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Bioactive compounds and antioxidant activity of *Pimpinella anisum* L. accessions at different ripening stages

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ABSTRACT

Chemical composition and antioxidant activity of four aniseed populations (Egyptian, Serbian, Tunisian and Turkish) were investigated during three developmental stages. The highest oil yield was achieved at full maturity in all aniseed accessions ranged from 11.93% (Serbia) to 13.80% (Tunisia). Fatty acid profile of aniseed oil was characterized by high proportions of palmitic (4.90–57.18%) and petroselinic (10.48–46.60%) acids which had an antagonist evolution during maturation. The essential oil yield reached its maximum at the beginning of ripening process in all aniseed accessions ranged from 1.94% (Serbia) to 3.09% (Tunisia). The main essential oil compound was *trans*-anethole (66.34–93.05%) during aniseed ripening in all accessions. Phenolic content patronized its maximum at the last stage of aniseed ripening ranged from 17.11 mg GAE/g DW (Serbia) to 25.16 mg GAE/g DW (Tunisia). The main phenolic compound of aniseed was naringin (17.55–32.49%) and its accumulation was followed by the reduction of gallic, rosmarinic, ellargic and syringic acids during aniseed ripening in all accessions. Concerning antioxidant activity, DPPH scavenging activity, chelating ability and reducing power were maximal at full maturity in all aniseed accessions. Our findings indicate that the determination of optimal periods and provenances for antioxidant accumulation can be used to evaluate the quality of aniseeds and could be important for industries.

1. Introduction

Several *Apiaceae* seeds are primarily grown for their use as spices such as anise (*Pimpinella anisum*), caraway (*Carum carvi*), coriander (*Coriandrum sativum*), cumin (*Cuminum cyminum*) and fennel (*Foeniculum vulgare*). These spices have been indispensable in the culinary art of seasoning and flavoring foods since ancient times (Bhagya et al., 2017). Additionally, they are also used in indigenous medicines, pharmaceuticals, nutraceuticals, aroma therapy, preservatives, natural colours, perfumes, cosmetics and biopesticides. These properties are due to diverse chemical arrays synthesized by these spices (Bhagya et al., 2017). Essential oils of spices are complex mixtures of volatile substances, ordinarily terpenes and terpene derivatives. They are the most important class of aroma compounds, especially monoterpenes which helps in contributing fragrance almost for about 90 per cent of spices (Lampe, 2003). Furthermore, spices are sources of antioxidants belonging to a large group of bioactive compounds which consist of flavonoids, phenolic compounds, sulfur-containing compounds, tannins, alkaloids, phenolic diterpenes, and vitamins. Some spices are sources of polyunsaturated fatty acids as the case of *Apiaceae* seeds (Charles, 2013; Yashin et al., 2017). Typical of the *Apiaceae* seeds, the major fatty acid component in oils is petroselinic acid, instead of oleic acid. Apart oleic acid, trace of another isomer *cis*-vaccenic acid is also detected (Nikolova et al., 1985; Denev et al., 2011).

Anise (*Pimpinella anisum* L.), one of the *Apiaceae* seeds, is commonly used as a spice in the Mediterranean area. Aniseeds have been also used as a traditional medicine in many cultures to treat various medical conditions and they are commonly recommended as antioxidant, antiseptic, antimicrobial, aperitif, digestive, antispasmodic, expectorant, galactogogue, estrogenic, anti-inflammatory and diuretic agents (Boskabady and Ramazani-Assari, 2001; Shojaii and Fard, 2012). Aniseeds contain 2–6% of essential oil consisting primarily of *trans*-anethole (Christaki et al., 2011) and also as much as 9.82–11.60% of oil rich in fatty acids, especially petroselinic acid (Bettaieb Rebey et al., 2017). Aniseeds also contain considerable amounts of phenolic com-

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Table 1
Physical characteristics of aniseeds (*Pimpinella anisum* L.) during maturity.

Harvest dates	Fruit colors, state of maturity				Dry weight (%)				Relative moisture content (%, w/w)			
	Turkey	*Serbia*	*Egypt*	*Tunisia*	*Turkey*	*Serbia*	*Egypt*	*Tunisia*	*Turkey*	*Serbia*	*Egypt*	*Tunisia*
20 June 2016	Immature, unripe, fully green-brown fruits				12.15^{cB}	10.82^{cC}	13.91^{cA}	13.08^{cA}	87.85^{aA}	89.18^{aA}	86.09^{aA}	86.92^{aA}
28 June 2016	Intermediate, half ripe, brownish fruits				58.28^{bA}	52.36^{bB}	56.44^{bA}	57.36^{bA}	41.72^{bB}	47.64^{bA}	43.56^{bB}	42.64^{bB}
06 July 2016	Full matured, brown and dried fruits				77.92^{aB}	81.72^{aA}	80.64^{aA}	82.57^{aA}	22.08^{cA}	18.28^{cB}	19.36^{cB}	17.43^{cC}

The data marked with the different small letter, for the ripening stage, and capital letter, for the provenance, in the table, value share significant differences at $P < 0.05$ (Duncan test).

Table 2
Changes in oil yield (%) and fatty acid composition during maturity of aniseed (*Pimpinella anisum* L.) accessions.

