quercetin.

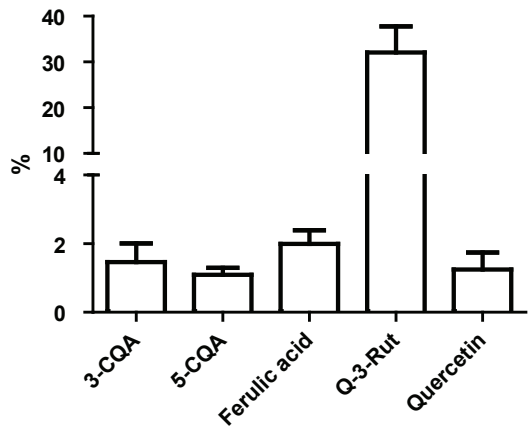

Figure 2. Phenolic profile (%) of Tunisian figs. 3-CQA, 3-*O*-Caffeoylquinic acid; 5-CQA, 5-*O*-caffeoylquinic acid; Q-3-Rut, quercetin-3-*O*-rutinoside.

In a recent study, Vallejo et al. (2012) determined the phenolic profile of dried figs of three cultivars by HPLC-MS, quercetin-3-*O*-rutinoside being reported as the main metabolite. Our results agree with this, once this compound represents ca. 59-93% of total phenolics (Table 1). The same was observed in fresh *F. carica* fruits of other varieties (Oliveira et al., 2009; Veberic et al., 2008; Vallejo

et al., 2012). On the other hand, with the exceptions of 5-*O*-caffeoylquinic acid and quercetin-3-*O*-rutinoside, the other phenolic compounds found in our varieties were different from those described by Vallejo et al. (2012). In addition, our varieties presented a slightly lower total phenolic content (Table 1) when compared with other dried cultivars (Vinson et al., 2005; Vallejo et al., 2012).

The phenolic compounds described above are important in the human nutrition context and, consequently, their bioavailability has been widely studied. Some reports have described that quercetin, 5-*O*-caffeoylquinic and ferulic acids are quickly absorbed in their intact form, whereas quercetin-3-*O*-rutinoside shows a slow absorption (Karakaya, 2004; Lafay & Gil-Izquierdo, 2008).

To assess the variation of the phenolic composition within Tunisian *F. carica* fruits, PCA was performed on the obtained data. Figure 3 shows the projection of chemical variables, grouped by phenolic compounds in all varieties, into the plane composed by the

Table 1. Phenolic composition of dried Tunisian *Ficus carica* varieties (mg/kg)[a].

