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Research Article

Oil quality characteristics of Chemlali olive tree (*Olea europaea* L.) grown under air fluoride pollution in arid region in Tunisia

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Abstract

This study's purpose was a comparison of oil quality characteristics from two adult olive orchards (Olea europaea L. cv. Chemlali) grown under two different environmental circumstances. The first is located around the main industrial factory in Sfax city, the Industrial Society of Phosphoric Acid and Fertilizers (polluted area) and the second is placed at 40km from the industrial unit and chosen as the control one (Control). Results of this study showed that olive production and oil content have decreased under polluted conditions. This decrease was accompanied with that of total phenol, oxidative stability, total chlorophyll and carotenoids. Nevertheless, this decrease was significant only for the photosynthetic pigments contents. For the acid composition of polluted olive plants, a significant decrease was observed in the case of oleic acid in parallel with an increase of the palmitic and linoleic acids. Nevertheless, this increase was significant only for the linoleic acid and oil samples of both treatments were classified as "extra virgin"

ABBREVIATIONS

VOO: Virgin Olive Oil; PAL: Phenylalanine Ammonia-lyase

INTRODUCTION

Environmental stresses such as extreme temperature, salinity, drought, flooding, heavy metals and air pollutants greatly affect plant metabolism and productivity. Nowadays, in Tunisia, face to the increase of the industrial activities, the olive tree development is facing to the combined effects of arid climate and air pollution. Indeed, in Sfax city, the main industrial region in Tunisia, it has been observed that recently the landscape around the main industrial factory "The Industrial Factory of Phosphoric Acid and Fertlizers" is characterized by the progressive degradation of vegetations Fluroide is among the most phytotoxic air pollutant emitted from this industry. Indeed, analysis of the air surrounding the factory showed that fluoride

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air contents oscillate between 3 and 12 µg dm⁻³ day⁻¹ [1].

Plant injury caused by pollutants released into the environment is most common near large cities and industrialized regions. Fluorides, the most stable of all chemical elements, result from the combination of fluorine with most chemical compounds [2,3] reported that fluoride pollutants taken up via root uptake and / or via direct foliar absorption are responsible of very acute damaging effects on vegetation.

The most common air pollutants are oxides of sulphur (especially SO_2), fluorides (especially HF), oxides of nitrogen, ozone, peroxyacetyl nitrate (PAN) and particulates [1]. Nevertheless, the effects of air pollutants on plants have been mainly studied in annual or forest species; and information on the response of woody plants is scarce. Furthermore, major investigations have been interested in the pattern of accumulation of toxic ions and physiological and morphological responses of plants.

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A great deal of research has been performed to evaluate visible symptoms and / or to examine the fluoride concentrations in the different plant parts [1,4]. However, few works deal with the changes in some biochemical traits resulting from alterations induced by this toxic substance. Indeed, to our best of Knowledge, there is no study on oil quality characteristics of adult olive tree subjected to air fluoride pollution under natural environmental conditions of arid climate in the south of Tunisia.

MATERIALS AND METHODS

Plant material and experimental design

The region is characterized by an arid climate of Mediterranean type. The annual rainfall and temperature averages over a 52-year period were 250mm and 23°C, respectively (Ben Ahmed et al., 2009) [5]. The studied olive tree (*Olea europaea* L. cv. Chemlali), were marked in land plots at 0.7 km from "Société Industrielle d'Acide Phosphorique et d'Engrais" (SIAPE) factory near to Sfax city. They consist of 35 years old trees planted on a loamy sand soil and served as the polluted trees. The control area is located in El Hencha region, at 40 km from the factory. The olive plants of both sites (polluted and control) were chosen to be similar in old and canopy. For each experimental site, ten trees from two adjacent rows (total 20 trees per treatment), with four replications of 5 trees each, were selected to be similar in potential yield and canopy.

