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Variations in fatty acid composition during maturation of cumin (*Cuminum cyminum* L.) seeds

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Changes in fatty acids were studied during maturation of cumin (*Cuminum cyminum* L.) seeds cultivated in the North-Eastern region of Tunisia (Menzel Temim). The fruits matured in 49 Days after flowering (DAF). The first results show a rapid oil accumulation started in newly formed fruits (8.2%) and continued until their full maturity (16.9%). During fruit maturation, fatty acid profiles varied significantly among the three stages of maturity. Fruits development resulted mainly in an increase of petroselinic acid and a decrease of palmitic acid (C16:0). At full maturity, the main fatty acids were petroselinic acid (55.9%), followed by palmitic (23.82%), linoleic (12.40%) and pamitoleic (2.12%) acids. Polyunsaturated and monounsaturated fatty acids increased significantly; however, saturated fatty acids decreased during ripening of cumin seed. Results of this study indicate that the variation in the fatty acid composition of cumin seeds during maturation may be useful in understanding the source of nutritionally and industrially important fatty acids in this fruit. Cumin seed is potentially an important source of petroselinic acid which has numerous industrial applications.

Key words: Cumin (*Cuminum cyminum* L.), Apiaceae, seed, fatty acids composition, petroselinic acid, maturation.

INTRODUCTION

Nowadays, the development of new crops for the production of industrial oils is an area of significant interest both scientifically and environmentally. While methods are being developed for modifying the fatty acid content and composition of oils produced by established crops such as oilseed rape and soya beans (Murphy, 1991), another approach is to investigate alternative species as potential sources of specialist oils. However, oils with different fatty acid composition are required depending on their use in industry or for human consumption. Oils with a high proportion of oleic acid are more stable than others and contribute to reduction in cardiovascular diseases in humans (Jacocot, 1995).

Recently, researchers have reported that seeds of some some Apiaceae genera should be regarded as a useful source for the extraction of petroselinic acid, which represents an important oleochemical raw material (Avato et al., 2001). For example, this acid can be used as a precursor of both lauric acid, which is a component of detergents and surfactants, and adipic acid, which is the monomeric component of nylon 66 (Murphy et al., 1994; Murphy, 1996). An example of such a crop is the herb plant cumin (*Cuminum cyminum* L.), a member of the Apiaceae family, which represent one of the best-known plant families, widely distributed in temperate climate regions where they are often used as spices, vegetables or drugs owing to the presence of useful metabolites (Olle and Bender, 2010). Cumin seeds

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Table 1. Harvest dates, days after flowering, fruit colour and state of maturity, relative moisture and oil contents of cumin seeds during maturation.

Harvest date	DAF	Fruit colour, state of maturity	Relative moisture content (%, w/w)	Oil content (%, w/w)
14 May 2011	5	Unripe, fully green	89.7 ± 0.9[a]	8.2 ± 0.1[a]
29 May 2011	20	Half ripe, green-brown	43.6 ±0.7[b]	13.8 ± 0.4[b]
27 June 2011	49	Fully ripe, brown	12.5 ±0.6[c]	16.9 ± 0.4[c]

Values in the same row with different superscript (a-c) are significantly different at $p < 0.05$.

contain oils with a high concentration of the monounsaturated fatty acid, petroselinic acid. This acid can be oxidatively cleaved to produce a mixture of lauric acid, a compound useful in the production of detergents, and adipic acid, a C_6 dicarboxylic acid which can be utilized in the synthesis of nylon polymer (Murphy, 1991).

Our recent studies on the compositional analysis of *C. cyminum* L. have described essential oil composition of different cumin parts (Bettaieb et al., 2010, 2011a), phenolic composition of different organs (Bettaieb et al., 2010; Bettaieb et al., 2011c) and biochemical changes of seed and aerial part under drought (Bettaieb et al., 2011b, 2012a, 2012b). Unlike fruits of other species, changes in lipids and fatty acids with respect to maturity of *C. cyminum* L. fruit have not yet been studied. The main objective of the work presented here was, therefore, to determine the influence of maturity stages on oil content and fatty acid composition of cumin seeds. The results will be important as an indication of the potential economic utility of *C. cyminum* L. as a raw material source for useful industrial oils components.

MATERIALS AND METHODS

Plant material

Cumin seeds were randomly collected at different ripening stages from cultivated plants in Menzel Temim (North-Eastern Tunisia; latitude 36° 44 29.12 N, longitude 10 40 51.26 E, altitude 163 m during May and June 2011. Menzel Temim region is characterized by low annual rainfall of 700 mm and mean annual temperature of 16.8°C. Harvest period was stretched from 5 Days After Flowering (DAF) to 49 DAF, the time required for complete maturity. The seed's colour and relative moisture content were adopted as a ripening criterion (Table 1). Indeed, only full green seeds were harvested at the initial stages of maturity. Green-brown seeds were considered as indicators of the intermediate stage. Only brown seeds were selected for analysis during the final stages of maturity. Moisture contents were determined by heating in an air-oven at 60°C to constant weight.