	Immature				Intermediate				Mature			
	Turkey	Serbia	Egypt	Tunisia	Turkey	Serbia	Egypt	Tunisia	Turkey	Serbia	Egypt	Tunisia
Oil yield (%)	**7.84^{cA}**	**7.15^{cA}**	**7.23^{cA}**	**6.77^{cB}**	**10.67^{bA}**	**9.62^{bA}**	**9.77^{bA}**	**10.09^{bA}**	**13.47^{aA}**	**11.93^{aB}**	**12.22^{aB}**	**13.80^{aA}**
C10:0	2.23^{aA}	2.18^{aA}	1.84^{aA}	1.77^{aA}	1.89^{aA}	1.75^{aA}	0.97^{bB}	0.83^{bB}	0.16^{bA}	0.10^{bA}	0.12^{cA}	0.16^{cA}
C12:0	1.17^{aA}	1.03^{aA}	1.09^{aA}	1.98^{aA}	0.99^{aA}	0.87^{aA}	0.67^{bA}	0.68^{bA}	0.56^{bA}	0.38^{bA}	0.40^{bA}	0.52^{bA}
C14:0	1.83^{aA}	1.62^{aA}	0.98^{aB}	0.87^{aB}	0.21^{bA}	0.34^{bA}	0.47^{bA}	0.09^{bA}	0.12^{bA}	0.10^{cA}	0.04^{cB}	0.07^{bB}
C16:0	57.18^{aA}	52.22^{aB}	50.93^{aB}	52.10^{aB}	34.18^{bA}	28.77^{bB}	29.52^{bB}	22.37^{bB}	5.66^{cB}	10.91^{cA}	12.16^{cA}	4.90^{cB}
C18:0	3.161^{aB}	4.88^{aA}	3.52^{aA}	4.01^{aA}	2.27^{bA}	3.46^{bA}	2.81^{aA}	3.08^{bA}	1.23^{cA}	0.94^{cA}	0.71^{bA}	0.85^{cA}
C20:0	0.31^{aA}	0.27^{aA}	0.09^{aA}	0.06^{aA}	0.22^{aA}	0.19^{aB}	0.03^{aB}	0.07^{aB}	0.11^{aA}	0.02^{aA}	0.04^{aA}	0.07^{aAcc}
C18:1 Δ6	10.48^{cB}	11.11^{cB}	14.46^{cA}	13.57^{cA}	28.51^{bC}	30.42^{bC}	33.51^{bB}	38.20^{bA}	45.17^{aA}	41.32^{aB}	40.22^{aB}	46.60^{aA}
C18:1 Δ9	7.38^{cB}	9.12^{cA}	9.23^{cA}	7.99^{cB}	10.17^{bB}	12.40^{bA}	13.04^{bA}	12.99^{bA}	19.44^{aA}	22.11^{aA}	18.32^{aB}	21.05^{aA}
C18:2	12.57^{cB}	12.23^{cB}	13.51^{bA}	12.52^{cB}	18.32^{bA}	18.66^{bA}	13.98^{bB}	19.17^{bA}	23.99^{aA}	20.40^{aB}	25.17^{aA}	22.99^{aA}
C18:3	0.33^{aB}	0.23^{aB}	0.95^{aAB}	1.72^{aA}	0.09^{bA}	0.02^{bA}	0.05^{cA}	1.02^{bA}	0.03^{bB}	0.12^{aB}	0.42^{bA}	1.07^{bA}
SFA (%)	65.88^{aA}	62.20^{aA}	58.45^{aB}	60.79^{aB}	39.76^{bA}	35.38^{bA}	37.28^{bA}	27.48^{bB}	7.84^{cB}	12.45^{cA}	13.47^{cA}	6.57^{cB}
UFA(%)	30.76^{cB}	32.69^{cB}	38.15^{cA}	35.80^{cA}	57.09^{bB}	61.50^{bB}	60.58^{bB}	71.38^{bA}	88.63^{aA}	83.95^{aA}	84.13^{aA}	91.71^{aA}
PUFA(%)	12.90^{cB}	12.46^{cB}	14.46^{bA}	14.24^{cA}	18.41^{bA}	18.68^{bA}	14.03^{bB}	20.19^{bA}	24.02^{aA}	20.52^{aB}	25.59^{aA}	24.06^{aA}
SFA/PUFA	5.10^{aA}	4.99^{aA}	4.04^{aA}	4.26^{aA}	2.15^{bA}	1.89^{bA}	2.65^{bA}	1.36^{bB}	0.32^{cA}	0.60^{cA}	0.52^{cA}	0.27^{cA}

Capric acid (C10:0) Lauric acid (C12:0) Myristic acid (C14:0) Palmitic acid (C16:0) Stearic acid (C18:0) Arachidic acid (C20:0) Saturated fatty acid (SFA) Petroselinic acid (C18:1 Δ6) Oleic acid (C18:1 Δ9) Linoleic acid (C18:2) Linolenic acid (C18:3) Saturated fatty acid (SFA); Unsaturated fatty acid (UFA); Polyunsaturated fatty acid (PUFA); The data marked with the different small letter, for the ripening stage, and capital letter, for the provenance, in the table, value share significant differences at $P < 0.05$ (Duncan test).

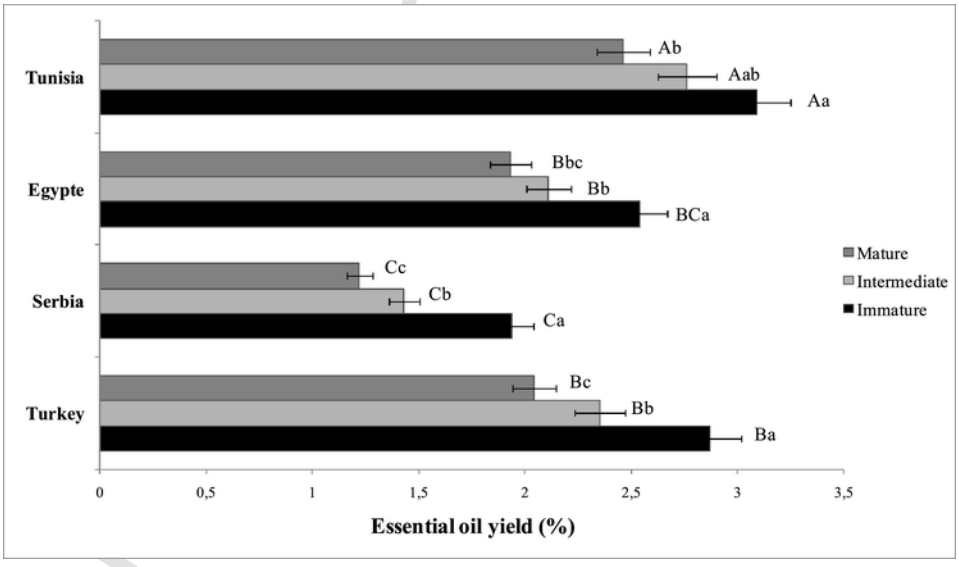

Fig. 1. Essential oil yield of *Pimpinella anisum* seed accessions at three stages of maturity. The data marked with the different small letter, for the ripening stage, and capital letter, for the provenance, in the table, values share significant differences at $P < 0.05$ (Duncan test).

pounds including phenolic acids (El-Wakeil et al., 1986; Bettaieb Rebey et al., 2017), flavonoids (Bettaieb Rebey et al., 2017) and flavonol glycosides (EI-Moghazi et al., 1979). In general, the function of these phytochemicals is to attract beneficial, keep harmful organisms and respond to environmental changes (Bhagya et al., 2017). So, they play a major role in the adaptation of plants to the environment and in overcoming stress conditions (Ramakrishna and Ravishankar, 2011; Mohammadi et al., 2018). The composition of these phytochemicals can be affected by internal (developmental and genetic) and external (environmental) factors. Fruit quality traits are crucial factors that influence fruit crop breeding programs (Shiraishi et al, 2018). Several investigations have reported the provenance effect on the chemical composition of several *Apiaceae* seeds such as *Caraway carvi* (Laribi et al., 2013), *Coriandrum sativum* (Laribi et al., 2015), *Cuminum cyminum* (Bettaieb Rebey et al., 2011), *Pimpinella anisum* (Bettaieb Rebey et al., 2017). There are some studies of *Apiaceae* seeds regarding the chemical composition variations as affected by maturation stages such as *Cuminum cyminum* (Bettaieb Rebey et al., 2013, 2014) and *Coriandrum*

Table 3
Essential oil composition (%) of aniseed (*Pimpinella anisum* L.) accessions during three stages of maturity.

