Anatomical features, fatty acid profile and tocopherol content of the Tunisian *Cakile maritima* subsp. *maritima* Scop. Fruit

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1 ABSTRACT

This study reported for the first time anatomical features, fatty acid composition and tocopherol content for the fruits of *Cakile maritima* subsp. *maritima* Scop. collected from two sites located at the coastal part of North Tunisia (Bizerte and Soliman). Anatomical investigations characterized the indehiscent siliqua of Soliman population. Transverse sections through fresh fruit show a large number of prismatic crystals located at the three first layers of the mesocarp, which parenchyma was characterized by the presence of large amounts of starch grains and solitary oil drops. A particular anatomical structure is identified at the valves junction. The endocarp is a thin tissue composed of 2-3 layers of small lignified wall cells and one layer of tangentially elongated and thin cells surrounding the seed. Moreover, the fruit oil from both populations are characterized by their richness in unsaturated fatty acids, particularly monounsaturated ones. The major identified fatty acids with GC/MS analysis of fatty acid methyl esters for Bizerte and Soliman populations are oleic (20.20 ± 1.42 and $23.9 \pm 2.87\%$, respectively), erucic (20.82 ± 1.60 and $22.04 \pm 2.65\%$, respectively) and linoleic (24.09 ± 2.47 and $21.34 \pm 2.76\%$, respectively) acids. Besides, analysis of tocopherols allowed the identification of two isoforms (α - and γ -tocopherols). The α -tocopherol was found as the prominent one in the two fruit oils and was most important in Soliman population than in Bizerte one (31.13 ± 2.45 mg/kg against 28.88 ± 2.21 mg/kg).

2 INTRODUCTION

The Brassicaceae Burnett family, also called Cruciferae or mustard family, includes 25 tribes, about 338 genera and some 3,709 species (Al-Shehbaz et al., 2006). *Cakile* Mill. genus belongs to the Brassicaceae DC. tribe (Gabr, 2018). The species number of this genus is undefined and varied from 6 to 15 ones (Warwick and Sauder,

2005). In Tunisia, according to Le Floch et al. (2010) and to Dobignard and Chatelain (2011), this genus is represented by one species *Cakile maritima* subsp. *maritima* Scop. synonym of *C. aegyptiaca* Willd., *C. latifolia* Poir., *C. edentulata* Jord., *C. littoralis* Jord. and *C. maritima* subsp. *maritima* Willd.) Nyman. It is a glabrous,

succulent annual herb. The segmented fruit is a silique consisting of 2 monospermous parts (Pottier-Alapetite, 1979; Cordazo, 2006; Davy *et al.*, 2006; Merchaoui *et al.*, 2016). It is known as sea rocket growing along sandy coasts and so reported as an obligate halophyte tolerant to salt spray (Munns and Termaat, 1986). In Tunisia, *C. maritima* subsp. *maritima* is typical of coastal dune vegetation, abundant around sandy beaches from North to South (Pottier-Alapetite, 1979). In addition to its ecological role in coastal dune and saline soil stabilization (Davy *et al.*, 2006), *C. maritima* subsp. *maritima* was reported having medicinal and therapeutic properties (Ksouri *et al.*, 2012; Merchaoui *et al.*, 2016). All parts of the plant have a high antioxidant capacity (Merchaoui *et al.*, 2016; Omer *et al.*, 2019). Previous studies reported its antibacterial, antifungal, and molluscicidal activities (Casal and Casal, 2004; Sellam *et al.*, 2007; Meot-Duros *et al.*, 2008; Radwan *et al.*, 2008; Cerniauskaite, 2010; Ksouri *et al.*, 2012; Omer *et al.*, 2016) and its nutritive values (Hedrick, 1972; Cornucopia, 1990; Guil-Guerrero *et al.*, 1999). Several research works were carried out concerning this species; in particular the effects of salinity on germination, growth and seed production (Debez *et al.*, 2004; Megdiche *et al.*, 2007; Ghars *et al.*, 2009; Debez *et al.*, 2018), its ecophysiology (Ellouzi *et al.*, 2011; Debez *et al.*, 2012, 2013; Ben Hamed *et al.*, 2014, 2016; Belghith *et al.*, 2018; Arbelet-Bonnin *et al.*, 2018, 2019), its antioxidant activity (Ben Amor *et al.*, 2006, 2007; Ksouri *et al.*, 2007; Meot-Duros *et al.*, 2008; Ellouzi *et al.*, 2011; Ben Mansour *et al.*, 2018; Fuochi *et al.*, 2019), polyphenol (Ksouri *et al.*, 2007; Ben Mansour *et al.*, 2018) and

