

TALC EFFECT ON THE VOLATILES OF VIRGIN OLIVE OIL DURING STORAGE

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ABSTRACT

The aim of the study was to assess the influence of talc on the extra virgin olive oil volatile profile during production and storage. The obtained results showed that talc used at 1% level did not cause significant differences, whereas at 2% level showed only slight differences were revealed compared to control. During storage a significant increase of the volatiles deriving from the oxidative process was observed and a concomitant significant decrease of the C6-LOX aldehydes. The evolution of the volatile compounds of extra virgin olive oils during storage was not significantly influenced by talc addition, with the exception of the sum of C6-LOX aldehydes, which showed a significant higher decrease.

Keywords: extra virgin olive oil, storage, talc, volatile compounds

1. INTRODUCTION

Volatile compounds, together with phenolics, are responsible for the sensory attributes of extra virgin olive oil (MORALES *et al.*, 1995; APARICIO *et al.*, 1996) and play a key role in the marketing process, by influencing the choice of the consumer. Volatiles arise during olive (*Olea europaea* L.) ripening (ANGEROSA and BASTI, 2001; APARICIO and MORALES, 1998) and are influenced by oil extraction processes (ANGEROSA *et al.*, 2000; KIRITSAKIS, 1998). Moreover, they may be altered during storage, especially if the main factors promoting product oxidation - such as heat, light, and air - are not well-managed (FRANKEL, 1985; MORALES and TSIMIDOU, 2000; BRKIĆ BUBOLA *et al.*, 2014).

It is well known that in fresh extra virgin olive oils the volatile compounds derive from the degradation of polyunsaturated fatty acids, through the lipoxygenase (LOX) pathway (ANGEROSA *et al.*, 2000; KALUA *et al.*, 2007), which produce C5 and C6 volatiles, mainly responsible for the "green" odor notes (ANGEROSA *et al.*, 2004). Among these volatiles, C6 unsaturated and saturated aldehydes, whose amount depends on the activity of the enzymes involved in LOX pathway (ANGEROSA *et al.*, 2001), are the most abundant compounds in high quality virgin olive oils (ANGEROSA *et al.*, 2004).

In recent years the use of coadjuvants in the oil mills has increased considerably, bringing increments of extraction efficiency during oil production and decreasing pomace moisture. It is common knowledge that the extraction systems can only recover about 80-90% of the oil contained in the starting olives, with the remainder being trapped in microgels or emulsified with the water phase (AGUILERA *et al.*, 2010). The extraction yield is further lowered in case of the so-called "difficult pastes" (CAPONIO *et al.*, 2014a), derived by batches of olives having a heterogeneous ripening index.

To increase extraction effectiveness, malaxation is the key phase of virgin olive oil production process and, generally, increasing the temperature determines an increase in oil extraction yield, due to a reduction of viscosity in the oily phase; however, the collateral effects of increasing temperature and time at this step are well known (ANGEROSA *et al.*, 2001). More recently, new technologies were also proposed for oil industries, among which ultrasounds, microwaves, and mechanical vibrations (BEJAOU *et al.*, 2016; TAMBORRINO *et al.*, 2014; TOSCHI *et al.*, 2013).

On the other hand, innovation in the field of olive oil extraction lead to the spread of a new generation of two-way decanters, which do not require the addition of water to the olive paste and result in obtaining high quality oils, without losing the minor compounds responsible for sensory, nutritional, and healthy features of olive oil. However, these decanters generate pomaces with a moisture content of higher than 55% (CAPONIO *et al.*, 2014a; TAMBORRINO *et al.*, 2015), which is an excessively high value for pomace refineries, necessitating to expensive preliminary drying.

Talc is a commonly used coadjuvant, not excluded by the EC Regulation No. 1513/2001 in the production of extra virgin olive oil, due to its exclusively physical action (CERT *et al.*, 1996). It is added during malaxation and has a positive effect on oil yield, especially in the case of difficult olive pastes (CAPONIO *et al.*, 2016). All investigations highlighted an effect of talc on the chemical parameters of extra virgin olive oil. Nevertheless, few studies are available regarding the influence of talc on the volatile compounds of extra virgin olive oil. In particular, CAPONIO *et al.* (2015) highlighted that talc addition did not significantly influence the sums of aldehydes, alcohols, and ketones; on the contrary, the sums of acids and esters significantly increased with talc addition. KOPRIVNJAK *et al.* (2016) did not find significant changes of C6 aldehydes and C5 compounds, whereas with 3% of talc a significantly higher value of C6 alcohols was observed. The authors concluded that correlations between the degree of talc addition and levels of volatile compounds are not clearly evident. Moreover, no information is available about the evolution of the volatile

compounds of extra virgin olive oil obtained with talc addition to olive paste malaxation during storage.

