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Effect of the seasonal climatic variations on the composition and quality of "Chemlali" virgin olive oil

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"Chemlali" is the main olive variety cultivated in Tunisia. Seasonal factors such as rainfall and temperature vary year to year influencing the composition and quality of virgin olive oil. This work aims to investigate the effect of the seasonal climatic variations on the quantity and the quality characteristics (free acidity, specific ultraviolet absorbance K_{232} and K_{270} , fatty acid composition, chlorophylls and total phenols) of Tunisian "Chemlali" olive oil. Water and oil content increased with increasing rainfall and temperature respectively. The seasonal variations of climatic factors within the growing region did not affect the main legal quality indices of all oil samples. K_{232} , total phenol contents and oxidative stability increased at high temperature, while K_{270} and chlorophyll content decreased.

Key words: Analytical quality parameters, chlorophyll, fatty acid composition, *Olea europaea* L., rainfall, temperature, total phenols.

INTRODUCTION

Mediterranean climatic conditions are favourable to the olive tree cultivation. The Tunisian olive trees number about 57 million units covering 16000 ha. "Chemlali" is the main variety cultivated in Tunisia, covering 60% of the olive growing surface, spread from the north-east of the country to the extreme south. This cultivar, appreciated for its sensory characteristics, contributes to 80% of the national olive oil production (Baccouri et al., 2007). It is a productive variety that is well resistant to severe environmental conditions. In fact, into the large agricultural areas of this region, the olive is primarily dry farmed and orchard density is tuned to average

precipitation.

Seasonal factors such as rainfall and temperature vary year to year influencing the composition and quality of virgin olive oil. Considering that virgin olive oil (VOO) is obtained from the olive fruit solely by mechanical and physical means, it retains more health beneficial phytochemicals as compared to other commercially available refined vegetable oils, which are generally solvent extracted (Visioli and Galli, 1998). Its potential beneficial effects to human health (Krichene et al., 2007) are due to the balanced fatty acid composition, rich in monounsaturated fatty acid (MUFAs), which are more

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■ 2003/2004 **■** 2004/2005 **■** 2006/2007

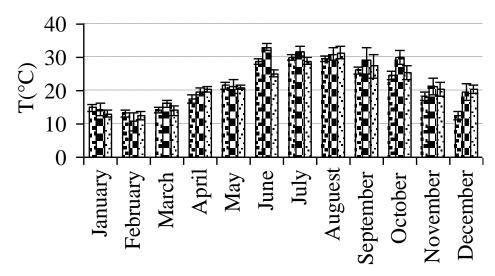


Figure 1. Monthly mean temperature (°C) registered at the experimental farm during three crop seasons. Bars indicate standard error.

resistant to lipid peroxidation than polyunsaturated fatty acid (PUFAs) (Kris-Etherton et al., 2002) coupled with the presence of polyphenols (Visioli et al., 2000), powerful antioxidant. The unique chemical and organoleptic characteristics of VOO depend on several factors, such as environmental (climate, soil), agronomic (irrigation, fertilization, harvesting time and methods), technological factors (fruit storage, extraction procedure) (Aparicio and Luna, 2002; Di Giovacchino, 2000; Salvador et al., 2000) and fruit health. Among these factors, the climate (humidity, rainfall, temperature) is a determinant parameter for fatty acid composition (Ben Temime et al., 2006; Aparicio and Luna, 2002; Stefanoudaki et al., 1999; Ranalli et al., 1997) and total phenol contents (Boskou, 2000).

Also the harvesting time can influence the VOO fatty acid from year to another probably because of the differences in the amount of summer rainfall in the growing region and temperature during olive fruit ripening and oil biosynthesis (Beltran et al., 2004).

Changes in chemical composition (fatty acid composition and polyphenols) of extractable olive oil according to macroclimatic factors have been studied by several authors in Spanish (Aranda et al., 2004) and other Tunisian cultivar (Ben Temime et al., 2006). Some reports include information about fatty acid composition (Angerosa et al., 1996; Montedoro et al., 1993) and changes of antioxidant compounds according to macroclimatic conditions (Ben Temime et al., 2006). Yet only a few studies took into account the effect of the macro and meso-climatic environments in olive water and oil contents (Mailer and Beckingham, 2006) and oil quality parameters (Spyros et al., 2004; Gutierrez et al.,

1999, 2000), fatty acid profile, polyphenols and induction time (Tura et al., 2007; Ranalli et al., 1999). Studies on the effects of seasonal climatic factors variations on "Chemlali" olive oil quality are scarce.