The SIAPE factory constitutes the main pollution source in Sfax city. It is a phosphate fertilizer producing factory, located in the southern suburb of Sfax, that converts crude phosphate with a high fluoroapatite [Ca5(PO4)3F] content into a granule phosphate fertilizer easily assimilated by plants. During the phosphate attack by sulphuric and phosphoric acids, fluoride compounds such as HF, $H_2SiF_{6^2}$ and CaF_2 are given off by the factory chimney [6]. According to Ben Abdullah [7], the air fluoride value in the polluted area was between 0.3 and 0.68 µg m-3. It exceeds the value tolerated by sensitive vegetation (0.25 µg m-3) as has been indicated by Doley [8]. In other term, vegetation developed around the SIAPE Society seems to be exposed continuously to the air fluoride pollution.

Oil mechanical extraction process and quality analysis

For oil analyses, three samples of 4Kg of fruits each per treatment were harvested at mid-December. Olive oil used for analysis was extracted using a laboratory olive Bench Hammer Mill (Abencor Analyzer, MC2 Ingenierias y Sistemas, Sevilla, Spain). Oil samples were filtered, transferred into amber glass bottles and stored at 4°C in darkness until analysis.

Oil quality indices, photosynthetic pigments contents and fatty acid composition: Free acidity (% oleic acid) and peroxide value (meq O_2 / kg) were measured following the analytical method as described in the European Regulation [9]. Extinction coefficients K_{232} and K_{270} were measured at 232 and 270 nm, respectively.

The chlorophyll fraction at 670 nm and the carotenoid fraction at 470 nm were evaluated from the absorption spectrum of each oil sample (7.5g) dissolved in cyclohexane as described by Mínguez - Mosquera et al [10].

Fatty acid composition was determined based on the European Regulations EEC 2586/91 method as described by Ben Ahmed et al [9,5].

Total phenols, phenolic compounds and oxidative stability: The concentration of total polyphenols was estimated with Folin-Ciocalteu reagent at 725 nm as described by Ben Ahmed et al [5]. Results were expressed as mg of caffeic acid per kg of oil. The different phenolic compounds analysed were determined from virgin olive oil according to Patumi et al [11] method. Oxidative stability is evaluated using a 679 Rancimat apparatus (Metrohm, Switzerland) at 120°C and 20 l h⁻¹ air flow. The oil stability is expressed as the induction time (hours) of hydroperoxide decomposition.

Statistical analysis

Statistical analyses were performed using the SPSS 10. Windows and treatment means were compared using Least Significant Difference (LSD) test at p < 0.05. At least three replicates were used for each analysis.

RESULTS AND DISCUSSION

Olive yield, free acidity, peroxide values, extinction coefficients and total chlorophyll and carotenoids contents

The average olive production of polluted plants during the experimental period (12.5kg tree⁻¹) was much lower (52%) than that of control ones (26kg tree⁻¹). This yield reduction could be due, at the same time, to the negative effects of pollutants on fruit development process, via limitation of water availability, and also to the alternate bearing phenomenon characterizing the olive tree production and/or to the effects of climatic conditions characterizing the experimental site. Generally, fruit development phase (from June to December) coincides with period of high temperature and radiations which can affect fruit growth, and hence, olive yield.

During the experimental period, the free acidity varied from 0.45 to 0.65 %, respectively, in control and polluted area and the peroxide value oscillated between 12.73 and 16.54 meq O_2 kg⁻¹, respectively (**Table 1**). The registered values were lower than the upper limits (0.8 % as oleic acid and 20 meq O_2 kg⁻¹ as the peroxide value) established by the EU legislation for extra virgin olive oil. More to the point, these oil quality indices were influenced by the air fluoride pollution, since differences between both treatments were statistically significant (p < 0.05).

According to Boskow [12], the increment of free acidity value under polluted conditions could be due to the increase of enzymatic activity of some lipolytic enzymes characterizing the damaged olive tissues. The increment of the peroxide value of oil from the polluted area could be explained, in accordance to Gutierrez et al [13], by the increase of the lipoxygenase activity. On the other hand, these values were higher than those recorded by Ben Ahmed et al [5] for the same olive cultivar irrigated with saline water.

The comparison of spectrophotometric absorption characteristics in the UV region at 232 and 270 nm between oils from the two treatments did not show significant differences.