In this framework, the aims of the present work were: i) to further investigate the influence of talc on the volatile compounds of extra virgin olive oils; ii) to evaluate whether the addition of talc to olive paste during malaxation influenced the evolution of the volatile fraction of extra virgin olive oils during storage. Olive fruits of Coratina, a cultivar that often leads to difficult olive pastes, were processed with and without talc addition at two different levels, and the corresponding oils were analyzed over a period of 6 months.

2. MATERIALS AND METHODS

2.1. Material

Olive fruits (*Olea europaea* L.) from Coratina cultivar, mechanically harvested in December 2013 were transported, immediately after harvesting, to a local plant (Andria, Bari, Italy) where, after leaf-removal, were milled within 24 h. Talc (hydrated magnesium silicate) was kindly furnished by Imerys Talc (Luzenac, France).

2.2. Oil extraction process

Three lots of about 2,000 kg of olives were considered in the trials. Each lot was divided into three homogeneous batches: one batch was processed without micronized natural talc addition (Co, control) and the remaining two batches were processed by adding 1% and 2% of talc (T1 and T2), respectively. Talc was added at the beginning of the malaxation phase.

For each batch, the olive paste after crushing was transferred into the malaxer, where it was mixed with talc only for the trials that provided its addition. After malaxation (50 min at $22\pm 1^\circ\text{C}$) the paste was pumped into a two-phase decanter, operating at 2,800 rpm, with a processing capacity of 3,000 kg h⁻¹. Finally, the oily phase was separated from any aqueous residue by centrifugation at 6,400 rpm.

Then, the obtained oil was poured into 1-L clear glass bottles and hermetically sealed with headspace of about 3 mL. Three bottles for each batch were sampled. One bottle for each batch was immediately analyzed (Time 0), whereas the others were placed in a carton box and stored in the dark to be analyzed after three (Time 3) and six (Time 6) months of storage. The samples were stored at room temperature.

2.3. Volatile compounds determination

For the determination of the volatile compounds, the oil samples (0.5 ± 0.005 g) were weighed into 20 mL vials, sealed with a screw aluminium cap and silicone/PTFE septa, and submitted to the SPME/GC-MS in the conditions reported in a previous paper (CAPONIO *et al.*, 2014b). In particular, the extraction was performed by exposing a 75 μm Carboxen/polydimethylsiloxane (CAR/PDMS) fiber (Supelco, Bellefonte, PA, USA) in the headspace of the sample at 40°C for 20 min. When the extraction process was completed, the fiber was inserted into the injector port (set at 230°C) of the gas chromatograph for thermal desorption of volatiles. The GC/MS instrumentation included an Agilent model 6850 gas chromatograph coupled to a mass spectrometer Agilent 5975. The volatile compounds were separated on a HP-Innowax (60 m \times 0.25 mm, 0.25 μm film thickness) polar capillary column (Agilent) under the following conditions: flow 1.5 mL min⁻¹; injector

temperature, 250°C; pressure of the carrier (helium), 30 kPa. The oven temperature was held for 5 min at 35°C, then increased by 5°C min⁻¹ to 50°C and held constant for 5 min, then raised to 230°C at 5.5°C min⁻¹ and finally held at 230°C for 5 min. The mass spectrometer was operated in the electron impact mode (electron energy = 70 eV), and the ion source temperature was 250°C. A continuous scan mode was employed with a scan time of 7.7 scans/s over a mass range of 33-200 amu. The volatile compounds were identified both by comparison of their mass spectra and retention times with those of authentic reference compounds and by their LRI and by comparison with the mass spectra present in the NIST and Wiley libraries. The volatile compounds were expressed as integrated area and its relative percentage.

2.4. Statistical analysis

Analysis of variance (one-way and two-way ANOVA) was carried out on the experimental data using the Minitab software (Minitab Inc., State College, USA). Two-way ANOVA was performed considering the amount of talc added (*talc*) and oil storage time (*time*), as well as the first order interaction (*talc*time*), as independent variables; Tukey's HSD test was applied for multiple comparisons.

3. RESULTS AND DISCUSSION

All the samples showed values of free fatty acids, peroxides, K₂₃₂, K₂₇₀, and DK which fulfilled the extra virgin olive oil marketing requirements according to current rules (OFFICIAL JOURNAL OF THE EUROPEAN COMMUNITIES, 2011) (data not shown). Table 1 reports the volatile compounds detected in the oil samples, obtained with and without the addition of talc during processing, immediately after their production (fresh oils). The volatiles were grouped on the basis of their most probable origin.