Oil extraction

Three samples of each cumin fruits variety were finely ground in an electric grinder (IKA-WERK. Type: A:10). 20 g of each ground sample were extracted using a soxhlet-apparatus with 100 ml hexane (Analytical Reagent, LabScan, Ltd., Dublin, Ireland) for 6 h, after a kinetic survey during 30 min, 1, 2, 4, 6 and 8 h. The extraction was protected against light. Oil was removed after mixture filtration and solvent evaporation under reduced pressure and temperature. After oil weighting, the oil content was determined.

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 (Douce, 1964) 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. Total lipid extraction was made in triplicate.

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. and 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.

Statistical analysis

Data were subjected to statistical analysis using statistical program package STATISTICA (Statsoft, 1998). 21 Percentage of each volatile compound and fatty acids were the mean of three replicates ± S.D. and the differences between individual means were deemed to be significant at $p<0.05$.

RESULTS AND DISCUSSION

Oil yield

The oil content of *C. cyminum* seeds at three stages of

Table 2. Variations in fatty acids composition (as a percent of TFA) of total lipids during cumin (*Cuminum cyminum* L.) seed maturation.

Fatty acid composition	Immature	Intermediate	Mature
C8:0 (caprylic acid)	1.29±0.05^{a}	1.80±0.01^{a}	1.63±0.03^{a}
C10:0 (capric acid)	2.02±0.02^{a}	1.56±0.01^{a}	0.92±0.01^{b}
C12:0 (lauric acid)	1.08±0.01^{a}	0.64±0.02^{a}	0.16±0.01^{b}
C13:0 (tridecanoic acid)	2.71±0.01^{a}	1.10±0.01^{b}	1.20±0.02^{b}
C14:0 (myristic acid)	0.93±0.01^{a}	0.50±0.01^{a}	0.15±0.00^{a}
C16:0 (palmitic acid)	52.86±0.52^{a}	44.45±0.12^{b}	23.82±0.10^{c}
C16:1n-7 (palmitoleic acid)	12.64±0.66^{a}	8.93±0.06^{b}	2.12±0.01^{c}
C18:1n-9 (oleic acid)	8.13±0.01^{a}	5.14±0.01^{b}	0.32±0.09^{c}
C18:1n-12 (petroselinic acid)	10.62±0.03^{c}	24.60±0.33^{b}	55.9±0.34^{a}
C18:2n-6 (linoleic acid)	3.90±0.01^{c}	7.81±0.24^{b}	12.40±0.11^{a}
C18:3n-3 (α-linolenic acid)	3.82±0.01^{a}	3.41±0.01^{a}	0.20±0.02^{b}
SFA	60.89±0.22^{a}	50.05±0.27^{b}	27.88±0.12^{c}
MUFA	31.39±0.35^{bc}	38.73±0.01^{b}	58.34±0.87^{a}
PUFA	7.72±0.09^{b}	11.82±0.09^{a}	12.61±0.11^{a}
SFA/ PUFA	7.88±0.08^{a}	4.23±0.04^{b}	2.21±0.03^{c}
n-6/n-3 ratio	1.02	2.29	62

Values in the same row with different superscript (a-c) are significantly different at $p < 0.05$.

ripening are given in Table 1. As shown, the transition of the seed from the immature (fully green) to mature (brown) was characterized by a significant increase in the total lipid content (from 8.2 to 16.9%, w/w on dry weight basis). The rate of accumulation of oil was approximately 1.14 fold higher during the transition from intermediate to mature fruits (2.1%) while it was relatively higher (6.6%) during the first episode of maturation (from immature to intermediate). There are no data on the dynamic of total lipid accumulation during the maturation process of *C. cyminum*, nevertheless, some literature data pointed out the influence of maturation stages on oil and total lipid content on some species. In good agreement with our results, a similar trend of oil accumulation was pointed out in *Coriandrum sativum* (Msaâda et al., 2009) and myrtle (Aidi et al., 2009). Conversely, Chahed et al. (2006) reported a low accumulation of total lipid during the earlier stage of the development of *Pistacia vera* fruits, while it increased rapidly during the last stage. Similarly, the similar trend of total lipid accumulation was also reported for rape seed oil (*Brassica napus*) (Vigeolas et al., 2003), *Perilla fretuscence* (Ichihara and Suda, 2003) and date *Plum persimmon* (Glew et al., 2005).

Oil accumulation in cumin seed may be regulated by the intervention of the enzymatic system ‘‘fatty acid synthetase’’ (FA-Synthetase), which operates differently during fruit ripening. Thus, at the first and the second phase, the FA-Synthetase system was induced, and oil accumulation was accelerated. At the stationary phase, enzymes were inactive, probably by retro-inhibition, and the rate of oil accumulation was reduced.