The aim of this work was to investigate the effect of the seasonal climatic variations on the quantity and the quality characteristics (free acidity, specific ultraviolet absorbance K_{232} and K_{270} , fatty acid composition, chlorophylls and total phenols) of Tunisian "Chemlali" olive oil.

MATERIALS AND METHODS

Experimental orchard and olives sampling

The trial was carried out in 2003-2004, 2004-2005 and 2006-2007 on olive trees (*Olea europaea L.*) of "Chemlali" variety at the experimental farm of "El Hajeb" in the region of Sfax (34° 43 N, 10° 41 E), Centre East of Tunisia. In order to determine the influence of the seasonal climatic variations on "Chemlali" virgin olive oil composition and quality.

The sandy soil had an organic matter content of 1.3%; 10.5% CaCO₃; 1.2% N and a pH of 7.8. The experimental orchard consisted of thirty mature olive trees planted at spacing of 24 m x 24 m and irrigated with drip irrigation system by well water (WW). Irrigation with WW was started in spring (April-May) and in autumnal period (October-December) when olive tree need a water supply for vegetative growth and fruit development. The amount of water received for each crop year was equal to 5.000 m³ ha⁻¹. The experimental plot was divided into three completely randomized blocks. Climatic conditions of the experimental site were obtained from a meteorological station installed within the experimental orchard. Averages of rainfall and air temperature were recorded. The monthly values of mean temperatures and rainfalls registered for the studied year were reported in Figures 1 and 2.

Healthy fruit samples were harvested in triplicate from all the

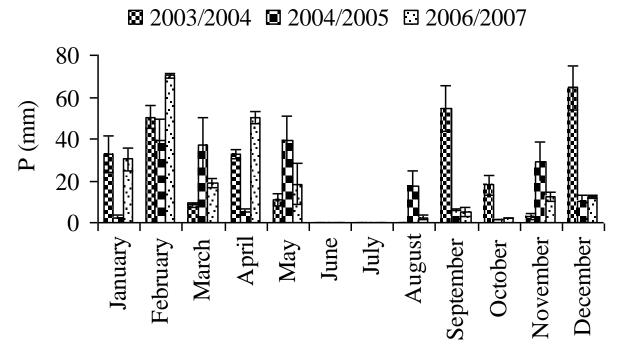


Figure 2. Monthly mean precipitation (mm) registered at the experimental farm during three crop seasons.

olive trees belonging to each block at the same ripening degree. The olive samples were brought to the laboratory for oil extraction. Fifty olives fruits were taken from each olive sample in order to determine water and oil contents.

Fruit water and oil yield estimation

Samples of approximately of 100 g (5 g \times 20 replications) of olive flesh from fruits were weighed and then dried for 24 h at 105°C, cooled for 30 min in desiccators and reweighed. The oil content was determined by Soxhlet extraction and was expressed as a percentage of dry olive paste weight.

Oil extraction

The oils were extracted using a laboratory olive mill (Sohxlet Analyzer, MC2 Ingenierias y Sistemas, Servilla, Spain) equipped with a metal crusher, a mixer whose internal partition is in rustproof steel, having a vertical or horizontal axis and a basket centrifuge. The crushed fruits were mixed for 30 min at 25°C, and then the oil was separated by a vertical centrifugation, collected and left to decant. The obtained oil was filtered and stored at -4°C in darkness using amber glass bottles until analyses.

Olive oil analyses

Quality indices

The free fatty acids (FFA), peroxide value were measured following the analytical methods described in European Economic Community regulation EEC 2568/91 (EEC Regulation 2568/91 and subsequent modifications ECC/796/02 and ECC/1991/03). K_{232} and K_{270} extinction coefficients, calculated from absorption at 232 and

270 nm respectively with UV spectrophotometer.