Varieties [b]	3-CQA	5-CQA	Ferulic acid	Q-3-Rut	Quercetin	Σ
Black phenotype						
MAG-k	nq	0.52 ± 0.06	0.64 ± 0.06	20.00 ± 0.55	0.35 ± 0.01	21.51
SWD	4.52 ± 0.16	0.40 ± 0.02	0.48 ± 0.01	39.60 ± 0.43	0.75 ± 0.01	45.75
ZID	0.34 ± 0.02	0.23 ± 0.02	1.79 ± 0.10	23.10 ± 1.53	0.21 ± 0.05	25.67
HAMI	1.08 ± 0.01	2.00 ± 0.12	2.83 ± 0.01	24.50 ± 0.16	0.72 ± 0.06	31.13
KAH	0.47 ± 0.01	0.82 ± 0.01	2.25 ± 0.05	17.50 ± 0.07	0.81 ± 0.01	21.85
Mean	1.28	0.79	1.60	24.94	0.57	29.18
SD	1.86	0.71	1.02	8.63	0.27	10.04
Max	4.52	2.00	2.83	39.60	0.81	45.75
Min	0.34	0.23	0.48	17.50	0.35	21.51
Green phenotype						
NAAS	8.44 ± 0.12	0.36 ± 0.13	1.93 ± 0.96	37.50 ± 2.69	1.38 ± 0.13	49.61
CHA	0.13 ± 0.01	0.12 ± 0.01	1.33 ± 0.02	5.22 ± 0.07	0.11 ± 0.01	6.91
MAG	1.03 ± 0.01	1.15 ± 0.35	5.88 ± 0.21	24.20 ± 1.49	0.45 ± 0.01	32.71
BTH	0.34 ± 0.01	0.50 ± 0.05	0.60 ± 0.04	4.33 ± 0.07	0.42 ± 0.01	6.19
BES	3.26 ± 0.25	0.59 ± 0.01	1.62 ± 0.07	8.06 ± 0.06	0.10 ± 0.02	13.63
BYD	0.17 ± 0.03	2.56 ± 0.02	1.50 ± 0.04	55.50 ± 7.85	1.90 ± 0.14	61.68
MKH	0.14 ± 0.01	1.94 ± 0.04	1.13 ± 0.09	45.00 ± 2.52	1.43 ± 0.02	49.79
Mean	1.93	1.03	1.98	23.63	0.66	31.50
SD	3.08	0.91	1.76	20.11	0.71	22.87
Max	8.44	2.56	5.88	55.50	1.90	61.68
Min	0.13	0.12	0.60	4.33	0.10	6.19
Red phenotype						
RGB	nq	2.38 ± 0.52	5.56 ± 0.02	69.50 ± 5.46	0.70 ± 0.05	78.14
CRS	3.06 ± 0.10	1.04 ± 0.13	3.38 ± 0.04	50.30 ± 2.14	8.76 ± 1.89	66.54
HAM	0.06 ± 0.01	0.18 ± 0.01	1.33 ± 0.03	12.30 ± 0.76	0.35 ± 0.04	14.22
WLD	0.17 ± 0.02	1.67 ± 0.01	0.23 ± 0.01	20.50 ± 0.08	0.12 ± 0.01	22.69
MIN	1.65 ± 0.15	2.13 ± 0.06	1.44 ± 0.11	88.40 ± 3.84	2.58 ± 0.24	96.20
Mean	0.99	1.48	2.39	48.20	2.50	55.56
SD	1.35	0.89	2.10	32.13	3.63	35.61
Max	3.06	2.38	5.56	88.40	8.76	96.20
Min	0.06	0.18	0.23	12.30	0.12	14.22

[a]Values are expressed as mean±standard deviation of three assays; nq, not quantified; Σ, sum of the determined phenolic compounds; 3-CQA, 3-*O*-caffeoylquinic acid; 5-CQA, 5-*O*-caffeoylquinic acid and Q-3-rut, quercetin-3-*O*-rutinoside. [b] MAG-k, "Magouli-Ekchine"; SWD, "Sawoudi"; ZID, "Zidi"; HAMI, "Hami"; KAH, "Kahli"; NAAS "Naasan"; CHA, "Chaâri"; MAG, "Magouli"; BTH, "Bither"; BES, "Besbassi"; BYD, "Bayoudi"; MKH, "Makbech"; RGB, "Ragoubi"; CRS, "Croussi"; HAM, "Hamouri"; WLD, "Weldeni"; MIN, "Minouri".