flavonoid constituents (Shams *et al.*, 2010). Other works have been focused on the allelopathic effects of its extracts (El-Amier and Abdullah, 2014). Besides, Clausing *et al.* (2000) and Kadereit *et al.* (2005) have studied its historical biogeography and phylogeography. Gandour *et al.* (2008) highlighted the genetic diversity of this species around Tunisian coasts. Zarrouk *et al.* (2003), Ghars *et al.* (2006) and Gandour *et al.* (2011) determined the composition of the lipid fraction isolated from the Tunisian *C. maritima* seed oil and mentioned it as a potential source of seed oil for industrial use. To the best of our knowledge no work has previously been done on *C. maritima* mature fruit oil except this of Gandour *et al.* (2011) where authors worked on immature fruits from plants cultivated in greenhouse and not on natural and spontaneous populations. Therefore, the present research was conducted, in the objective to determine fatty acid and tocopherol contents of the oil extracted from fruits collected on two wild populations of the Tunisian *C. maritima* growing in two localities in Tunisia (Soliman and Bizerte). In addition, this present research work gives anatomical characteristics of the *C. maritima* fruit knowing that previous histological studies of this species have only concerned the leaf anatomy of *C. maritima* Scop. subsp. *maritima* (Ciccarelli *et al.*, 2010), the stem and the leaf anatomy of *C. maritima* Scop. subsp. *aegyptiaca* (Willd.) (Gabr, 2017) and the root, stem and leaf anatomy of *C. maritima* Scop. subsp. *euxina* (Pobed.) Nyár. (Jianu *et al.*, 2014). Our results would add valuable information to the existing knowledge on the phytochemistry and the anatomical features of fruit.

3 METHODOLOGY

3.1 Plant material: Fresh fruits of *C. maritima* were collected at maturity in July 2018 from two salty Tunisian coastal sites; Soliman (North East of Tunisia, semi-arid bioclimatic stage, 36°42' N, 10°29' E) and Bizerte (Extreme North of Tunisia, sub-humid bioclimatic stage, 37°16' N, 9°52' E). Voucher specimens (*Cmm*1-

5) identified by the botanist Prof. Fethia Harzallah-Skhiri were deposited in the herbarium of the Laboratory of Bioresources: Integrative Biology and Valorization (LR14-ES06), High Institute of Biotechnology of Monastir, Tunisia. The harvested material was shade-dried then grounded into a

homogenous fine grade powder using an electric grinder (Duronc CG 250 Premium 250 W). Powder samples were stored at 2 °C until further analyses. Also, some siliques collected on Soliman population are kept aside for fruit anatomical studies.

3.2 Anatomical studies: Anatomical investigations were carried out on transversal sections (20-30 µm in thickness) of fresh fruits prepared previously using a razor blade for a free hand cutting, according to the usual techniques (Cutler *et al.*, 2008). All the sections were bleached in sodium hypochlorite solution (15%) for 10-15 min, washed three times in water and successively rapidly in water/acetic acid 5% (v/v) and stained in alun carmine-green iodine combination for 15 min. The best sections were washed in distilled water and mounted on glass slides using glycerine gel. Sections were examined under a light VWR Microscope and representative fields were selected and photographed with the camera attached to the microscope at a magnification of $\times 100$ and $\times 400$. For the description purpose, we used some help books (Esau, 1977; Fahn, 1979, 1990).