Organoleptic evaluation

Sensory analysis (fruitiness) of virgin olive oil samples were carried out by an analytical panel of 12 assessors according to European Official Methods of Analysis ECC/1991/03, and a sensory laboratory were used. Test Room was consistent to the standard COI/T.20/Doc. no. 6. All assessors had more than 8 years of experience in evaluating virgin olive oil. Oil samples were heated at 30 °C by a thermostat before sensory analyses and were presented fully randomized to the tasters. Dark-blue glasses were used as no colour evaluation was to be made. Each taster on the panel melled and then taste the oil under consideration. They then entered the intensity with which they perceive each of the negative and positive attributes on the 10 cm scale in the profile sheet provided. Regarding the fruity taste, each oil sample was graded on a scale from 1 to 10 cm, where 1 is a low intensity of perception of positive attributes.

Total chlorophylls

Total chlorophyll compounds were determined by the method described by Minguez et al. (1991). Chlorophyll pigments were determined by measuring the absorbance at 630, 670 and 710 nm with spectrophotometer (Perkin-Elmer UV/VIS Spectrophotometer, Norwalk, CT). The results were expressed in ppm and obtained from the following formula (Wolf, 1958).

Total chlorophylls (ppm) = $[A_{670}-(A_{630}+A_{710})/2]/0.10786*L$

Where L is vat thickness (1 cm) and 0.10786 is the variable coefficient according to the spectrophotometer used.

Total phenols

Total phenols were extracted three times from an oil-in-hexane solution using water: methanol buffer (60:40) according to the method described by Vazquez-Roncero et al. (1973). The amount of total phenols was determined colorimetrically according to the Folin-Ciocalteau procedure (Singleton and Rossi, 1965). The Folin-Ciocalteau reagent was added to a suitable aliquot of the combined extracts, and the absorption of the solution was measured at 725 nm with a spectrophotometer (Perkin-Elmer UV/VIS Spectrophotometer, Norwalk, CT). The results are expressed as mg of caffeic acid per kilogram of oil.

Oxidative stability

The oxidative stability was measured using a Rancimat apparatus (model 743 Metrohmn CO., Basel, Switzerland). Stability was expressed as the oxidation induction time (Hours) using 3.5 g of oil. The temperature was set at 102°C, and the air flow rate was 10 L per hour (Oueslati et al., 2009).

Fatty acid composition

Fatty acids were converted to fatty acid methyl esters (FAMEs) before analysis by shaking a solution of 0.2 g of oil and 3 ml of hexane with 0.4 ml of 2N methanolic potassium hydroxide and analysed by Shimadzu 17 Autosystem gas chromatograph equipped with flame ionisation detector (FID) and a fused silica capillary column (15 m length × 0.25 mm × 0.15 μm film thickness). Nitrogen was used as carrier gas with a flow through the column of 1 ml/min. The temperature of the injector was 220°C; detector and oven temperatures were 250 and 180°C respectively. Fatty acids were identified by comparing retention times with those of standard compounds. The results are expressed as relative area percentage of the total.

Statistical analysis

ANOVA analyses were performed for the obtained results, considering the temperature and rainfall as independent variables; results are presented as means of three repetitions. All statistical analyses were carried out with the program SPSS 10 for Windows, statistical software (SPSS Inc., Chicago, IL, USA). LSD test was used to determine significant difference for each parameter among seasonal weather variations.

RESULTS AND DISCUSSION

Climatic conditions

Figures 1 and 2 show the trends of annual temperatures and rainfalls. The crops seasons (2003/2004, 2004/2005, 2006/2007) are characterized by relatively mild winters with abundant precipitation and by hot and dry summers. The rainfall amount was significantly (p < 0.01) high at autumnal period in 2003-2004 crop season as compared to both other crop seasons (10 to 25 mm at 2004/2005 and 5 to 20 mm at 2005/2006). The summer and autumnal periods of 2004-2005 crop season (May-September) were characterized by a significant high temperature (p < 0.01).

Fruit water and oil contents

The mean values of fruit water content ranged from 51.83 to 58.53%, for 2006 to 2007 and 2003 to 2004 crop seasons respectively (Table 1), coherent with the values reported by Mailer and Beckingham (2006), which reported that the fruit moisture content is often about 55% or higher in developing fruit. The significant difference (p < 0.05) in water content between crop seasons is ascribable to higher rainfall values (75.40 mm) at autumnal period in 2003-2004 crop season as compared to other crop seasons, involving an increase of water in fruits.

