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Characterization of Marsa Matrough figs (flesh and pulp and jam): evaluation of polyphenols, anthocyanins and antiradical activity

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ABSTRACT
The identification of polyphenols and anthocyanins and the determination of the antiradical activity in the fig (flesh and pulp) of Marsa Matrough was evaluated. Furthermore, an innovative jam production chain, suited to the climate and to the general condition of the region, has been developed within local population and the nutritional characteristics, polyphenol content, and antiradical activity of jam samples has been determined.

INTRODUCTION
Fig (*Ficus carica* L.) is a deciduous tree of the Moraceae and is recognized as one of the oldest fruits along with apple and grape. The fruits of fig are eaten raw and are processed into some products such as dry fruit and jam, whereas fig leaves have been used in folk medicine or as materials for Chinese medicine. Figs are intensively cultivated in semi-arid climate in the Mediterranean area. In Egypt, notwithstanding the many possibilities of its adaptation to the climate, its cultivation follows traditional agriculture techniques. Figs are generally fresh consumed; however, they exhibit a very short post-harvest life especially in the semi-arid Mediterranean regions.
In Egypt several fig cultivars are planted in different areas (North coast, Sinai, oases and South of Egypt). Some of these cultivars were introduced from ancient time to Egypt and vegetative propagated, rending, therefore, the distinction among the different cultivars difficult (Sabet N. et al., 2013). The fig trees growing in the Marsa Matrouh area are bush-shaped, with a low scaffolding at the soil level, with 4-5 branches often forming a large irregular canopy. The spacing is large and irregular with high planting density in wadi or in irrigated areas. Notwithstanding the warm weather throughout most of the year with temperatures frequently exceeding 40 °C during the day and the low rainfall, most trees show a good vegetative and also productive state. The trees belong to the Sultany variety, which is one of the most common and widely spread in Egypt. The figs are of the common type with red-violet skin, deep red pulp, late ripening. Figs are low in sodium and have no fat or cholesterol, and their functional food properties include significant amounts of vitamins, amino acids, sugars, and antioxidant compounds (Solomon A. et al., 2006). The richness of a fig's nutrient contents depends on the cultivar that produces it. The black- and purple-fruited cultivars have 2-fold greater total antioxidant capacity, 15-fold greater total anthocyanins, and 2.5-fold greater total phenolics than green- and yellow-fig cultivars (Çalişkan O. et al. 2011). Phenolic compounds are distributed in fruits and vegetable and their importance grew in the last years especially for the increased antiradicalic and antioxidant activity that has been associated to the presence of hydroxycynnamic acid and flavonols. In fact polyphenols play a role in contrasting oxidative stress, which has been identified as cause of aging and various human diseases (Dudonne´ S. et al., 2009). In fig fruits and flesh, polyphenols have been characterized in the case of many cultivars (Solomon A. et al., 2006, Veberic R. et al., 2008, Del Caro A. et al. 2008, Vallejo F. et al., 2012, Russo F. at al., 2014). The lipid oxidation is a well-known factor responsible for atherosclerosis. Epidemiological studies have correlated diets rich in fruits and vegetables with a low incidence of coronary heart disease, and several researches have been stimulated on the antioxidant properties of biomolecules in fruits, vegetables and their processed products (juice, jam)(Dai Q. et al., 2006, Silva et al., 2004, Mellano et al., 2010) . A healthy and regular diet, respectful of traditional recipes, using only territorial food, can be guaranteed choosing from the market a series of products naturally strengthened in metabolites of biological

interest. Typical agricultural and food goods often represent added value products for a specific geographical area, as a part of the culture of food, related to the history and the environment, that overcome the simple nutritional function, and face the increasingly demand of discerning consumers, particularly mindful to the protection of their own health (Romani A. et al., 2006). The food demand is increasingly influenced by considerations related to the health and natural foods, requiring higher levels of quality, safety, certification, traceability and product recall. The significance of "quality", then, includes several aspects, closely related to each other, the main of which are: nutritional or functional properties, commercial quality and technological safety.

The aim of this research is the identification of polyphenols and anthocyanins and the determination of the antiradical activity in the fig (flesh and pulp) of Marsa Matrough. Furthermore, an innovative jam production chain, suited to the climate and to the general condition of the region, has been developed within local population and the nutritional characteristics, polyphenol content, and antiradical activity of jam samples has been determined.

MATERIAL AND METHODS

Samples were collected during June 2015 within *Matrouh Rural Development Project*; the fruits (6 units) were manually peeled and the skin separated from the pulp.

Extraction

15 g of fig skin, 50 g of pulp, and 2 g of jam were extracted with 50 mL of 70% ethanol, adjusted to pH 2 with formic acid overnight and then filtered to eliminate residues.