3.3 *Cakile maritima* fruit oil extraction: Fifty g of each sample fruit powder (dried fruits containing seeds) were placed into a cellulose paper cone and extracted using hexane in a Soxhlet apparatus for 8 h (18–22 cycles/h). Extractions were performed at least three times. The hexane was removed with a rotary vacuum evaporator in a water bath at 40°C. The total oil recovered was weighed and stored at -20°C until analysed. The oil yield obtained from 100 g of fruit powder was determined to calculate the lipid content. The result is expressed as the lipid percentage in the mature fruits containing seeds powder.

3.4 Fatty acid Methyl Esters (FAMES) analysis: Fatty acid methyl-esters (FAMES) of the total lipids were prepared according to the IUPAC 2.301 IUPAC 2.301 (1992) standard procedure. The lipid extract was esterified to form FAMES, which are quantified by gas chromatography-mass spectrometry (GC/MS). Thus, 200 µL aliquot of the oil was dissolved in

2 mL of hexane and introduced into a test tube. Then, 0.5 mL of sodium methylate (CH_3ONa , 3% in methanol) was added. The resulting mixture was stirred and 0.2 mL of 1 N H_2SO_4 and 1.5 mL of sodium chloride were added. The reaction mixture was agitated for 1 min. After decantation, 1 µL of the recovered hexane phase containing FAMES was analysed in splitless mode.

3.5 Chromatographic analysis: The gas chromatography mass spectrometry (GC/MS) analyses were performed on a gas chromatograph HP 6890N interfaced with an HP 5975B mass spectrometer (Agilent Technologies, Palo Alto, California, USA) with electron impact ionization (70 eV). A Trace-FAME capillary column (100 m $\times$ 0.25 mm $\times$ 0.25 µm film thickness; Thermo Scientific, Waltham, MA, USA) was used for the FAMES separation. The oven temperature was programmed to 100°C for 5 min, raised to 240°C at a rate of 5°C/min and held isothermal for 15 min. The carrier gas was helium with a flow rate of 1.2 ml/min. Scan time and mass range were 1s and 45-500 m/z, respectively. Identification of FAMES was made by using the NIST and Wiley GC/MS library and by comparing their retention times with those of 37 authentic standards purchased from Sigma-Aldrich (Steinheim, Germany). The percent of FAMES were calculated with reference to the total fatty acids.

3.6 Tocopherol analysis: Tocopherols composition was determined by High Performance Liquid Chromatography (HPLC) according to the standard method ISO 9936 (2012). Sample solutions were prepared by dissolving 4 g of oil in 25 mL of *n*-heptane and filtered through 0.45 µm PTFE filter (Polytetrafluoroethylene) prior to injection. The HPLC system was equipped with an HP Agilent 1200 pump (Palo Alto, CA), coupled with an Agilent 1100 Series fluorescence detector set at the wavelengths $\lambda = 295$ and 330 nm for excitation and emission, respectively. A 20 µL of each sample solution and calibrated standard solutions (containing α -, β -, γ -, δ -tocopherols with varied concentrations (3-6

µg/mL) were injected. The column was a normal phase YMC-Pack SIL column (250 × 2 mm ID, 5 µm; YMC Co., Kyoto, Japan). Eluent used was a 3.85% (volume fraction) of THF solution in *n*-heptane at a flow rate of 1ml/min.

3.7 Statistical analysis: The analyses are made in triplicate and the results are expressed as mean value ± standard deviation. The data

were analysed using analysis of variance (ANOVA). The significance of the differences between means were determined at *p* values < 0.05 using Duncan's multiple range test and by calculating Standard Error (SE) of various treatments. All analyses were performed using SPSS version 18.0 for windows.

4 RESULTS AND DISCUSSION

4.1 Anatomical features of *Cakile maritima*: *Cakile maritima* fruit is an indehiscent heteroarthrocarpic siliqua in an oblong shape with a joint that divides the fruit laterally into two superposed asymmetric segments supporting 2 lateral horns. The joint region has been defined as the articulating surface between the two fruit segments which may abscise at maturity containing the seed (Al-Shehbaz, 1985). The proximal and the distal segments of the indehiscent siliqua are monospermous (each one contains only one viable seed) and sizes of the distal segment and of its corresponding seed are more important than those of the proximal one. Transverse section through the siliqua of *C. maritima* shows the two valves of the ovary which margins are retained in the replum, and separated by the occurrence of the seed in the center (Fig. 1a). Ovary pericarp surrounding developing seed is differentiated into three major tissue types: an external exocarp, a central mesocarp and an internal endocarp. The exocarp is constituted of a one layer of arranged rectangular, or circular cells. Most of them are large and isodiametric. A thin cuticle covers the surface of this tissue (Figs. 1b, c). The mesocarp is composed of 8-12 layers of thin-walled parenchymous cells. The four first layers constitute the chlorenchyma