The oil content constitutes a determinant element from an economic point of view, in fact, an increment in oil content determines an increase of the yield. As shown in Table 1, oil content, expressed as a percentage of dry matter, ranged from 44.28 to 45.50%. The mean values of fruit flesh were comparable to those reported by Abaza et al. (2003) in the same cultivar. Results showed that oil content varies year to year. This variation is probably due to the differences in temperature in the growing region during olive fruit ripening which may be stimulating some enzymes responsible for oil biosynthesis. Data showed a significant increase of oil content with increasing autumnal temperature (p < 0.05) and rainfall decrease. All the oil content increments occurred simultaneously to a decrease in water content of fruit ($r^2 = 0.86$) (Table 1).

Free fatty acid, peroxide value and specific ultraviolet absorbance (K_{232} and K_{270})

In all the examined samples, the free fatty acid (FFA) contents were much lower than the upper limit of 0.8% established for the best commercial olive oil quality, designated as "extra" virgin (EC Regulation 2568/91 and subsequent modifications ECC/796/02 and ECC/1991/03). The peroxide values (PV) of the oils analyzed were below the limit of 20 meq O₂ kg⁻¹ of oil, which is accepted as the limit for extra-quality virgin olive oil (International Olive oil Council, 2003). As shown in Table 1, the analytical parameters (FFA and PV) are not significantly influenced by the seasonal climatic variations. This findings on "Chemlali" cultivar behaviour are in contrast with previous results reported by Gutierrez et al. (1999, 2000) on Spanish cultivars (Picual and Hojiblanca). In fact, they found that FFA is a parameter negatively correlated to the temperature into an olive orchard characterized by a conventional cropping system. Indeed, the analytical parameters depend on several factors, such as olive fly attacks or improper systems of harvesting, transport and storage of olives.

In the studied crop seasons K_{232} and K_{270} ranged from 1.61 to 1.87 and from 0.15 to 0.19 respectively (Table 1). The values were lower than the limits established for extra virgin olive oil (2.5 and 0.22 for K_{232} and K_{270} , respectively). The high K_{232} value was associated to high

Parameter	2003/2004	2004/2005	2006/2007
WC	58.53 ^a	53.53 ^a	51.83 ^b
OC	44.28 ^a	45.50 ^b	44.75 ^b
FFA	0.27 ^a	0.26 ^a	0.26 ^a
PV	6.70 ^a	6.80 ^a	6.80 ^a
K ₂₃₂	1.61 ^a	1.87 ^b	1.81 ^b
K ₂₇₀	0.19 ^a	0.15 ^a	0.16 ^a
Fruitiness	26 ^a	27 ^a	29 ^a
TP	41.53 ^a	91.53 ^b	110 ^c
IT	30 ^a	50 ^b	62 ^c
CC	1.57 ^a	0.66 ^b	0.46 ^c

Table 1. Standard quality parameters, polyphenols, chlorophyll and oxidative stability of olive oils obtained in three crop seasons.

Data represents mean value (m). Different letters within column indicate the significant difference (p<0.05) between both crop seasons. WC, water content (%); OC, oil content (% of dry olive paste weight); fruit wt, fruit weight (g); FFA, free fatty acid (% of oleic acid); PV, peroxide value (meq of oxygen/kg of oil); fruitiness (mm); IT, induction time (h at 98°C); TP, total phenols (mg/kg); CC, chlorophyll content (mg/kg).

autumnal temperature, while minimum K_{270} value was found in oils obtained from fruits grown at dry conditions. Data showed that the high autumnal temperature values determined an increase of K_{232} (2004 to 2005 crop season), while a high amount of autumnal rainfall caused an increment of K_{270} value (2003-2004 crop season).

Fatty acid composition

Table 2 shows the results of fatty acid methyl esters (FAME) contents for all the VOO samples collected during three crop seasons (2004 to 2006). The most abundant fatty acid was the oleic acid (C18:1) with contents varying from 57.49 to 59.22%. Furthermore, the levels of palmitic (C16:0) and palmitoleic (C16:1) acids ranged from 18.95 to 19.74% and from 2.57 to 2.61%. The variability of fatty acid composition of the oil samples covered the normal range expected for olive oils (Codex Stan, 1989). However, the oil samples had high monounsaturated fatty acid (MUFA) content and a moderate level of linoleic acid (18%), in accordance with the general observations for oils from the south of producing countries like Spain, Greece and Italy (Giansante et al., 2003; Psomiadou et al., 2003).