Fatty acid composition

Changes in fatty acids are of special importance to the quality of the oil. In the present study, fatty acid accumulation patterns resulting from seed ripening were observed. A total of 11 different fatty acids were identified in percentages of the TFA of the seed oil. The fatty acid profiles were qualitatively similar and showed that fatty acids composition changed significantly during cumin seed development (Table 2). During cumin seed ripening, monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) increased; whereas, saturated fatty acids (SFA) decreased significantly ($p < 0.05$). Thus, in newly formed seed (immature), saturated fatty acids (SFA) formed 60.89% of TFA with a palmitic acid (C16:0) contribution of 52.86% of TFA. This proportion decreased progressively until 23.82% of TFA at the final stage (Table 2). However, caprylic (C8:0), capric (C10:0), lauric (C12:0), tridecanoic (C13:0) and myristic (C14:0) acids belong to minor fraction of SFA. Monounsaturated fatty acids (MUFA) represent 31.39% of TFA with a palmitoleic, oleic and petroselinic acids contribution of 12.64, 8.13 and 10.62%, respectively. During the seed ripening, the proportion of petroselinic acid increased significantly by 2.30 and 5.26 folds, respectively, for intermediate and mature episode. In addition, fatty acid composition at the last stage had a high amount of petroselinic acid and thus, could have a large spectrum of usage in industry. This fatty acid has been shown to be biosynthesized of an unusual pathway from palmitoyl-ACP (C16:0) by two steps, catalysed by a

dedicated Δ^4-desaturase and an elongase (Guiet et al., 2003) and is metabolized and accumulated in the developing endosperm of the Apiaceae family (Cahoon and Ohlrogge, 1994). It can be oxidatively cleaved to produce a mixture of lauric acid, a compound useful in the production of detergents, and adipic acid, a C_6 dicarboxylic acid which can be used in the synthesis of nylon polymer (Murphy, 1994). However, we noticed a considerable decrease of palmitoleic and oleic acids estimated by 29.35 and 36.77, and 83.22 and 96.06%, correspondingly, for intermediate and mature stage.

It was also noticed that petroselinic and palmitic acids follow evolutionary changes in opposite directions, their amounts during seed ripening were negatively correlated (r = -0.78532). This is in agreement with the results obtained by Lakshminarayana et al. (1981) and Msaada et al. (2008). The data, however, elucidate that palmitic acid is the precursor of petroselinic acid as reported by Cahoon et al. (1992). Concerning polyunsaturated fatty acids (PUFA), they were linoleic acid (C18:2 n-6) which constituted only 3.82% of TFA at first stage and increased progressively to reach 12.40% of TFA in full ripe fruit. Linoleic acid is known as essential fatty acids and is precursor of omega-3 and -6 polyunsaturated fatty acids, and it is detected with a considerable level during the second and last four stages of maturity occurring to the cumin seed, an important nutritional value. The second PUFA was the linolenic acid (C18:3 n-3), which proportions decreased from 3.90% of TFA to 0.20% of TFA during the cumin seed ripening. Peiretti et al. (2004) and Msaada et al. (2009) also reported variations in the proportions of PUFA during the growth cycle of borage and coriander, respectively. Interest in the PUFA, as health-promoting nutrients, has expanded dramatically in recent years. A rapidly growing literature illustrates the benefits of PUFA in alleviating cardiovascular, inflammatory, heart diseases, atherosclerosis, autoimmune disorder, diabetes and other diseases (Finley and Shahidi, 2001; Riemersma, 2001). The ratio of saturated fatty acids to unsaturated fatty acids (SFA/PUFA) decreased during seed maturation to reach 2.21 in fully ripe fruit. Similar results were also found in ripe coriander and niger seeds with a ratio of 0.379 and 0.370, respectively (Ramadan and Mörsel, 2006).

To evaluate the nutritional value of the present composition, the ratio n-6 to n-3 was used (Table 2). As can be seen, this ratio varied from 1.02 to 62 in the immature and the mature seeds, respectively. However, the oil from mature seeds seemed not appropriate for human nutrition due to its higher n-6/ n-3 ratio and the appreciable amount of linolenic acid which make it susceptible to oxidation (Labuschagne and Hugo, 2010). Furthermore, this oil could find industrial applications especially in the manufacture of oil based paints (Mondal et al., 2010).

Conclusion

The highest oil content was reached at full fruit ripeness. Petroselinic acid was the main fatty acid at the second and last stage of seed maturity. Percentages of fatty acids varied significantly among the three stages of maturity which indicates potential for selection of industrial and nutritional fatty acid profiles. The fatty acid profiles at the last stage of maturity had a large spectrum of applications in industry due to their high amounts in petroselinic acid and have a high healthy nutritional value. During fruit maturation, palmitic and petroselinic acids follow evolutionary changes in opposite directions suggesting metabolic link.

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