Compounds*	RI[a]	RI[b]	Immature				Intermediate				Mature			
			Turkey	Serbia	Egypt	Tunisia	Turkey	Serbia	Egypt	Tunisia	Turkey	Serbia	Egypt	Tunisia
Terpene hydrocarbons			**1.12^{aA}**	**0.12^{cA}**	**0.09^{aA}**	**0.05^{bB}**	**1.65^{bA}**	**0.89^{bB}**	**0.08^{aC}**	**0.10^{aC}**	**2.06^{aA}**	**1.25^{aB}**	**0.04^{bD}**	**0.12^{aC}**
Linalool	1097	1557	1.12^{cA}	0.12^{cA}	0.09^{aA}	0.05^{bB}	1.65^{bA}	0.89^{bB}	0.08^{aC}	0.10^{aC}	2.06^{aA}	1.25^{aB}	0.04^{bD}	0.12^{aC}
Oxygenated Monoterpene			**0.09^{bA}**	**0.03^{bB}**	**0.12^{aA}**	**0.11^{aA}**	**0.11^{bA}**	**0.04^{bB}**	**0.01^{bB}**	**0.09^{aA}**	**0.18^{aA}**	**0.08^{aB}**	**0.03^{bC}**	**0.07^{aB}**
α-Terpinene	1018	1249	0.09^{bA}	0.03^{bB}	0.12^{aA}	0.11^{aA}	0.11^{bA}	0.04^{bB}	0.01^{bB}	0.09^{a}	0.18^{aA}	0.08^{aB}	0.03^{bC}	0.07^{a}
Phenylpropanoids			**80.68^{cD}**	**88.58^{cB}**	**90.08^{cA}**	**86.48^{cC}**	**85.65^{bC}**	**91.14^{bA}**	**91.94^{bA}**	**90.26^{bB}**	**87.83^{aB}**	**94.65^{aA}**	**93.89^{aA}**	**94.63^{aA}**
Anisole	918	1720	2.73^{aA}	2.33^{aA}	2.85^{aA}	1.92^{aB}	2.48^{aA}	1.22^{bC}	1.74^{bB}	1.45^{bC}	2.33^{aA}	1.09^{cB}	0.52^{cC}	1.01^{cB}
Estragole	1197	1430	8.14^{cA}	4.12^{aB}	5.71^{aB}	1.31^{aC}	10.25^{bA}	3.75^{bC}	5.62^{aB}	0.62^{bD}	12.70^{aA}	2.64^{cB}	3.82^{bB}	0.23^{cC}
trans-Anethole	1253	1740	66.34$^{c\ D}$	70.63^{cC}	79.25^{cB}	81.25^{cA}	70.24^{bC}	84.21^{bA}	83.38^{bB}	87.24^{bA}	72.80^{aC}	88.49^{aB}	89.21^{aB}	93.05^{aA}
p-Anisaldehyde	1250	1718	1.58^{aB}	1.15^{aC}	2.15^{aA}	1.95^{aA}	0.65^{bC}	0.89^{bB}	1.05^{bA}	0.85^{bB}	0.32^{cB}	0.44^{cA}	0.10^{cC}	0.18^{cC}
Cis-Isoeugenol	1359	2180	1.89^{cA}	0.35^{cB}	0.12^{bC}	0.05^{bC}	2.03^{bA}	1.07^{bB}	0.15^{bC}	0.10^{bC}	2.34^{aA}	1.99^{aB}	0.24^{aC}	0.16^{aC}
Sesquiterpene hydrocarbons			**4.28^{bC}**	**3.60^{aD}**	**5.02^{aB}**	**7.00^{aA}**	**4.27^{b}**	**3.02^{b}**	**3.60^{b}**	**5.10^{b}**	**4.40^{a}**	**2.75^{c}**	**2.43^{c}**	**4.48^{c}**
β-Elemene	1388	1465	0.01^{bC}	0.09^{aB}	0.12^{aA}	0.02^{cC}	0.02^{bC}	0.06^{bC}	0.09^{bA}	0.05^{bB}	0.09^{aA}	0.01^{cC}	0.07^{bB}	0.09^{aA}
γ-Himachalene	1484	1690	3.22^{aB}	2.45^{aC}	3.33^{aB}	3.95^{aA}	2.86^{bA}	2.22^{bA}	2.45^{bA}	2.78^{bA}	2.15^{cB}	2.04^{cB}	1.11^{cC}	2.58^{cA}
Zingiberene	1494	1672	0.35^{cA}	0.25^{aA}	0.09^{cB}	0.21^{cA}	0.65^{bA}	0.30^{aB}	0.25^{bC}	0.37^{bB}	0.94^{aA}	0.36^{aB}	0.32^{aB}	0.41^{aB}
β-Himachalene	1505	1942	0.14^{cA}	0.08^{aA}	0.05^{bB}	0.01^{bB}	0.22^{bA}	0.09^{aB}	0.08^{aB}	0.09^{aB}	0.45^{aA}	0.09^{aB}	0.10^{aB}	0.10^{aB}
β-Bisabolene	1506	1832	0.03^{cC}	0.64^{aB}	1.26^{aA}	0.01^{bC}	0.06^{bC}	0.22^{bB}	0.64^{cA}	0.22^{aB}	0.09^{aC}	0.05^{cC}	0.74^{bA}	0.23^{aB}
Isolongifolene	1532	2003	0.36^{aB}	0.03^{aC}	0.09^{aC}	1.52^{aA}	0.20^{bB}	0.03^{aC}	0.03^{bC}	0.56^{bA}	0.15^{bA}	0.02^{aB}	0.03^{bB}	0.09^{cA}
Diepi-α-cedrene	1575	2020	0.17^{bB}	0.06^{bC}	0.08^{aC}	1.28^{aA}	0.26^{bB}	0.10^{bC}	0.06^{bC}	1.03^{bA}	0.53^{aB}	0.18^{aC}	0.06^{bD}	0.98^{bA}
Total identified			**86.17^{cD}**	**92.33^{cB}**	**95.31^{bA}**	**93.64^{cC}**	**91.68^{bB}**	**95.05^{bA}**	**95.63^{bA}**	**95.55^{bA}**	**94.47^{aC}**	**98.73^{aB}**	**96.39^{aC}**	**99.30^{aA}**

Values are means of six replications (N = ±6 SD); The data marked with the different small letter, for the ripening stage, and capital letter, for the provenance, in the table, values share significant differences at $P < 0.05$ (Duncan test); RI[a] Order of elution in apolar column (HP-5); RI[b] Order of elution in polar column (HP Innowax), MS: mass spectrum; * Order of elution in HP-5 column.

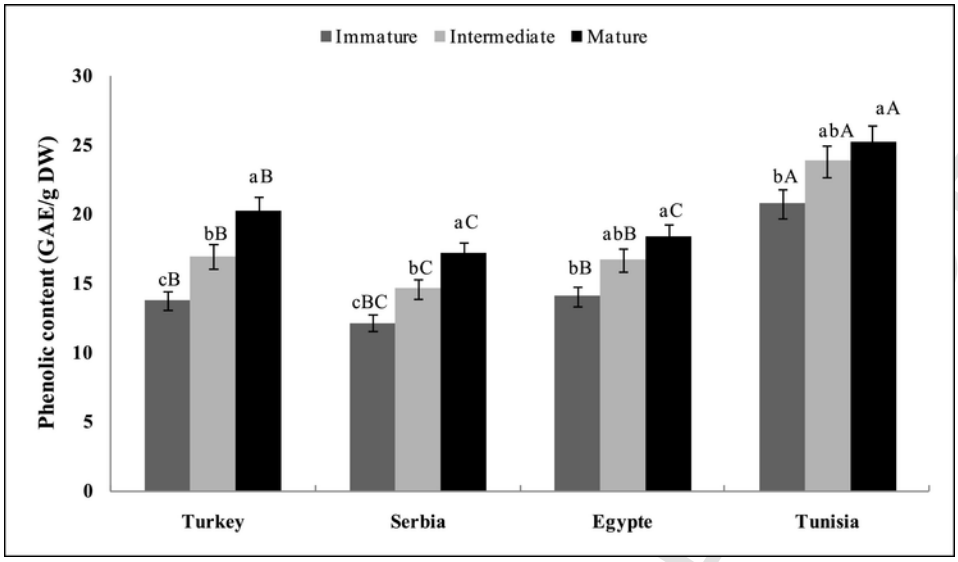

Fig. 2. Phenolic content of *Pimpinella anisum* seed accessions as affected by ripening stages. Values are means of six replications (N = ±6 SD); The data marked with the different small letter, for the ripening stage, and capital letter, for the provenance, in the table, values share significant differences at $P < 0.05$ (Duncan test).