The overall volatile profile agreed with the findings of other authors for Italian extra virgin olive oils (ANGEROSA *et al.*, 2004; KALUA *et al.*, 2007), which were found to be richer in C6 volatile compounds and poorer in esters than Spanish and Moroccan extra virgin olive oils (REINERS and GROSCH, 1998). The low amounts of volatiles generated by auto-oxidation and/or sugar fermentation evidenced the high quality level of the starting olives. The most represented volatile compound was *trans*-2-hexenal, accounting for more than 80% headspace. It derives from the LOX activity involving polyunsaturated fatty acids and is responsible for bitter, almond, and green notes (MORALES *et al.*, 1997; BENDINI *et al.*, 2009). CAVALLI *et al.* (2004) analyzing seven French Cailletier olive oils, found a content of *trans*-2-hexenal ranging from 37-64%.

The volatile profile of the oils obtained with talc addition generally corresponded to data reported in literature (BRKIĆ BUBOLA *et al.*, 2014; CAPONIO *et al.*, 2015; CAVALLI *et al.*, 2004; KOPRIVNJAK *et al.*, 2016). In particular, talc used at 1% level did not cause significant differences, whereas at 2% level a significant increase of ethanol (responsible for alcoholic, ripe apple, and floral notes) (REINERS and GROSCH, 1998; SERVILI *et al.*, 2001) and a decrease of 1-penten-3-one (associated with the green and pungent notes), *trans*-2-penten-1-ol (responsible for green notes), and ethyl acetate (responsible for sweet and aromatic notes) (ANGEROSA *et al.*, 2004; BENDINI *et al.*, 2009) were observed.

Table 1: Integrated area mean value and results of one-way ANOVA ($p < 0.05$) of the volatile compounds detected in oil samples obtained without (Co) and with addition of 1% and 2% of talc during processing (T1 and T2, respectively), analyzed immediately after production (fresh oils).

Volatile compounds	Co		T1		T2	
	Area	%	Area	%	Area	%
C₆ LOX pathway						
Hexanal	6.84E+07	a 3.26	6.96E+07	a 3.38	6.59E+07	a 3.24
<i>trans</i> -2-hexenal	1.72E+09	a 81.90	1.67E+09	a 81.31	1.66E+09	a 81.59
Hexan-1-ol	2.61E+07	a 1.25	2.45E+07	a 1.19	2.59E+07	a 1.28
<i>cis</i> -3-hexenal	1.12E+07	a 0.53	9.06E+06	a 0.44	9.02E+06	a 0.44
<i>trans</i> -3-hexen-1-ol	1.01E+06	a 0.05	1.07E+06	a 0.05	1.03E+06	a 0.05
<i>cis</i> -3-hexen-1-ol	4.15E+07	a 1.98	4.42E+07	a 2.15	4.42E+07	a 2.18
<i>cis</i> -2-hexen-1-ol	4.40E+07	a 2.10	4.52E+07	a 2.20	4.63E+07	a 2.28
C₅ LOX pathway						
1-penten-3-one	4.54E+07	a 2.17	4.48E+07	a 2.18	3.92E+07	b 1.93
<i>trans</i> -2-pentenal	6.26E+06	a 0.30	6.67E+06	a 0.32	5.99E+06	a 0.30
1-penten-3-ol	9.99E+06	a 0.48	8.72E+06	a 0.42	9.08E+06	a 0.45
<i>trans</i> -2-penten-1-ol	2.50E+06	a 0.12	2.30E+06	a 0.11	1.85E+06	b 0.09
<i>cis</i> -2-penten-1-ol	2.47E+07	a 1.18	2.69E+07	a 1.31	2.47E+07	a 1.22
Carbohydrate fermentation						
Ethyl acetate	1.46E+07	a 0.70	1.26E+07	a 0.61	7.63E+06	b 0.38
Ethanol	3.22E+07	a 1.54	4.06E+07	ab 1.97	4.49E+07	b 2.21
Acetic acid	1.74E+07	a 0.83	1.44E+07	a 0.70	1.55E+07	a 0.76
Other origins						
Methyl acetate	3.34E+06	a 0.16	3.06E+06	a 0.15	3.11E+06	a 0.15
Pentan-3-one	1.02E+07	a 0.49	8.53E+06	a 0.41	8.58E+06	a 0.42
<i>cis,cis,cis</i> -1,3,6-Octatriene, dimethyl	3,7- 3.14E+06	a 0.15	3.17E+06	a 0.15	3.00E+06	a 0.15
Nonanal	3.75E+06	a 0.18	4.20E+06	a 0.20	3.95E+06	a 0.19
<i>trans,trans</i> -2,4-hexadienal	5.36E+06	a 0.26	5.79E+06	a 0.28	5.78E+06	a 0.28
<i>trans,trans</i> -2,4-heptadienal	8.28E+05	a 0.04	1.01E+06	a 0.05	7.34E+05	a 0.04
Benzaldehyde	1.85E+06	a 0.09	2.29E+06	a 0.11	1.90E+06	a 0.09
Propanoic acid	6.20E+05	a 0.03	5.87E+05	a 0.03	7.29E+05	a 0.04
Hexanoic acid	1.44E+06	a 0.07	1.32E+06	a 0.06	1.53E+06	a 0.08
Phenylethyl alcohol	3.56E+06	a 0.17	4.02E+06	a 0.20	3.45E+06	a 0.17

With the aim of evaluating the effect of talc on the volatiles, the variations of groups of compounds having the same origin, and belonging to the same chemical class, have been monitored during storage in the oils with or without talc addition. Moreover, some specific compounds considered to be effective indices of oil oxidations have been focused.