with isodiametric large cells. The successive parenchyma tissue was formed by large radially elongated thin wall cells, in which are diffused the vascular bundles (Fig. 1d). The innermost layer was rather constituted by large tangential cells. The first large parenchyma cells were characterized by the presence of large amounts of starch grains and solitary oil drops (Fig. 1e). A conspicuous observation was made at the occurrence of large idioblasts constituted by prismatic crystals (Figs. 1c, f) refracting and shining in the subepidermic cells of this halophyte plant. Those appeared as dense globular form, and the constituent units are like glass flakes. They generally form within cells, in our study, they may be extracellular. He *et al.* (2014) reported the occurrence of a large categories of biominerals in plants such as calcium oxalate crystals, calcium carbonate, and silica. Those authors mentioned that functions of biominerals may depend on their shape, size, quantity, and localization. Calcium oxalate is the most abundantly biomineral present in higher plants, found in a variety of defined shapes such as raphide, druse, styloid, prismatic and sand crystals (Nakata, 2003, 2012). Their occurrence in *C. maritima* has not been reported earlier.

Fig. 1. Cross sections of *Cakile maritima* immature fresh fruit. (a) Cross section of the fruit derived from silique valves and the one immature seed in the loculus. Two fused carpels with replum formed from persistent placenta and connected by false septum (valves are equivalent to the mature ovary walls) ($\times 100$), (b, c) Valve tissues under increased magnification, showing endocarp1, endocarp2 and mesocarp. The last tissue contains the vascular bundles. The replum or the region of valve joint, showing parenchyma lignified wall cells, vascular bundles in arch disposition, located in inner

region of the joint zone. The exocarp and the subepidermic cells containing crystal idioblasts ($\times 400$), (d) Detail of the vascular bundle ($\times 400$), (e) Magnified view of chlorenchyma cells showing oil drops and some starch grains ($\times 600$), (f) Magnified view of the crystal idioblasts shining and birefringent ($\times 600$) (g) The unique seed in one loculus limited by the endocarp2. The seed is protected by layers forming the testa. Oil drops and starch grains are identified in testa and cotyledon ($\times 100$), (h) Detail of the testa, endocarp and disposition of vascular bundles ($\times 400$).

arc of vb. - vascular bundle of the joint region, ci. - crystals idioblasts, cot. - cotyledon, em. - embryo, en. - endocarp, en1. - endocarp1, en2. - endocarp2, ex. - exocarp, fsep - false septum, lp. - lignified parenchyma, loc. - loculus, mc. - mesocarp, mvb. - middle vascular bundle, odr. - oil drops, ph. - phloem, rep. - replum, se. - seed, s. - starch, t. - testa, - v. - valve, vb. - vascular bundle, xy. - xylem

The sole work of Ciccarelli *et al.* (2010) reported the observation of idioblasts in leaves of *Cakile maritima* subsp. *maritima* Scop., but their shapes and size are different from our find. Anitha and Sandhiya (2014) and Leme and Scremin-Dias (2014) identified also in leaves from studied plants structure crystals which looks like those observed in our fruit samples. Côté (2009) studied the diversity and distribution of idioblasts producing calcium oxalate crystals in *Dieffenbachia seguine* and observed scattered among pollen, prismatic crystals which are also difficult to see strongly birefringent under crossed polarizers. Contrariwise, the same structures are reported in *Euonymus* sp., Celastraceae shrub (Schweingruber *et al.*, 2012) and in parenchyma cells for *Mangifera indica* L. wood samples (Gupta *et al.*, 2017). Those authors think that their occurrence can be accounted to the adaptation of the plant to the local microclimatic conditions or side effect due to mining stress. Abd Elhalim *et al.* (2016) noted an increase in the number of calcium oxalate crystals in *Zygophyllum album* L. organs and reported that this feature is related to xerophytic adaptation and that calcium ions increase plant salt tolerance. Plants induce calcium oxalate crystal formation in the aim to remove excess of biologically active calcium when other mechanism has become saturated (Borchert, 1985, 1986; Franceschi and Horner, 1980; Pennisi and McConnell, 2001; Volk *et al.*, 2002; Faheed *et al.*, 2013). A characteristic anatomy was distinguished for the tissues at the valves junction (replum). In fact, this region is constituted by a cluster of wall lignified cells directed to the exocarp. On the opposite side, 5-6 vascular bundles were observed arranged in the form of an arch, constituted by phloem