Table 4
Antioxidant activity of aniseed (*Pimpinella anisum* L.) extracts as affected by maturity stages.

		Antioxidant assays		
		DPPH (IC_{50} µg/mL)	Chelating ability (IC_{50} mg/mL)	Reducing power (EC_{50} µg/mL)
Turkey	**Immature**	103.45^{cA}	108.08^{cA}	1234.28^{cB}
	Intermediate	42.97^{bA}	57.81^{bA}	947.05^{bB}
	Mature	18.97^{aAB}	12.46^{aAB}	684.73^{aB}
Serbia	**Immature**	153.18^{cB}	122.76^{cAB}	982.27^{cA}
	Intermediate	58.04^{bB}	68.09^{bB}	687.41^{bA}
	Mature	20.18^{aB}	10.89^{aA}	565.11^{aA}
Egypt	**Immature**	103.02^{cA}	130.55^{cB}	1080.39^{bA}
	Intermediate	62.17^{bC}	68.51^{bB}	764.22^{abAB}
	Mature	16.77^{aB}	13.17^{aB}	618.82^{aB}
Tunisia	**Immature**	96.21^{cA}	97.34^{cA}	1154.33^{cAB}
	Intermediate	35.27^{bA}	52.84^{bA}	755.88^{bAB}
	Mature	12.87^{aA}	9.23^{aA}	523.47^{aA}

Each value in the table was obtained by calculating the average of six experiments; The data marked with the different small letter, for the ripening stage, and capital letter, for the provenance, in the table, values share significant differences at $P < 0.05$ (Duncan test).

sativum (Msaada et al., 2009). Fewer studies have determined the effect of both provenance and maturation factors on the biochemical composition of *Apiaceae* seeds as the case of *Foeniculum vulgare* (Salami et al., 2017).

However, to the best of our knowledge, no report was found concerning the biochemical characterization of *Pimpinella anisum* populations originated from different countries at different maturity stages. In aniseeds, petroselinic acid and *trans*-anethole are considered as the most important components. For molecular studies, finding the variation of these compounds in different developmental phases is of great importance. Thus, this study aimed to investigate the variation of fatty acids, essential oils, phenolics and antioxidant activities of four aniseed accessions (Egypt, Serbia, Tunisia and Turkey) during three maturity stages in order to support its technological development.

2. Materials and methods

2.1. Plant material

Four anis (*Pimpinella anisum* L.) seed ecotypes of cultivated origin were used in this study. The first one (Tunisian aniseed) was harvested in June 2016 from the region of Korba in the northeast of Tunisia (latitude 38°22″(N); longitude 29°63″(E) and the altitude is 637 m). The precipitation average was 400–500 mm/year and the monthly average temperature was 17.7 °C. Whilst, the other seeds were imported, one from Serbia (Vojvodina, latitude 45°18″(N), longitude 20°09″(E), and altitude is 111 m), one from Egypt (Luxor, latitude 32°39″ (N); longitude 25°41″(E) and altitude is 82 m) and the other from Turkey (Burdur, latitude 37°43″(N), longitude 30°17″(E), and altitude is 950 m). Plant identification was carried by Professor Abderrzek Smaoui (Biotechnology Center in Borj-Cedria Technopole, Tunisia). A voucher specimen was deposited at the herbarium of the Laboratory of Bioactive Substances, Biotechnology Center in Borj-Cedria Technopole under the "BC2011-2002" number. The four ecotypes were cultivated under the same environmental conditions. The experiment was carried out in a greenhouse at day light (photoperiod varying from 12 to 16 h) and at a temperature varying from 16 to 22 °C during the day and from 10 to 12 °C during the night at the Biotechnology Center of Borj-Cedria Technopole (36°49″(N), 10°11″(E) and altitude is 28 m). The experimental design was the complete random blocks with six replications. Each ecotype sown area was of 10 m^2 (10 m × 1 m). Seeds were sown on December 12, 2016 with row spacing of 0.20 m and by respecting a density of 80 plants m^{-2}. Fertilization consisted of 200, 150 and 80 kg $ha^{-1}$of P_2O_5, K_2O and N, respectively, incorporated uniformly to the soil before sowing, and supplemented by 80 kg $ha^{-1}$of N brought twice during the crop cycle. Pre-irrigation was done immediately after sowing for uniform emergence and establishment of seedlings. Irrigation was done by submersion twice to three times per week. In addition, weeds were controlled by hand when needed. The fruits were harvested at three developmental stages including unripe fruit, half ripe and full ripe fruits. The samples were air-dried in the dark for 3 days at room temperature (25 °C, 30% relative humidity) and then reduced to a fine powder. Anise powder was then vacuum-packaged in nylon linear low density polyethylene pouches and stored in the dark at ambi-

Table 5
Changes of phenolic composition (%) during aniseed (*Pimpinella anisum* L.) maturation.