The palmitic, palmitoleic, linoleic and linolenic acid contents remained unchanged in three crop seasons. No significant differences were found in ANOVA, that is different to say the growing region have no effect in major fatty acid. Results are in contrast with the findings of previous studies in other varieties, which reported that olive fatty acid composition changed in relation to climatic factors variations (Allalout et al., 2009; Ben Temime et al., 2006; Beltran et al., 2004; Stefanoudaki et al., 1999). Differences can be explained by cultivar which an essential factor determining olive fatty acid composition (Baccouri et al., 2007; Ben Temime et al., 2006).

The stearic acid level was lower than the upper limit 5% established for high extra virgin olive oil (EEC, 2003). At 2004-2005 crop season, the stearic acid content significantly increased, whereas oleic acid content showed an opposite trend (Table 2). The decrease of oleic acid content is due to the transformation of oleic acid into stearic acid by stearoyl-ACP $\Delta 9$ desaturase activity which is active during triacylglycerol biosynthesis accentuated by high summer rainfall (August).

The percentages of saturated, monounsaturated and polyunsaturated fatty acids (PUFA) and the oleic acid/linoleic acid ratio (O/L) in the studied olive oils were also evaluated. It was observed that "Chemlali" oil was rich in total saturated fatty acids (SFA) (21.98%) essentially due to its higher content in palmitic acid which represents the major acid of the SFA fraction. The total monounsaturated fatty acids (MUFA) are low for olive oil, considering the most abundant varieties, such as Picual. The olive oil was rich in total polyunsaturated fatty acids (18.82%) because of its high contents in linoleic acid representing the major fatty acid component of that fraction. The oleic acid/linoleic acid ratio varies between 3.23 and 3.38 according to crop season. This ratio can be useful to characterize olive cultivars and to have a marked relationship with stability (Ouselati et al., 2009). As shown in Table 1, the olive fruit from cooler areas contained oil with more unsaturated fatty acids than the fruit from warm areas. This finding agrees the result reported by Ben Temime et al. (2006).

Organoleptic evaluation

All the virgin olive oils obtained in the three crop seasons were characterized by the absence of defects and a typical "fruity" flavour particularly preferred by consumers. All the oil samples were classified as 'extra virgin'. Table

Table 2. Fatty acid composition	of oils obtained in three crop seasons.
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Percentage (%)	2003/2004	2004/2005	2006/2007
Palmitic acid (C16:0)	19.74 ^a	18.95 ^a	19.47 ^a
Palmitoleic acid (C16:1)	2.61 ^a	2.57 ^a	2.40 ^a
Steraic acid (C18:0)	1.76 ^a	1.87 ^b	2.10 ^b
Oleic acid (C18:1)	59.22 ^a	57.49 ^b	59.22 ^a
Linoleic acid (C18:2)	18.00 ^a	17.75 ^a	17.50 ^a
Linolenic acid (C18:3)	0.80 ^a	0.74 ^a	0.71 ^a
Arachidic acid (C20:0)	0.36 ^a	0.37 ^a	0.36 ^a
Σ SFAs	21.86 ^a	21.98 ^a	21.93 ^a
Σ PUFAs	18.80 ^a	18.49 ^a	18.21 ^a
Σ MUFAs	61.97 ^a	60.22 ^b	61.62 ^a
Oleic/linoleic	3.29 ^a	3.23 ^b	3.38 ^a
MUFAs/PUFAs	3.29 ^a	3.25 ^a	3.38 ^a

Different letters within column indicate the significant difference (p<0.05) between three crop seasons.

1 shows the organoleptic evaluations made by an IOOC (International Olive Oil Council) recognized olive oil taster panel, which reveal no significant differences were found in the fruity flavour of all oil samples.

Chlorophyll contents

The colour of olive oil, even if it is not a parameter legally established, is an important quality characteristic which can play a key role as a factor of acceptability among consumers. In fact, many consumers associate a deep green colour with the idea of a good quality virgin olive oil (Baccouri et al., 2007). The mean values of chlorophyll contents in all oil samples obtained during the crop seasons are reported in Table 1. Chlorophyll values were lower than the mean contents previously reported by Allalout et al. (2009) in Arbequina and Koroneiki cultivars. Differences into the pigment contents can be attributed both to the cultivar (Psomiadou and Tsimidou, 2001) and the ripening degrees at the harvesting date.