superposed by xylem (Figs. 1b, c). The last tissue was surrounded by lignified wall parenchyma cells. A single bundle is observed towards the inside near the loculus. Bundles are collateral and in primary type. The endocarp is a thin tissue composed of 2-3 layers of small lignified cells (end1) and one layer of tangential elongated and thin cells surrounding the seed (end2) (Figs. 1g,h). The seed coat typically consists of 8-9 layers constituted by cellulosic walled cells including the outer and inner testa (Figs. 1g, h). The first 3 layers are broad thin-walled, the outermost one is distinguished by its isodiametric large cells recovered with a sinuous wall. The following 5 layers are less wide and tangentially elongated. The innermost layer is larger.

4.2 Fruit oil content and fatty acid composition: Expressed on the dry weight basis, fruit oil content was $9.89 \pm 0.06\%$ for *C. maritima* population from Soliman and $8.41 \pm 0.04\%$ for those from Bizerte. The fruit oil contents from the both Tunisian coastal sites are slightly different. GC analysis of FAMES from the two fruit oils revealed the presence of eleven saturated, monounsaturated and polyunsaturated fatty acids with some differences according to the locality of harvest (Table 1). The fruit oils from both Bizerte and Soliman populations, are characterized by their richness in unsaturated fatty acids (UFA) (82.16 and 77.9% , respectively), particularly monounsaturated ones (MUFA) (45.57 ± 4.06 and $49.74 \pm 5.21\%$, respectively). *C. maritima* fruit oils were characterized by an unsaturated/saturated (U/S) ratio of 4.61 ± 0.51 and 3.53 ± 0.34 , respectively. A high ratio of U/S is regarded favourable for the reduction of serum cholesterol and atherosclerosis and

prevention of heart diseases (Oomah *et al.*, 2002). The most relative abundant saturated fatty acid (SAFA) detected in fruit oil from the two populations of *C. maritima* is palmitic acid (11.57 ± 1.34 and $16.38 \pm 2.05\%$, respectively). The other three SAFA are at less important percentages varying from 0.19 ± 0.02 to $3.61 \pm 0.35\%$ and from 0.14 ± 0.02 to $4.72 \pm 0.61\%$, respectively. The two oils are rich in unsaturated fatty acids represented particularly by oleic (C18:1 n-9), erucic (C22:1, cis-13), and linoleic (C18:2 n-6) acids varying from 20.20 ± 1.42 to $24.09 \pm 2.47\%$. Oleic acid is 20.20 ± 1.42 and $23.9 \pm 2.87\%$, respectively, while linoleic acid is 24.09 ± 2.47 and $21.34 \pm 2.76\%$, respectively. The erucic acid is also abundant (20.82 ± 1.60 and $22.04 \pm 2.65\%$, respectively). Fruit oil from Soliman is richer in palmitic, stearic, oleic, 11-eicosenoic and erucic acids. Fruit oil from Bizerte is particularly characterised by palmitoleic, linoleic, arachidic, and α -linolenic acids. Comparing our results with the sole work done on fruits (Gandour *et al.* 2011), but immature ones, we reported differences in the percent of the majority of the fruit oil fatty acids, particularly palmitic, oleic, 11-Eicosenoic, α -Linolenic and erucic acids. Linoleic and α -linolenic acids prevent deficiency symptoms and cannot be synthesized by humans (Paola Benatti *et al.*, 2004). According to Russo (2009),