	Immature				Intermediate				Mature			
	Turkey	Serbia	Egypt	Tunisia	Turkey	Serbia	Egypt	Tunisia	Turkey	Serbia	Egypt	Tunisia
Phenolic acid	**43.24^{cA}**	**39.93^{bB}**	**40.25^{bB}**	**39.94^{cC}**	**40.52^{bA}**	**34.22^{bC}**	**31.67^{cD}**	**38.35^{bB}**	**36.83^{aB}**	**35.24^{aB}**	**37.49^{aAB}**	**37.15^{aA}**
Gallic acid	13.72^{bA}	12.33^{bA}	8.23^{aB}	–	18.89^{aA}	14.69^{aB}	10.08^{aC}	–	0.46^{cA}	0.34^{cA}	0.17^{bB}	0.08^{aB}
Chlorogenic acid	0.24^{cC}	0.47^{cC}	5.78^{cB}	13.54^{bA}	9.60^{bBC}	11.38^{bB}	12.86^{bB}	26.29^{aA}	22.18^{aA}	16.81^{aB}	17.56^{aB}	24.97^{abA}
Syringic acid	2.22^{aC}	2.93^{aB}	3.89^{aA}	2.19^{aC}	0.30^{bAB}	0.51^{bA}	0.82^{bA}	1.93^{bA}	0.15^{bB}	0.69^{bA}	0.85^{abA}	0.25^{bB}
p-Coumaric acid	1.23^{bB}	2.23^{bA}	1.00^{bB}	2.41^{bAB}	5.31^{aA}	2.38^{bB}	1.74^{aB}	2.27^{abB}	5.54^{aA}	3.63^{aC}	2.39^{aCD}	3.42^{aB}
Rosmarinic acid	16.56^{aA}	10.81^{bD}	12.34^{bC}	14.06^{aB}	1.83^{cB}	1.75^{cB}	2.88^{cA}	3.62^{cA}	8.43^{bB}	13.08^{aA}	15.33^{aA}	9.27^{bB}
Ellargic acid	9.27^{aB}	11.16^{aA}	9.01^{aB}	7.74^{aC}	4.59^{bA}	3.51^{bAB}	3.29^{bAB}	4.24^{bA}	0.07^{cB}	0.69^{cA}	1.19^{cA}	0.16^{cB}
Flavonoids	**44.11^{cB}**	**47.68^{bcA}**	**39.13^{bD}**	**42.28^{aC}**	**46.75^{bB}**	**45.22^{bB}**	**50.39^{aA}**	**43.24^{aC}**	**48.64^{aA}**	**49.01^{aA}**	**45.11^{aB}**	**43.83^{aB}**
Epicatechin-3-Ɵ-gallate	0.12^{bA}	–	0.22^{aA}	0.25^{bA}	0.30^{aB}	–	0.51^{aA}	0.93^{aA}	0.46^{aB}	0.08^{aBC}	0.59^{aB}	1.16^{aA}
Coumarin	2.84^{cA}	1.99^{cAB}	2.00^{bA}	1.67^{cB}	4.59^{bA}	5.07^{bA}	4.21^{aA}	2.48^{bB}	5.78^{aA}	5.89^{aA}	4.79^{aB}	5.34^{aA}
Rutin	1.85^{bB}	0.70^{bC}	1.22^{bB}	2.83^{bA}	2.65^{aC}	3.00^{aC}	4.32^{aB}	6.00^{aA}	2.50^{aA}	2.16^{bA}	1.62^{bB}	1.00^{cB}
Quercetin	15.45^{aB}	21.15^{aA}	12.68^{aB}	12.51^{aB}	8.58^{bA}	6.93^{bB}	8.02^{bA}	6.93^{bB}	4.68^{cA}	2.85^{cB}	3.51^{bB}	4.26^{cA}
Naringin	17.55^{cD}	19.03^{cC}	20.57^{cB}	22.70^{bA}	24.71^{bB}	25.15^{bB}	30.04^{abA}	22.25^{bBC}	30.07^{aA}	32.49^{aA}	30.41^{aA}	27.31^{aB}
Apigenin	6.30^{aA}	4.81^{bB}	2.44^{cC}	2.32^{bC}	5.92^{aA}	5.07^{bA}	3.29^{bAB}	4.65^{aA}	5.15^{aA}	5.54^{aA}	4.19^{aB}	4.76^{aAB}
NI	12.65^{bC}	12.33^{bC}	20.57^{aA}	18.70^{bB}	12.66^{bC}	20.49^{aA}	17.90^{bB}	19.35^{aA}	14.45^{aA}	15.68^{cA}	17.30^{aA}	16.95^{cA}

Values are means of six replications (N = ±6 SD); (-): not detected; The data marked with the different small letter, for the ripening stage, and capital letter, for the provenance, in the table, values share significant differences at $P < 0.05$ (Duncan test).

ent temperature for a maximum of 1 month until used for further analysis.

2.2. Oil extraction

Ten g of each ground sample were extracted using a soxhlet-apparatus with 100 ml hexane (Analytical Reagent, LabScan, Ltd., Dublin, Ireland) for 6 h. The extraction was protected against light. Oil was removed after mixture filtration and solvent evaporation under reduced pressure.

2.3. Total lipid extraction

Triplicate sub-samples of 0.5 g were extracted using the modified method of Bligh and Dyer (1959). Thus, fruit samples were kept in boiling water for 10 min to inactivate phospholipase and then ground manually using a mortar and pestle. A chloroform/methanol/hexane (Analytical Reagent, LabScan, Ltd., Dublin, Ireland) mixture (1: 2: 1, v/v) was used for total lipid extraction. After washing with water and centrifugation at 3000 × g for 10 min, the organic layer containing total lipids was recovered and dried under a nitrogen stream. Then, the residue was dissolved in a known volume of toluene–ethanol (4:1, v/v) at −20 °C for further analyses.

2.4. Fatty acid methylation and analysis

Total fatty acids were converted into their methyl esters using 3% sodium methylate in methanol according to the method described by Cecchi et al. (1985). Heptadecanoïc acid (C17:0) methyl ester was used as an internal standard in order to quantify fatty acids. The superior phase that contains fatty acid methyl esters (FAMEs) was aspired and the solvent volume reduced under a stream of nitrogen, prior to analysis. FAMEs were analysed by gas chromatography using a Hewlett-Packard 6890 chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame-ionization detector (FID) and an electronic pressure control (EPC) injector. They were separated on a RT-2560 capillary column (100 m length, 0.25 mm i.d., 0.20 mm film thickness). The oven temperature was kept at 170 °C for 2 min, followed by a 3 °C min^{-1} ramp to 240 °C and finally held there for an additional 15 min period. Nitrogen (U) was used as carrier gas at a flow rate of 1.2 mL min^{-1}. The injector and detector temperatures were maintained at 225 °C A comparison of the retention times of the FAMEs with those of co-injected authentic standards (Analytical Reagent, LabScan, Ltd., Dublin, Ireland) was made to facilitate identification.

2.5. Essential oil extraction

Fifty grams of ground aniseeds material were subjected to hydrodistillation in 1 L of deionized water using a Clevenger apparatus for up to 4 h, time which was necessary for a complete extraction (Bettaieb Rebey et al., 2017).

2.6. Gas chromatography (GC-FID)

GC analysis of volatile compounds was carried out according to Bettaieb Rebey et al. (2017) using an Agilent 6980 gas chromatograph equipped with a flame ionisation detector (FID) and an electronic pressure control (EPC) injector attached to HP-INNOWAX polyethylene glycol capillary column (30 m 0.25 mm). The flow of the carrier gas (N^2) was 1.6 mL min^{-1}. The split ratio was 60:1. The analysis was performed using the following temperature program: oven temps isotherm at 35 °C for 10 min, from 35 to 205 °C at the rate of 3 °C min^{-1} and isotherm at 205 °C during 10 min. Injector and detector temperature were held, respectively, at 250 and 300 °C. One micro-liter of the sample (dissolved in hexane as 1/50 v/v) was injected into the system. Individual peaks were identified by comparison of their retention indices relative to (C6-C22) n-alkanes with those of literature and/or with those authentic compounds available in our laboratory. Percentage compositions of samples were calculated according to the area of the chromatographic peaks using the total ion current

2.7. Gas chromatography-mass spectrometry

GC–MS analysis was performed on a gas chromatograph HP 5890 (II) interfaced with a HP 5972 mass spectrometer with electron impact ionization (70 eV). A HP-5MS capillary column (30 m x 0.25 mmoL, 0.25 μm film thickness) was used (Neffati et al., 2010). The carrier gas was helium at 1.2 mL min1. Each sample (1 μL) was injected in the split mode (1:20), the program used was isothermal at 70 °C, followed by 50–240 °C at a rate of 5 °C min1, then held at 240 °C for 10 min. The mass spectrometer was an HP 5972 and the total electronic impact mode at 70 eV was used. The components were identified by comparing their relative retention times and mass spectra with the data from the library of essential oil constituents, Wiley, Mass-Finder and Adams GC–MS libraries.