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Taking into account the values of free acidity, peroxide value and $\rm K_{_{232}}$ and $\rm K_{_{270}}$ the oil samples obtained from both treatments met the European Union requirements for the extra virgin olive oil category.

Total chlorophyll and carotenoids contents in the virgin oils were enormously affected by the air pollution level around the industrial society. Indeed, both of the photosynthetic pigments contents were reduced at more than 50% in oil coming from plants exposed to air pollution if compared to the control ones (**Table 1**) and differences between both treatments were significant. This reduction could be attributed to the pigments' degradation by pollutants particularly the fluoride one. Furthermore, the decrease of these pigments contents confirms the oxidative stress induced by the pollutants and could be considered as a toxicity sign caused by environmental stress.

Fatty acid composition

Results displaying the fatty acid composition of virgin olive oil (VOO) characterizing both of the experimental sites is represented in **Table 2**. For both oil samples, the most abundant acid was the oleic one with values recorded in oil obtained from polluted plants were statistically lower (p = 0.0031) than those in oil of control ones. The unsaturated- saturated acid ratio did not appear to be influenced by the air pollution characterizing the polluted experimental site. However, the oil obtained from control plants would be nutritionally better than that obtained in the case of polluted ones. According to Salvador et al [14], the reduction of oleic acid and the slight increase of the palmitic

one in the case of polluted plants could be due to disturbance occurring in the triacylglycerol biosynthesis induced by the air pollutants characterizing the polluted area. On the other hand, the higher amounts of linoleic acid recorded in polluted plants and the low level of oleic acid found in the case of Chemlali olive oil, in comparison to other cultivars such as Arbequina [15] and Cornicabra [16] may be due, according to Sanchez and Harwood [17], to the transformation of oleic acid into linoleic one by the oleate desaturase activity and / or probably to the disturbance of the activities of enzymes involved in the oleic acid synthesis chain by fluoride pollution characterizing the SIAPE site.

Phenolic compounds, total phenols and oxidative stability

Table 3 reports the concentrations of the major phenolic compounds, total phenols and oxidative stability of VOO samples under both environmental conditions. Total phenols contents of VOO were not significantly influenced by the air pollution level. They were of 281 and 277 mg / kg, respectively in control and polluted plants. The three phenolic compounds in highest concentrations in both oil samples are hydroxytyrosol, tyrosol and glycoside oleuropein. The maintenance of high phenols contents in polluted oil samples could be involved in the antioxidative mechanisms developed by the olive tree in response to oxidative stress induced by air pollution as was suggested by several papers under different environmental constraints [18].

On the other hand, the higher polyphenol contents recorded in oils of stressed plants could be due to the acceleration of

Table 1: Free acidity, peroxide values, extinction coefficients, total chlorophyll (Chl) and carotenoids (Car) contents of oils and olive yield of olive trees (Cv. Chemlali) grown under non polluted and fluoride polluted area.									
	Free Acidity (%)	Peroxide Value (meq O ₂ / kg)	K232	K270	Chl (mg/ kg)	Car (mg/kg)	Olive yield (Kg/ tree)		
Control	0.45±0.05ª	12.73±1.21ª	2.33±0.21ª	0.25±0.08ª	27.30±2.45ª	12.05±1.85ª	26±3.85ª		
Polluted	0.65±0.09 ^b	16.54±1.56 ^b	2.27±0.25ª	0.24±0.06ª	13.27±1.76 ^b	5.89±1.01 ^b	12.5±2.65 ^b		
Values are means of three samples $(n = 3) \pm$ standard deviations. Different letters (a, b) indicate significant differences $(p < 0.05)$ between treatments.									