they have an important cardioprotective effect in the secondary prevention of sudden cardiac death due to arrhythmias. While, oleic acid has been described as a regulator of immune function and health (Miles and Calder, 1998). Sales-Campos *et al.* (2013) demonstrated that oleic acid, which is naturally found in olive oil and is a major component of the Mediterranean diet, presents different properties that can be useful both in the immunomodulation, treatment and prevention of different types of disorders such as cardiovascular or autoimmune diseases, metabolic disturbances, skin injury and cancer. However, fruit oil of *C. maritima* from Bizerte and Soliman contains high proportions of erucic acid (20.82 and 22.04%, respectively), a compound naturally produced in green plants, and especially in members from the Brassicaceae (Sahasrabudhe, 1977; Economic Research Service USDA Crambe, 1996; Anneken, 2006). It is considered as anti-nutritional for both humans and animals (Rajcan *et al.*, 1999), but widely used in the industry (Domergue *et al.*, 1999; Kaushik and Agnihotri, 2000). According to Karleskind (1996), Zarrouk *et al.* (2003) and Ghars *et al.* (2006), seeds of *C. maritima* produce an oil which contains a high level of erucic acid exceeding 25%.

Table 1: Fatty acid profile (as % of total fatty acids) of fruit oil from two Tunisian populations of *Cakile maritima* (Bizerte and Soliman).

Fatty acid	RT (min)*	Fatty acid area (%)	
		Bizerte	Soliman
Saturated			
Palmitic acid (C16:0)	24.42	11.57 ± 1.34 ^a	16.38 ± 2.05 ^b
Stearic acid (C18:0)	28.06	2.42 ± 0.20 ^a	4.72 ± 0.61 ^b
Arachidic acid (C20:0)	30.23	3.61 ± 0.35 ^a	0.81 ± 0.11 ^b
Behenic acid (C22:0)	34.9	0.19 ± 0.02 ^a	0.14 ± 0.02 ^a
Monounsaturated			
Palmitoleic acid (C16:1 n-7)	25.17	2.37 ± 0.19 ^a	0.23 ± 0.03 ^b
<i>cis</i> -Vaccenic acid (C18:1 n-7)	29.23	0.95 ± 0.08 ^a	0.64 ± 0.07 ^a
Oleic acid (C18:1 n-9)	28.77	20.20 ± 1.42 ^a	23.9 ± 2.87 ^a
11-Eicosenoic acid (C20:1 n-9)	31.95	1.23 ± 0.04 ^a	2.93 ± 0.26 ^b
Erucic acid (C22:1, <i>cis</i> -13)	35.14	20.82 ± 1.60 ^a	22.04 ± 2.65 ^a
Polyunsaturated			
Linoleic acid (C18:2 n-6)	29.68	24.09 ± 2.47 ^a	21.34 ± 2.76 ^a
<i>α</i> -Linolenic acid (C18:3 n-3)	30.95	1 2.5 ± 1.28 ^a	6.82 ± 0.75 ^b
SAFA		17.79 ± 0.97 ^a	22.05 ± 2.07 ^b
MUFA		45.57 ± 4.06 ^a	49.74 ± 5.21 ^b
PUFA		36.59 ± 2.65 ^a	28.16 ± 2.42 ^b
U/S		4.61 ± 0.51 ^a	3.53 ± 0.34 ^b

Means ± SD with the same letter (s) in the same line are not significantly different ($p > 0.05$)

* Retention time on TRACE TR-225 GC Column, **SAFA** - saturated fatty acids, **MUFA** - monounsaturated fatty acids, **PUFA** - polyunsaturated fatty acids, **U/S** - unsaturated/saturated ratio.

Content in oil for fruit was less important than this reported for seeds by Zarrouk *et al.* (2003), Ghars *et al.* (2006) and Gandour *et al.* (2011). In fact, the fruit contains a low number of seeds. In our study we have counted a mean number of 2 per silique. The amount of oil found corresponds to the seeds and the fruit tissues that were not reported elsewhere. The occurrence of oil drops in mesocarp parenchyma cells and in seed teguments support our results. Then, based on these results, we considered that the *C. maritima* fruit oil can be used for nonedible purposes, and it might compete successfully with other plant oils as a source of fatty acids essentially for industrial applications.