2.8. Polyphenol extraction and analyses

2.8.1. Preparation of extracts

Seed extracts were obtained by stirring 1 g of dry material powder with 10 mL of 80% acetone for 30 min. Extraction was carried out using maceration at room temperature for 24 h followed by filtration through Whatman No. 4 filter paper and after evaporation to dryness. The yield (%) of evaporated dried extracts was calculated as 100 x DWextr/DWsamp, where DWextr is the weight of extract after evaporation of solvent, and DWsamp is the dry weight of original sample. Samples were stored at 4 °C until analysis.

2.8.2. Total phenolic amounts

The total phenolic amount of the acetone extracts was determined by using Folin–Ciocalteu reagent (Merck), according to the procedure described by Dewanto et al. (2002). Briefly, 125 µl of sample extract were dissolved in 500 µl of distilled water and 125 µl of Folin–Ciocalteu reagent. The mixture was shaken, before addition of 1.25 ml of 7% Na_2CO_3, adjusting with distilled water to a final volume of 3 ml, and mixed thoroughly. After incubation in the dark for 90 min, the absorbance at 760 nm was measured versus the prepared blank. Total phenolic amounts were expressed as mg of gallic acid equivalents per gram of dry weight (mg GAE/g DW), through a calibration curve with gallic acid. All samples were analysed in six replicates.

2.8.3. RP-HPLC evaluation of phenolic compounds

Diluted samples from *P. anisum* seeds were injected to RP-HPLC. The separation of phenolics was performed with an Agilent 1100 series HPLC system equipped with on-line degasser (G 1322 A), quaternary pump (G 1311 A), a thermostatic auto sampler (G 1313 A), column heater (G 1316 A) and diode array detector (G 1315 A). Instrument control and data analysis were carried out using Agilent HPLC Chemstation 10.1 edition through Windows 2000. The separation was carried out on a reverse phase ODS C18 (4 µm, 2509 4.6 mm, Hypersil) column used as stationary phase at ambient temperature. The mobile phase consisted of acetonitrile (solvent A) and water sulphuric acid (0.2%) (solvent B). The flow rate was kept at 0.5 mL $min^{-}1$. The gradient program was as follows: 15 A/85% B 0–12 min, 40% A/60% B 12–14 min, 60% A/40% B 14–18 min, 80% A/20% B 18–20 min, 90% A/10% B 20–24 min, 100% A 24–28 min. The injection volume was 20 µl and peaks were monitored at 280 nm. Peak identification was obtained comparing the retention time and the UV spectra of the *P.anisum* phenolics chromatogram with those of pure standards which were purchased from Sigma (St. Louis, MO, USA). Analyses were performed in triplicates

2.8.4. DPPH radical scavenging assay

Radical-scavenging activity was determined according to Hanato et al. (1998). Two millilitres of the extract at different concentrations were added to 0.5 ml of a 0.2 mM DPPH methanolic solution. After shaking, the mixture was incubated at room temperature in the dark for 30 min, and then the absorbance was measured at 517 nm. BHA was used as positive reference while methanol was used as negative reference. DPPH radical-scavenging activity was expressed as the inhibition percentage (I%) and was calculated using the following formula:

$$I\% = 100 \times (Ablank - Asample)/Ablank$$

where Ablank is the absorbance of the control at 30 min reaction (containing all reagents except the test compound), and Asample is the absorbance of the sample at 30 min. Antiradical activity was expressed as IC50, defined as the concentration of the extract generating 50% inhibition.

2.8.5. Chelating effect on ferrous ions

The ferrous ion chelating activity of different organ extracts and essential oils was assessed as described by Zhao et al. (2006). Different concentrations of the sample were added to 0.05 mL of $FeCl_2.4H_2O$ solution (2 mM) and left for incubation at room temperature for 5 min. Then, the reaction was initiated by adding 0.1 ml of ferrozine (5 mM), and the mixture was adjusted to 3 ml with deionized water, shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was calculated using the formula given below:

$$\text{Metal chelating effect (\%)} = [(A_0 - A_1)/A_0] \times 100$$

where A_0 is the absorbance of the ferrozine-Fe^{2+} complex and A_1 is the absorbance of the test compound. Results were expressed as IC_{50}, efficient concentration corresponding to 50% ferrous iron chelating. EDTA was used as a positive control. Samples were analyzed in six replicates.

2.8.6. Reducing power

The method of Oyaizu (1986) was used to assess the reducing power of different seed extracts. 1 ml of different concentrations of organ extracts and essential oils in acetone 80% were mixed with 2.5 mL of a 0.2 M sodium phosphate buffer (pH = 6.6) and 2.5 mL of 1% potassium ferricyanide ($K_3Fe(CN)_6$), and incubated in a water bath at 50 °C for 20 min. Then, 2.5 ml of 10% trichloroacetic acid were added to the mixture that was centrifuged at 650 *g* for 10 min. The supernatant (2.5 ml) was then mixed with 2.5 ml distilled water and 0.5 ml of 0.1% ferric chloride solution. The intensity of the blue-green colour was measured at 700 nm. The EC_{50} value (mg/ml) is the extract concentration at which the absorbance was 0.5 for the reducing power and was calculated from the graph of absorbance at 700 nm against extract concentration. Ascorbic acid was used as a positive control.

2.9. Statistical analysis

Data were subjected to statistical analysis using statistical program package STATISTICA. Analysis of variance (ANOVA) followed by Duncan's multiple comparison test ($p < 0.05$) were used. Two-factor ANOVA was applied to analyse differences among four ecotypes and three maturation variants. Mean values were calculated from six plants per ecotype at each maturation stage. Therefore, mean values for the traits detected by standard analytical methods are based on six replicates.

3. Results and discussion

3.1. Seed oil content

The dynamic of oil accumulation from *P. anisum* seeds at three stages of maturity are given in Table 2. Significant changes ($P < 0.05$) were observed among the studied accessions for oil content during maturity. In fact, the changeover of the seed from the immature (fully green) to mature (brown) stage, was characterized by a gradually increase in the oil yield. As demonstrated, the lowest oil content (6.77%) was detected in Egyptian aniseed accession at immature stage while the highest one (13.81%) was in Tunisian aniseed accession at full ripening stage. No reported literature was found concerned the variation of oil content of aniseeds during ripeness. It seems likely that the biosynthesis of oil is very active at the later stage of seed maturation and that the regulation of oil accumulation seems to be associated with the activation of ''fatty acid synthetase'' as previously reported by (Bettaieb Rebey et al., 2013).

This developmental pattern of oil content was similar to that reported for other oleaginous fruits and seeds such as *Cuminum cyminum* (Bettaieb Rebey et al., 2013), *Coriandrum sativum* (Nguyen et al., 2015), and *Pistacia lentiscus* (Trabelsi et al., 2015), but opposite to that observed for other species such us *Rhus tripartitum* (Tlili et al., 2014), *Persea Americana* (Villa-Rodríguez et al., 2011) and *Olea europeae* (Bengana et al., 2013). Currently, it appears that the dynamic of oil accumulation is species dependent and that the implication of genetic factors is suggested too (Alignan et al., 2009).