HPLC/DAD Analysis. Analyses of flavonols, hydroxycinnamic acids and anthocyanins were carried out using an HP 1100L liquid chromatograph equipped with a DAD detector and managed by an HP 9000 workstation (Agilent Technologies, Palo Alto, CA, USA). Compounds were separated by using a 250 × 4.6 mm i.d, 5 μm LUNA C18 column (Phenomenex, USA). UV/Vis spectra were recorded in the 190-600 nm range and the chromatograms were acquired at 250, 280, 330, 350 and 520 nm. The samples were analyzed by gradient elution at a flow rate of 0.8 ml/min. The mobile phase was a multi-step linear solvent gradient system, starting from 95% H_2O (adjusted to pH 2 by HCOOH) up to 100% CH_3CN in 53 min.

HPLC-TOF Analysis. The HPLC system was interfaced with an Agilent TOF MS equipped with an ESI source (Agilent Corp, Santa Clara, CA, USA). The TOF/MS analysis worked using full-scan mode and the mass range was set to *m/z* 100–1500 in both positive and negative modes. The conditions of the ESI source were as follows: drying gas, high purity nitrogen (N_2); drying gas temperature, 350°C; drying gas flow-rate, 6 L/min; nebulizer, 20 psi; capillary voltage, 4000 V (negative) 4000 V (positive); fragmentation, 80-150 V, and skimmer, 60 V. The acquisition and

Identification and quantification of individual compounds. The identity of polyphenols was ascertained using data from HPLC-DAD and HPLC-TOF analyses, by comparison with bibliographic data and combination of retention times, UV/Vis and mass spectra with those of authentic standards. The quantification of individual polyphenolic compounds was performed directly by HPLC-DAD using a five-point regression curve ($r^2 \geq 0.998$) in the range of 0-30 μg on the basis of authentic standards. In particular, flavonols like the quercetin derivatives were determined at 350 nm using quecetin 3-*O*-glucoside as a reference compound, hydroxycinnamic acid derivatives were determined at 330 nm using chlorogenic acid as the reference compound, while anthocyanins were determined at 520 nm using oenin as reference compound. In all cases, actual concentrations of the derivatives were calculated after applying corrections for differences in molecular weight. Each sample was analyzed in triplicate, so as to express the analytical results as an average.

Antiradical activity. Free radical scavenging activity was evaluated with the DPPH• (1,1-diphenyl-2-picrylhydrazyl radical) assay. The antiradical capacity of the sample extracts was estimated according to the procedure reported by Brand-Williams, 1995, and slightly modified. Two mL of the sample solution, suitably diluted with ethanol, was added to 2 mL of an ethanol solution of DPPH• (0.0025g/100mL) and the mixture kept at room temperature. After 20 min, the absorption was measured at 517 nm with a Lambda 25 spectrophotometer (Perkin-Elmer) versus ethanol as a blank. Each day, the absorption of the DPPH• solution was checked. The antiradical activity is expressed as IC_{50}, the antiradical dose required to cause a 50% inhibition. IC_{50} was calculated plotting the ratio: $(A_{blank}\text{-}A_{sample}/A_{blank}) \; x \; 100$, where A_{blank} is the absorption of the DPPH• solution and A_{sample} is the absorption of the DPPH• solution after addition of the sample, against the concentration of the sample. IC_{50} is expressed as mg sample /mg DPPH•.

Fig jam manufacturing. After fig washing and figs necks removing, for 1 kg fruit, 600 g sugar, 3 g citric acid, and 5 g pectin were added. The mixture was heated and boiled for about 20 minutes.

RESULTS AND DISCUSSION

Flavonoids and anthocyanins were identified according to their MS fragmentation pattern (Table 1).

Table 1:Main fragmentation pattern of identified compounds in fig skin and pulp

	$[M-H]^+$ m/z	fragment m/z	$[M-H]^-$ m/z	fragment m/z
anthocyanins				
cyanidine glycoside				295
cyanidine-3-rutinoside	595	449	593	295
flavonoids				
apigenin xilosyl glucoside	565		563	431
quercetin rutinoside	611	465-303	609	
quercetin glucoside	465	303	463	
kaempferol derivative	595	287	549	
quercetin derivative	595	549	551	303

Table 2 reports the quali-quantitative data of fig skin and pulp.

Table 2: Anthocyanins, flavonoids, and hydroxycinnamic acid content (mg/kg, fresh weight) of skin and pulp.