4.3 Tocopherol content: Fruit oil content in tocopherol for the two *C. maritima* populations is presented in Table 2. Two isoforms, α -tocopherol and γ -tocopherol are detected and identified in all samples. δ -tocopherol and β -tocopherol are never found. Individual tocopherol contents exhibited some variations among the two populations. The α -tocopherol is the main one in the two fruit oils, moreover its content in Soliman population is higher than in Bizerte one (31.13 ± 2.45 mg/kg against 28.88 ± 2.21 mg/kg). Whereas, γ -tocopherol is better represented in fruit oil from Bizerte (4.48 ± 0.49 mg/kg) than in this from Soliman (3.27 ± 0.36 mg/kg) plant population.

Table 2. Tocopherol content (mg/kg) of fruit oil from two Tunisian populations of *Cakile maritima* (Bizerte and Soliman).

Identified Tocopherols	Bizerte	Soliman
	Concentration (mg/kg)	
α -Tocopherol	28.88 $\pm$ 2.21 ^a	31.13 $\pm$ 2.45a
γ -Tocopherol	4.48 $\pm$ 0.49 ^a	3.27 $\pm$ 0.36a
Total tocopherols (mg/kg)	33.37 $\pm$ 2.78a	34.42 $\pm$ 2.82a

Means $\pm$ SD with the same letter (s) in the same line are not significantly different ($p > 0.05$)

To treat with oxidative stress, plants have developed among others, two protective mechanisms, enzymatic and non-enzymatic detoxification. The latter involves vitamin E, namely tocopherols (α -tocopherol, β -tocopherol, γ -tocopherol and δ -tocopherol) and tocotrienols known by their antioxidant power (Alscher and Heath, 2002). In response to abiotic stress, plants accumulate α -tocopherol and γ -tocopherol in leaves (Munné-Bosch, 2005). According to Horvath *et al.* (2006), α -tocopherol is the predominant tocopherol form in photosynthetic tissues, while γ - and δ -tocopherols are predominant in seeds, fruits, and storage organs of Dicots. Vitamin E is an essential micronutrient found mainly in vegetable oils and in products derived from those oils (Wenneras *et al.*, 2013). Published data related to the tocopherol contents in the fruit oil of *C. maritima* are not available. Although, in Tunisia, the studies of Ellouzi *et al.* (2011, 2013) showed the positive role of tocopherols in stress tolerance. *C. maritima* can rapidly evolve physiological and antioxidant mechanisms to adapt to salt and manage the oxidative stress. They also specify that *C. maritima* has a large pool of α -tocopherols. In contrary, the pool of γ -tocopherols is less important. γ -tocopherols seem to respond to osmotic stress but not to

salt induced oxidative stress unlike the α -tocopherols. Besides, Goffman and Mollers (2000) revealed that the tocopherol content of *Brassica napus* L. oil seed consists of 64% γ -tocopherol, 35% α -tocopherol, and less content in δ -tocopherol. Likewise, α - and γ -tocopherols were the major isomers determined by Scialabba *et al.* (2010) in dry seeds of nine *Brassica* species, δ -tocopherol was present in traces and β -tocopherol was absent. Then, this study reported for the first time the anatomical features of *C. maritima* fruit with the occurrence of large crystal idioblasts, oil drops and some starch grains in the first layers of the mesocarp. Those biominerals should be calcium oxalate crystals produced by *C. maritima* to remove excess oxalate or calcium and so increase its salt tolerance. Furthermore the composition of *C. maritima* fruit oil is characterized by relatively high unsaturated fatty acids particularly oleic, erucic and linoleic acids. The cultivation of this plant can have significant industrial applications and become profitable economically. Indeed, the higher content of *C. maritima* fruit oil in tocopherols; specially α -tocopherol, permit to speculate that this species might be considered as a potential source of natural antioxidant. The oil must be extracted directly from the fruit without the procedure of extracting the seeds.

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