3.2. Fatty acid composition during Pimpinella anisum seed maturation

The qualitative and quantitative changes in fatty acid profile during seed maturation were also depicted in Table 1. Comparison of the analytical data revealed that a total of 10 different fatty acids (%) were identified in the seed oils. During ripening, different trends were observed for the fatty acid accumulation. Thus, in newly formed seeds saturated fatty acids (SFA) formed 65.88, 62.20, 58.45 and 60.79% of TFA, respectively for Turkish, Serbian, Egyptian and Tunisian aniseeds. The representative fatty acid of this group in *Pimpinella anisum* was palmitic acid (C16:0), which followed the same trend as the saturated fatty acids, in all the accessions. Proportions of SFA progressively declined ($p < 0.05$) until 5.66, 10.91 12.16 and 4.90% of TFA at the full maturity. Capric, lauric, myristic and stearic acids were also present in higher amounts at immature stage and decreased gradually with maturity process (Table 2).

On the contrary, unsaturated fatty acids (UFA), represented mainly by petroselinic acid, in all the provenances, were present in low proportions (about 32%) at the beginning of seed formation and significantly increased ($p < 0.05$) with ripening process, reaching its highest level at fully mature seeds (more than 90%). The level of petroselinic acid was in accord with values previously reported for aniseed ranging from 51.6% to 90.7% (Bettaieb Rebey et al., 2017). During maturation, the biosynthesis of petroselinic and palmitic acids were in opposite directions, which could be elucidated by the role of palmitic acid as a precursor of petroselinic acid (Nguyen et al., 2015). Generally, the proportion of saturated to polyunsaturated fatty acids reduced noticeably during seed maturation. Comparable results have been reported in other oilseed species (Ramadan and Morsel, 2003; Vuorinen et al., 2014; Nguyen et al., 2015). Furthermore, changes in fatty acids are of special importance to the quality of the oil (Msaada et al., 2009), thus, fully ripe aniseeds could have a large spectrum of usage in industry.

Given the nutritional importance of lipids, unsaturated fatty acids, insight into their accumulation during maturation might be used to inform the timing of seed management practices, such as irrigation and fertilization, in order to enhance fruit quality (Zhu et al., 2017).

3.3. Essential oil yield and composition

Essential oil (EO) yield of aniseeds was affected by both ripening stages as well as seed provenances (Fig. 1). During the seed development, essential oil yield varied from 1.22% to 3.09% and reaching a maximum at immature stage in comparison with fully matures seeds. There were significant differences ($p < 0.05$) in EO yields between different aniseed accessions and maturity grades. Thus, at the earlier stage, the highest EO yield was observed in Tunisian accession (3.05%). At fully mature period the seed oil content dropped and the maximum yield was obtained, also, in Tunisian aniseeds (2.46%). On the whole, the EO content of aniseed diminished with the ripening process which can be explained by the fact that the number of fruits in the first stage was higher than the last one (Özel, 2009). In agreement with Özel (2009), EO production of aniseed has been considered as associated with early growing period. Besides, ours findings were in harmony with the findings of other researchers on some umbel plants such as coriander (Msaada et al., 2007), dill (Callan et al., 2007) and fennel (Salami et al., 2017). These authors demonstrated that ripening process affected the EO biosynthesis and that the EO content in premature period was higher than in the mature period.

The composition of the EOs extracted from *Pimpinella anisum* accessions at different maturity stages are listed in Table 3. Independently of the ripening stage and provenances, the EO compounds were grouped in four chemical classes according to their functional groupings. Indeed, phenylpropanoides were represented in high amount, followed by sesquiterpene hydrocarbons. Conversely, oxygenated and terpenic hydrocarbons were the minor class in aniseed essential oil. As reported by Morshedloo et al. (2017), terpene synthases are the key enzymes in the biosynthesis of terpenes. In fact, they catalyze the oxidation and cyclization steps of the precursors for each class of terpenes (Tholl, 2015).

A total of 14 components were identified. Separately of the maturity stage and accession, *Trans*-anethole was the main component of the EOs. Hence, in immature stage, it ranged from 66.34% in Turkish seeds to 81.25% in Tunisian population. In full mature stage, *trans*-anethole increased considerably until 72.80%, 88.49%, 89.21% and 93.05% respectively for fruits from Turkish, Serbia, Egypt and Tunisia.

Other major EO compounds were estragole, anisole, *p*-anisaldehyde and γ-himachalene in all studied ripening stages. These compounds decreased in fruits and they were less than 2.50% in all studied populations and fruit developmental stages. The rest of fruit essential oil compounds represented approximately one-tenth of the total essential oil components at each sampling in all stages.

For industrial purposes the developmental stage is very important to obtain higher essential oil yield as well as desirable active constituents. In aniseed, *trans*-anethole is considered as the most important component of the oil. For molecular studies, finding the *trans*-anethole variation in different developmental phases is of great importance. Thus, at full maturity EO were composed mainly of *trans*-anethole/ estragole (72.50-12.70%; 88.49-2.64% and 89.21-3.82%), respectively, for Turkish, Serbian and Egyptian provenances. Nevertheless, EO of Tunisian aniseeds was distinguished by the highest proportion of *trans*-anethol (93.05%) and a considerably proportions of γ-himachalene (2.58%). These components, listed above, characterize *P. anisum* essential oils (Ullah and Honermeier, 2013; Bettaieb Rebey et al., 2017).

In fact, Özel (2009), reported the modification in EO composition during Turkish aniseeds maturation but without variety mention. They found that the changeability in the *trans*-anethole level during the ripening may have resulted from the growing properties of anise plant. The umbels of anise become mature at different periods from top to bottom. Therefore, aniseeds harvested at different maturation stages eventually include fruits having different maturation levels. For instance, even the same main compounds were present in the four varieties; there was a great difference in their percentages and this can be due to environmental and genetic factors (Bettaieb Rebey et al., 2017). Moreover, in agreement with Salami et al. (2017) who studied the geographical variability of fennel seeds during maturation, the strong chemical variability in essential oils could be ascribed not only to the geographical origin of the sample and its environmental conditions but also to the variety and genetic factors.

3.4. Total phenol content

The contents of total phenols found in aniseed extracts from four accessions (Egypt, Turkey, Tunisia and Serbia) during three ripening stages are shown in Fig. 2. There were significant differences ($p < 0.05$) in total phenolic contents between different aniseed accessions and ripening stages. In fact, the highest phenolic content (25.16 mg GAE/g DW) was detected in Tunisian aniseed accession at full ripening stage while the lowest one (13.22 mg GAE/g DW) was in Serbian

aniseed accession at immature stage. In this way, Selami et al. (2017) also noted higher amount of total phenolics in fennel fruit extract from Iran population than the other ones (England, Poland and Spain) and they deduced that could be due to its environmental condition. In our study, the four aniseed accessions were cultivated under the same environmental conditions, so the observed differences in their TPC were closely related to genetic factors as reported by Bettaieb Rebey et al. (2011) in the case of Tunisian cumin seeds.