Chlorophyll contents decreased from 1.57 mg kg⁻¹ (2003 to 2004) to 0.46 mg kg⁻¹ in 2005 to 2006 crop season. This significant decrease of chlorophyll contents could be due to the peroxidase activity stimulation by high autumnal temperature. In fact, the maturation of fruit is associated to the chlorophyll degradation (Gandul-Rojas et al., 2004; Johnson-Flanagan and Spencer, 1996). Simultaneously with the decrease of chlorophyll contents, a photosynthetic activity decrease was observed (data not shown). The highest chlorophyll contents, reported at the beginning of the study period, was positively correlated with a high rainfall that could inhibit the peroxidase activity.

Total phenois

Total phenols (TP), the main antioxidants present in

VOO, are major responsible of the product stability and taste (Servili et al., 2003; Abaza et al., 2002; Kellie and Petter, 2002). TP contents presented a great variability depending on the crop season ranging from 41.53 to 110 mg kg⁻¹ (as caffeic acid). As reported by different authors, the amount of total phenols is ranging between 50 and 1000 mg kg⁻¹, depending on various factors such as cultivar (Salas et al., 1997; Bruni et al., 1994), climate (Salvador et al., 1998), location, degree of maturation, type of crushing machine and oil extraction procedures among others (Aquilera et al., 2005).

The oils from the last crop year (2005-2006) had a higher mean TP contents in comparison to the first and the second crop years. The latter is probably related to low rainfall accumulation of that year, as it is known that water shortage tends to generate a stress situation in the olive tree that induces phenol production in the olive fruit (Tovar et al., 2002). The higher TP content found in oils obtained in 2004 to 2005 crop season was associated to a low fruit water content which during ripening, while the lowest oil total phenol contents at 2003 to 2004 crop season was associated to the highest fruit water content (58.53%) This finding is in agreement with results reported by Salvador et al. (2001) for some Spanish cultivar.

A significant increase of TP content was reported in oils coming from fruits grown at high autumnal temperature (Table 1), as described previously by Ben Temime et al. (2006). The increase could be probably due to $_{\rm L}$ -phenylalanine ammonia-lyase activity stimulation by high autumnal temperature.

Oxidative stability

The oxidative stability of VOO samples was evaluated by the Rancimat test. Results are reported in Table 1. The mean values of oxidative stability ranged from 30 to 62 h in 2003 to 2004 and 2006 to 2007 crop seasons, respectively. The induction time was lower than those reported by several authors for others varieties (Aguilera et al., 2005; Beltran et al., 2005). The major reason can be a lower oleic acid in Chemlali, regarding the widespread varieties.

A significant increase of oxidative stability was reported in 2006-2007 crop season. In accordance with many other authors (Amirante et al., 2006; Andrews et al., 2003; Gomez-Alonso et al., 2002), a positive correlation between TP contents and oxidative stability was recorded ($r^2 = 0.98$), showing that the oil rich in TP are characterized by the higher stability. The higher stability was also into relation with fatty acid composition. In fact, samples characterized by the lower content in polyunsaturated fatty acids showed a high oxidative stability (Peres et al., 2004).

Conclusion

The study of the effect of the seasonal climatic variations on the composition and quality of "Chemlali" virgin olive oil is of great interest to the local industrial sector, the international olive oil business, and the final consumer since, despite the economic importance of this olive oil variety in Tunisia, complete and reliable data are still no available. The preliminary results revealed that the high autumnal temperature affected positively the olive oil content, with a positive increment of economical profits. No substantial differences in FFA and PV were noticed, while the K₂₃₂ and K₂₇₀ values were significantly different. Regard to the organoleptic evaluation and the other legally required parameters, all the VOO samples were defined as extra virgin olive oil. The seasonal climatic variations within the region influenced strongly total phenol and chlorophyll contents. A significant decrease of chlorophyll contents was found in oils coming from fruits grown at high temperature. However, the total phenol contents and the oxidative stability increased. Further studies of the effects of macroclimatic environments on "Chemlali" virgin olive oil composition are under investigation.

Conflict of interest

The authors have not declared any conflict of interest.

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