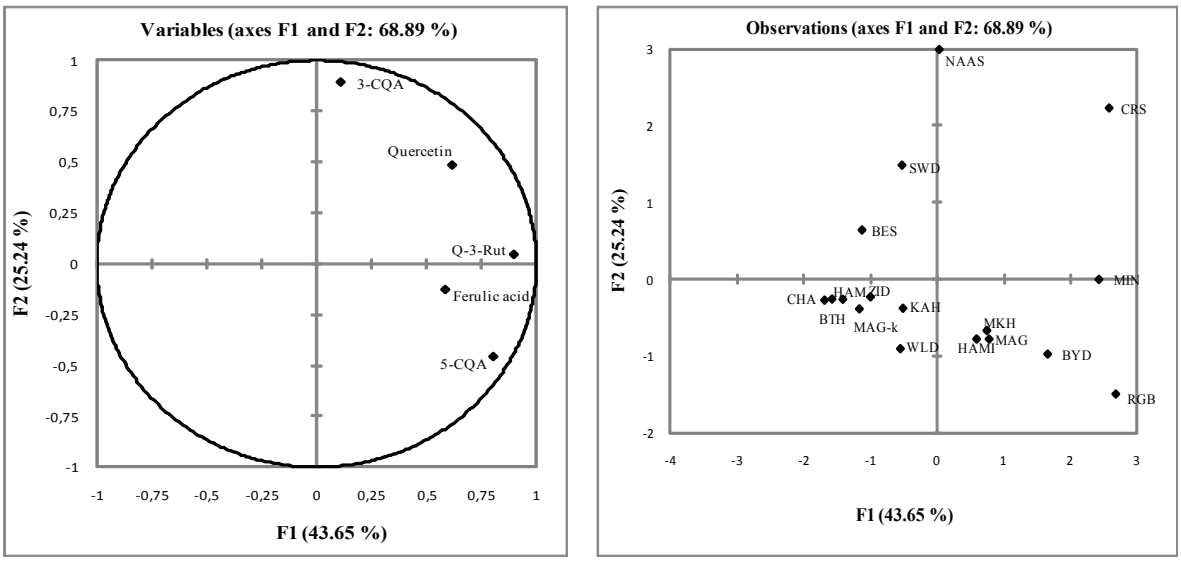

Figure 3. PCA of all phenolic compounds in Tunisian *Ficus carica* fruits: projection of phenolic compounds (variables: 3-CQA, 3-*O*-caffeoylquinic acid; 5-CQA, 5-*O*-caffeoylquinic acid; ferulic acid; Q-3-Rut, quercetin-3-*O*-rutinoside; quercetin; BES, "Besbassi"; BTH, "Bither"; SWD, "Sawoudi"; WLD, "Weldeni"; ZID, "Zidi"; BYD, "Bayoudi"; MAG-k, "Magouhi-Ekchine"; HAMI, "Hami"; MIN, "Minouri"; KAH, "Kahli"; MKH, "Makbech"; CHA, "Chaâri"; HAM, "Hamouri"; RGB, "Ragoubi"; CRS, "Croussi"; MAG, "Magouli"; NAAS, "Naasan") into the plane composed by the principal axes F1 and F2 (68.89%).

principal axes F1 and F2 containing 68.89% of the total variance. We can observe that NAAS (green phenotype), CRS and MIN (red phenotypes) are positively correlated to their higher concentrations of 3-*O*-caffeoylquinic acid, quercetin and quercetin-3-*O*-rutinoside, respectively. HAMI (black phenotype), MKH, MAG, BYD (green phenotypes) and RGB (red phenotype) are projected into the plane formed by F1 positive axis and F2 negative axis due to their low amounts of 3-*O*-caffeoylquinic acid and quercetin. In contrast, the other varieties are presented into the planes formed by F1 and F2 negative axes, corresponding to the samples with lower quantities of all phenolic compounds. These results confirmed that the phenolic composition does not allow to distinguish the several Tunisian *F. carica* phenotypes, since all varieties are composed by the same compounds, at similar amounts, with no significant differences between them.

Antioxidant activity

The DPPH• assay provides basic information on the antiradical activity of extracts (Fukumoto & Mazza, 2000). The extracts of the representative phenotypes exhibited DPPH• scavenging capacity, in a concentration-dependent way (Figure 4A), BYD and HAM being the most effective varieties (Table 2).

The different samples also revealed superoxide scavenging ability, which was concentration-dependent (Figure 4B), BYD being the most active against this free radical (Table 2). Superoxide radical is one of the most effective free radicals that can arise from physiological processes, such as purine metabolism (Borges et al., 2002) or electron leakage from the respiratory chain that will reduce oxygen (Halliwel, 1991). Although necessary in many physiological processes, its augmentation can be in the genesis of several pathological conditions (Halliwel, 1991), such as cell damage in cardiovascular and neurological systems and inactivation of enzymes and other proteins by oxidation and nitration (Pacher et al., 2007).

In what concerns to nitric oxide, HAM was the only variety displaying a protective effect, having an inhibitory capacity of ca. 30% for the highest tested concentration (1.67 mg/mL). Nitric oxide exerts important biological functions, including blood pressure control, neural signal transduction and antimicrobial protection (Ignarro, 1999). Nevertheless, this nitrogen reactive species can be responsible for oxidative damage, once it reacts rapidly with superoxide radical to form peroxynitrite, a major damaging oxidant produced *in vivo* (Beckman, 1996).