Abbreviations: Chl: total chlorophyll; Car: carotenoids

Table 2: Fatty acid composition (%) of virgin olive oils from olive trees (Cv. Chemlali) grown under non polluted and fluoride polluted area.						
	Control area	Polluted area				
Palmitic acid (C16)	20.45 ± 1.40^{a}	21.88 ± 1.38^{a}				
Palmitoleic acid (C16')	2.69 ± 0.49^{a}	3.54 ± 0.46^{a}				
Heptadecanoic acid (C17)	0.14 ± 0.01^{a}	0.17 ± 0.03^{a}				
Heptadecenoic acid (C17')	0.19 ± 0.02^{a}	0.16 ± 0.02^{a}				
Stearic acid (C18)	3.08 ± 0.28^{a}	2.94 ± 0.21^{a}				
Oleic acid (C18')	66.48 ± 5.03ª	55.74 ± 4.78^{b}				
Linoleic acid (C18")	14.15 ± 3.46^{a}	20.16 ± 3.02 ^b				
Linolenic-eicosanoic acid (C18''')	0.86 ± 0.08^{a}	0.84 ± 0.06^{a}				
Eicosanoic acid (C20)	0.69 ± 0.09^{a}	0.64 ± 0.05^{a}				
Eicosenoic acid (C20')	0.37 ± 0.04^{a}	0.30 ± 0.04^{a}				
Insat / Sat ratio	3.49 ± 0.64^{a}	3.14 ± 0.42^{a}				
Values are means of three samples $(n = 3)$	+ standard deviations. Different letters	s(a, b) indicate significant differences ($p < 0.05$) between treatments.				

Values are means of three samples (n = 3) ± standard deviations. Different letters (a, b) indicate significant differences (p < 0.05) between treatments. **Abbreviations:** Insat / Sat: Insaturated-Saturated acids ratio.

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 Table 3: Phenolic composition concentrations (mg / Kg of oil), total phenols contents (mg/kg of oil) and oxidative stability (h) of olive oils from olive trees (Cv. Chemlali) grown under non polluted and fluoride polluted area.

	Control area	Polluted area			
Tyrosol	41.53 ± 2.36 ^a	48.273.21 ^b			
Hydroxytyrosol	79.52±3.56ª	81.362.06ª			
Oleuropein	10.23±2.84ª	11.452.56ª			
glycoside oleuropein	23.23±2.56ª	22.492.47ª			
Vanillic	10.62±1.56ª	19.091.04ª			
Caffeic	7.87±1.24ª	9.37±1.03 ^b			
Syringic	10.27±1.2ª	9.261.24ª			
p-coumaric	6.12±1.32ª	5.561.42ª			
o-coumaric	5.19±1.02ª	6.761.25ª			
Ferulic	4.27±0.43ª	4.88±0.65ª			
Total phenols (mg/kg)	281.74±4.96ª	277.09±4.45ª			
Oxidative stability (h)	16.02±1.02ª	15.61±1.45ª			
Values are many of three complex (r. 2) - standard deviations					

Values are means of three samples $(n = 3) \pm$ standard deviations. Different letters (a, b) indicate significant differences (p < 0.05) between treatments.

maturation of the olives which could account for the higher levels of phenols. Furthermore, as it is known, the air pollution, and per consequent high accumulation of pollutants and dehydration of plant tissues could result in water deficit and pollution toxicity. Per consequent, the increase of phenols contents in stressed plants might be due to the effects of water deficit on the activation of Phenylalanine ammonia-lyase (PAL), a key enzyme in the biosynthetic pathway of phenolic compounds, which is directly involved in the accumulation of polyphenols in the virgin olive oil [19] as recently reported in cv. Leccino olive by Servili et al [18]. Moreover, periods of severe conditions could influence PAL activity in olive fruit [20].

The oxidative stability of VOO samples did not show a significant variation between treatments. Furthermore, values recorded were comparable to those obtained by José-Motilva et al [15] in the case of Arbequina olive cultivar but lower than those characterizing the Cornicabra virgin olive oil [14]. The same authors have stated that oxidative stability of VOO depends on a multitude of factors such as the extraction system, climate, latitude and stage maturity of collected olive fruits. Moreover, the higher oxidative stability of VOO obtained in polluted plants could be due to the higher phenols contents as has been suggested by several papers [14,15]

CONCLUSION

In conclusion, our results are further evidence of direct effects of air pollution on qualitative parameters of olive oil, particularly the fatty acid and phenolic composition of virgin olive oil. Nevertheless, oil samples obtained from both treatments met the European Union requirements for the extra virgin olive oil category.

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