On the other hand, the maturity stages had a significant influence on TPC of all aniseed accessions. In fact, there was a progressive increase of TPC in all aniseed populations from unripe to ripe stages. Likewise, Bettaieb Rebey et al. (2014) reported that variation of TPC in cumin seeds was clearly dependent on the ripening stages with an increase of TPC during maturity progression. Selami et al. (2017) also reported an increase of TPC in all fennel fruit populations as maturity progressed. These authors illustrated that the process of maturation progresses in plant appears to be an ordered expression of the genome to activate specific enzymes necessary for phenol biosynthesis at a particular stage of development. So, the observed increase of TPC at full ripe stage could be due to higher production of phenylalanine ammonialyase, key enzyme of phenolic biosynthesis.

In general, plants need phenolic compounds for pigmentation, growth, reproduction and resistance to pathogens (Sytar et al., 2012). So, phenolic compounds are found to protect plants against bacterial damage (Konaté et al., 2013, 2015). Moreover, they also act in defense against external agents such as light, temperature and humidity (Cunha et al., 2012). Considering that high temperatures may have promoted an increase in the respiratory rate and due to the formation of free radicals during the development of seeds, an increase in the phenolic compounds will have to take place in order to combat the reactive compounds generated by the respiration. However, Marin et al. (2015) found that phenolic production in the Red Oak cultivar was negatively correlated with increasing temperature. As reported by Sytar et al. (2018), these effects are more complex and can be genotype specific.

3.5. Antioxidant activity

Antioxidant activities of aniseed extracts from four accessions (Egypt, Turkey, Tunisia and Serbia) during three ripening stages were evaluated by DPPH, chelating ability and reducing power assays (Table 4). Based on DPPH assay, mature aniseed extracts of four accessions showed higher ability to scavenge DPPH radical than the other stages with IC_{50} = 12.87, 16.77, 18.97 and 20.18 μg/mL for Tunisian, Egyptian, Turkish and Serbian accessions, respectively. Additionally, the antiradical potential of mature aniseed extracts from these four accessions was more strong than the well-known synthetic antioxidant BHT (IC_{50} = 24.12 μg/mL). It is also interesting to mention the presence of high negative correlation between total phenolic amounts and DPPH IC_{50} values (r = -0.9; $p < 0.05$) during aniseed maturation for all accessions. In fact, the decrease of DPPH IC_{50} values was followed by an increase of phenolic content during aniseed maturation. So, the high antiradical potential of mature aniseed in all accessions was strongly related to high phenolic contents. Similar results were obtained by Selami et al. (2017) in the case of fennel fruit. Besides, Bettaieb Rebey et al. (2014) also compared total phenolic amounts and DPPH IC_{50} values during cumin seed maturation and they deduced that antioxidant activity depends on the content of total phenolics.

Based on chelating and reducing power assays, the higher antioxidant potential was detected in aniseed extracts at mature stage than the other stages for all accessions similarly to the results obtained for DPPH assay. In addition, mature aniseed extract of Tunisian accession had the highest chelating (IC_{50} = 9.23 mg/mL) and reducing power (EC_{50} = 523.47 μg/mL) abilities. During maturation, chelating and reducing power measurements were negatively correlated to total phenolic amounts (r = -0.9; $p < 0.05$). So, as observed in DPPH assay, the high chelating and reducing power abilities of mature aniseed in all accessions were attributed to high phenolic contents. However, contrarily to DPPH assay, chelating and reducing power abilities were unable to compete with the positive controls, namely ascorbic acid in iron reducing (EC_{50} = 40 μg/mL) and EDTA in iron chelating (IC_{50} = 0.03 mg/mL), independently of maturation and accession factors. Gülçin et al. (2003) also reported that Turkish aniseed extract had lower chelating and reducing power properties than the positive controls (BHT, BHA and α-tocopherol).

3.6. Identification of phenolic compounds using HPLC

As antioxidant potential of aniseed extract depends on total phenolic content, it is very interesting to determine aniseed phenolic composition. Qualitative determination of aniseed phenolic compounds from four accessions (Egypt, Turkey, Tunisia and Serbia) during maturation was performed by RP-HPLC coupled with an UV–vis multi-wavelength detector. As can be seen in Table 5, a total of 12 phenolic compounds were identified during aniseed maturation in all accessions. The main phenolic compound of aniseed was naringin (17.55–32.49%), followed by chlorogenic acid (0.24–26.29%), quercetin (2.85–21.15%), gallic acid (0.08–18.89%) and rosmarinic acid (1.75–16.56%) in all accessions. So, aniseed accessions had the same composition in phenolic compounds but in different proportions according to the stage of maturity. During aniseed maturation, a strong negative correlation (r = -0.9; $p < 0.05$) was detected between phenolic acid and flavonoid proportions, independently of accession factor. In fact, the decrease of phenolic acid proportions was mainly expressed by the reduction of gallic, rosmarinic, ellargic and syringic acids during aniseed maturation and it was followed by an increase of flavonoid proportions, especially due to naringin accumulation. Bettaieb Rebey et al. (2017) reported that naringin was the main phenolic compound of ethyl acetate aniseed extract from Tunisian and Egyptian accessions. Naringin is a flavanone glycoside found in grapes and citrus fruits (Alam et al., 2014) and it is known as a strong antioxidant and scavenger of free radicals (Cavia-Saiz et al., 2010). So, the high antiradical activity of mature aniseeds, observed in our study, could be probably due to the high biosynthesis of naringin at this stage. However, Bar-Peled et al. (1993) reported that naringin biosynthesis and accumulation decreased during *Citrus* fruit maturation. A part naringin, mature aniseed was also characterized by an accumulation of chlorogenic acid which also had a strong ability to scavenge free radicals (Raudonis et al., 2009). However, Bettaieb Rebey et al. (2014) reported a decrease of chlorogenic acid proportions during cumin seed maturation. So, accumulation of phenolic compounds strongly varied in relation to the ripening stage and it is a result of equilibrium between their biosynthesis and degradation (André et al., 2009). This equilibrium is typically controlled via enzyme activity/gene expression (Qu et al., 2013). It is interesting to mention that other factors, related to environmental conditions, may significantly influence the content and composition of phenolic compounds in plants. In the case of lettuce plants, Sytar et al. (2018) found that the accumulation of total phenolics, flavonoids, anthocyanins, and phenolic acids (benzoic acid derivatives and cinnamic acid derivatives) increased in direct sunlight (high UV radiation, moderate temperature) conditions outdoors as compared to the greenhouse conditions (low UV radiation, high temperature).

4. Conclusion

The data in this study revealed great differences in biochemical composition of aniseed with both provenances as well maturity stage. It may be suggested that these differences could be due to the effect of harvesting time and genetic factors. Also, our findings indicated that

aniseeds are considered valuable, as they provide components with potential for industrial and pharmacological applications as antioxidants. Moreover, the significant changes of the biochemical composition during ripening suggest that aniseeds are a promising source for the extractions of petroselinic acid, *trans*-anethole as well as naringin, which is responsible for high antiradical activity, at the full maturity. Nevertheless, further analysis should be done for achieving more perceptive facts.

Uncited references

Adams (2001), Marzouk and Cherif (1981), Milanez et al. (2018) and Ramadan and Wahdan (2012).

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