The high scavenging capacity verified with the variety chosen from the green phenotype (BYD) can be, at least partially, related with its higher phenolic content (Tables 1 and 2). On the other hand, although ZID variety presented higher quantities of phenolics than HAM (Table 1), it had lower antioxidant capacity (Table 2). This might be explained by the existence of possible

interactions between phenolics and other compounds present in the tested extract and not determined herein, namely antagonisms, which may result in the decrease of activity.

Overall, the results obtained in the different assays revealed *F. carica* dried figs good capacity to scavenge free radicals. This ability is likely to be partially related to the presence of phenolic compounds, since they are recognized as able to act as antioxidants by different ways: as reducing agents, hydrogen donators, free radicals scavengers, and singlet oxygen quenchers and, therefore, as cell saviours (Halliwell et al., 2000; Fattouch et al., 2007). Additionally, these compounds are known to inhibit some enzymes involved in radical generation (Parr & Bolwell, 2000) and to up-regulate endogenous antioxidant enzymes (Zhan & Yang, 2006). In fact, our research group has demonstrated the strong antioxidant activity of several natural extracts characterized by high contents of quercetin-3-*O*-rutinoside as these ones, namely those obtained from *Cydonia oblonga* Mill., *Dracaena draco* (L.) L. or *Lycopersicon esculentum* Mill. (Oliveira et al., 2010; Ferreres et al., 2010; Silva et al., 2011; Santos et al., 2011). Furthermore, we can also highlight the presence of 5-*O*-caffeoylquinic and ferulic acids and quercetin, which are known to be excellent *in vitro* antioxidants, namely against DPPH•, nitrogen and oxygen reactive species (Rice-Evans et al., 1996, 1997; Cai et al., 2004; Boots et al., 2008). On the other hand, as we referred above, we cannot ignore the existence of other non-determined compounds with antioxidant activity, such as those resulting from Maillard reaction (Billaud et al., 2005).

Conclusion

This is the first study involving these fig varieties and contributes to the knowledge of their phenolic composition and antioxidant properties. A similar qualitative phenolic profile was observed and the data obtained indicate that, in a general way, phenolics composition does not depend on the phenotype or the variety. Dried figs revealed good antiradical capacity against DPPH• and $O_2^{\bullet-}$, which might be partially explained by their phenolic compounds. Thus, the consumption of these dried figs in a balanced diet may provide beneficial health effects.

Table 2. IC25 values of dried Tunisian *F. carica* varieties obtained in antioxidant activity evaluation assays (μg/mL)[a].

Assay	Variety		
	HAM	BYD	ZID
DPPH•	14.12±4.72a	10.32±0.58a	155.19±14.56b
$O_2^{\bullet-}$	14.00±2.09a	2.89±0.94b	100.69±20.97c

[a]In the same line, means with different letter are significantly different (p<0.05).

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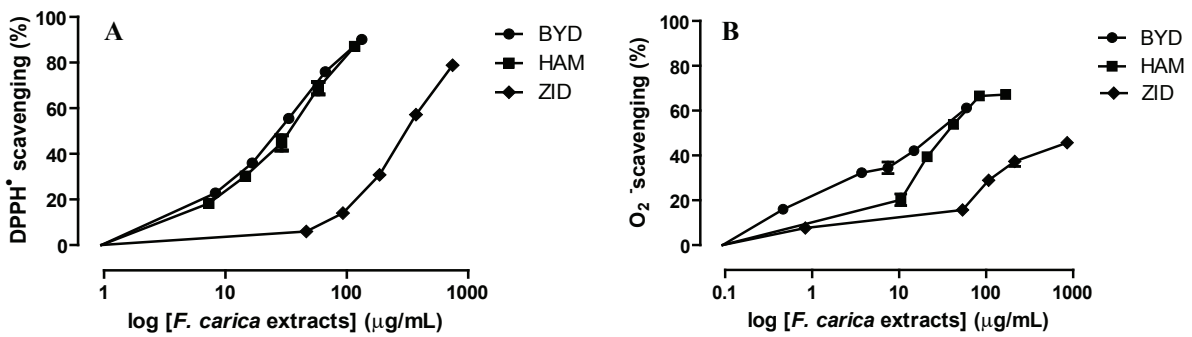

Figure 4. Effect of three Tunisian *F. carica* dried fruits phenotypes against (A) DPPH•, and (B) superoxide radical. Values show mean±SE of three experiments performed in triplicate.

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