	PULP	**SKIN**
flvonoids		
flavonoid	-	7.67
apigenin xilosyl glucoside	-	16.46
quercetin rutinoside	-	434.16
quercetin glucoside	-	44.10
kaempferol derivative	-	11.98
quercetin derivative	-	79.26
TOTAL	-	**593.64**
anthocyanins		
Cyanidine glycoside	0.82	5.07
Cyanidine-3-rutinoside	32.06	125.12
TOTAL	**32.88**	**130.23**
HYDROXYCINNAMIC ACIDS		
TOTAL (caffeic acid derivatives)	**2.10**	**27.48**

As already pointed out, phenolic content was substantially higher in the skin than in the pulp and total content was of the same magnitude order than what previously found (Solomon A. et al., 2006, Del Caro A. et al. 2008, Vallejo F. et al., 2012, Russo F. at al., 2014). Hydroxycinnamic acids could not be identified; however all of them (4 compounds) are caffeic acid derivatives and their total amount was evaluated as caffeic acid content.

In table 3 the nutritional parameters of figs jam are reported

Table 3: Main nutritional parameters of fig jam.

Citric acid	2.790 ± 0.3410	g/100g
Malic acid	0.080 ± 0.011	g/100g
Humidity	44.26 ± 0.38	g/100g
Proteins	0.58 ± 0.07	g/100g
Fats	0.16 ± 0.04	g/100g
Ashes	0.41 ± 0.01	g/100g
Carbohydrates	51.49 ± 0.60	g/100g
Energetic value	914 ± 8	kJ/100g
Sodium	0.009 ± 0.004	g/100g (as NaCl)
Saturated fat acids	0.04 ± 0.01	g/100g
High molecular fraction alimentary fiber	3.10 ± 0.46	g/100g
Fructose	6.28 ± 0.41	g/100g
Glucose	7.43 ± 0.55	g/100g
Sucrose	35.1 ± 3.4	g/100g

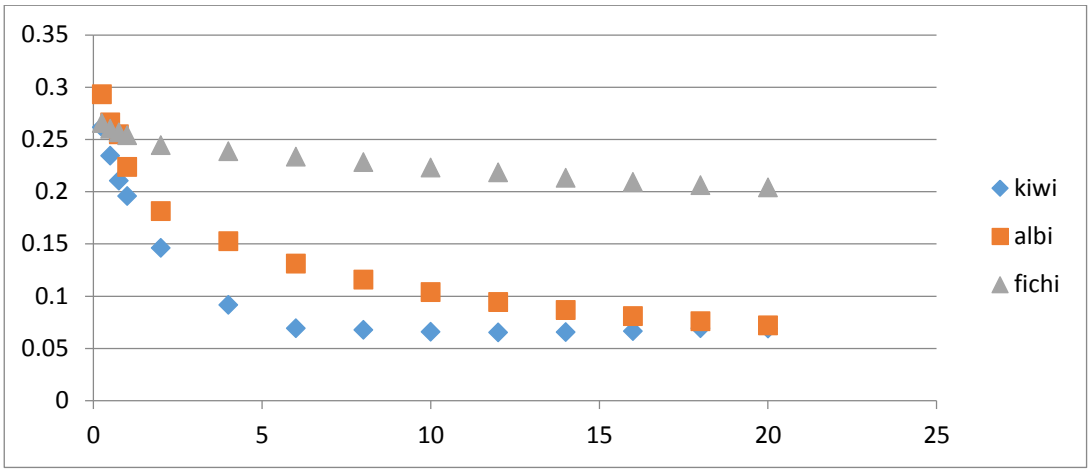
In the all jam samples, the following classes of polyphenols were identified: caffeic acid derivatives, flavonols and catechins; the compounds were quantified using chlorogenic acid, quercetin 3-O-glucoside, kaempferol 3-O-glucoside and catechin as references compounds. Table 4 reports the quantitative data of fig jam compared to commercial jams.

Table 4: hydroxycinnamic acid derivatives, catechins, flavonoids, and total polyphenols content (mg/g) of fig, apricot and kiwi jams. n.d. = not determined

jam	Hydroxycnnamic acid derivatives	Catechins	Flavonoids	Total polyphenols
Fig	n.d.	n.d.	0.01	0.01
Kiwi, plums, dog rose and hawthorn	0.27	n.d.	0.34	0.61
Apricot	0.08	0.20	0.03	0.31

The kinetics of the reduction of the DPPH radical is showed in Figure 1, in order to compare the efficiency of the individual extracts in quenching the stable DPPH radical.

Figure 1. kiwi= kiwi, plums, dog rose and hawthorn jam, albi= apricot jam, fichi= fig jam



From the figure it comes out that according to the polyphenols low content of fig jam, the antiradical activity is the lowest. The collected data can be a suitable base for the definition of commercial quality, and nutritional/functional properties of processed food containing fruit, spices and officinal plants.

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