Figures 1 and 2 show the evolution of C6 and C5 volatile compounds, respectively, both derived from the LOX pathway.

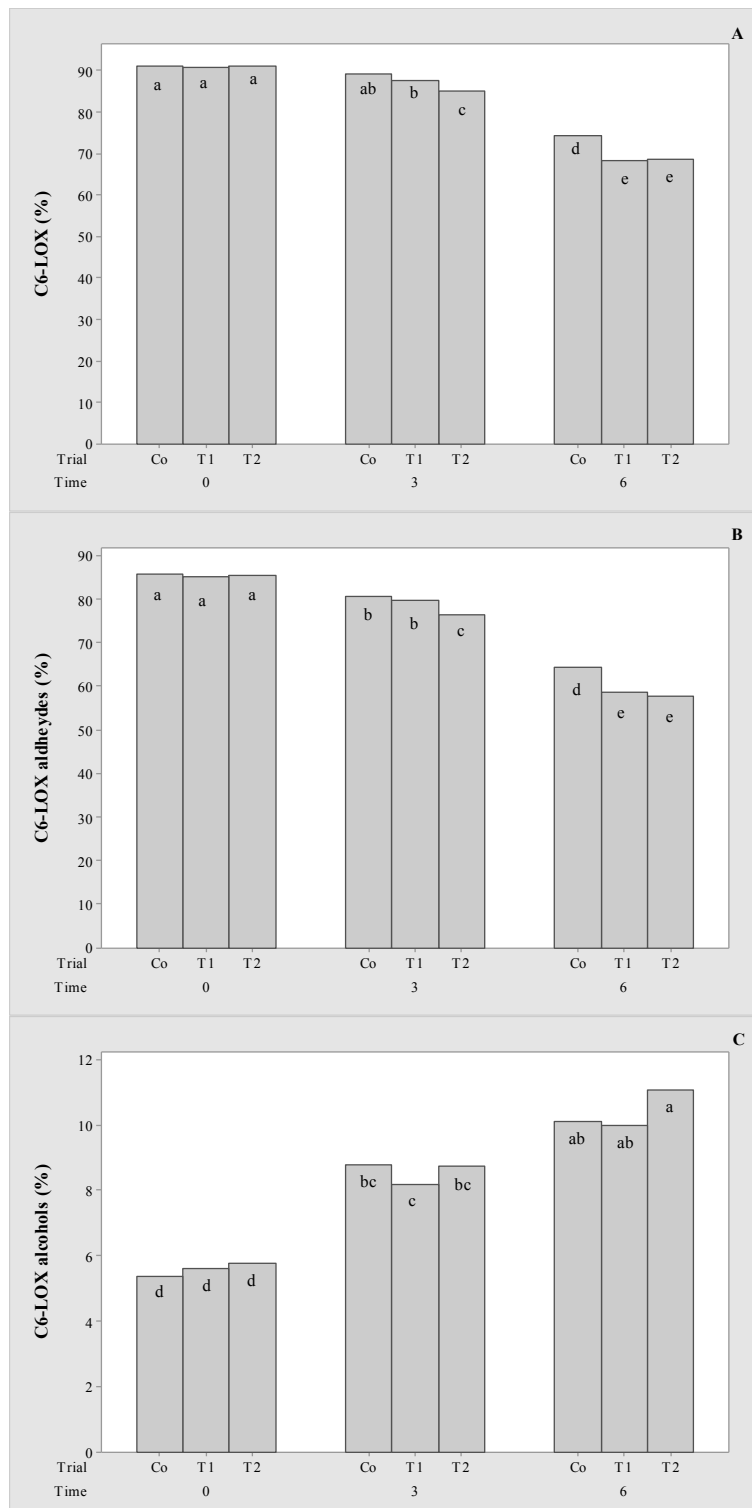


Figure 1: Percent mean value and results of two-way ANOVA ($p < 0.05$) of C6 volatile compounds deriving from the lipoygenase (LOX) pathway of the oils obtained without (Co) and with addition of 1% and 2% of talc (T1 and T2, respectively) during storage: total (A), aldehydes (B), and alcohol (C). 0, fresh oils; 3, oil stored for three months; 6, oil stored for six months.

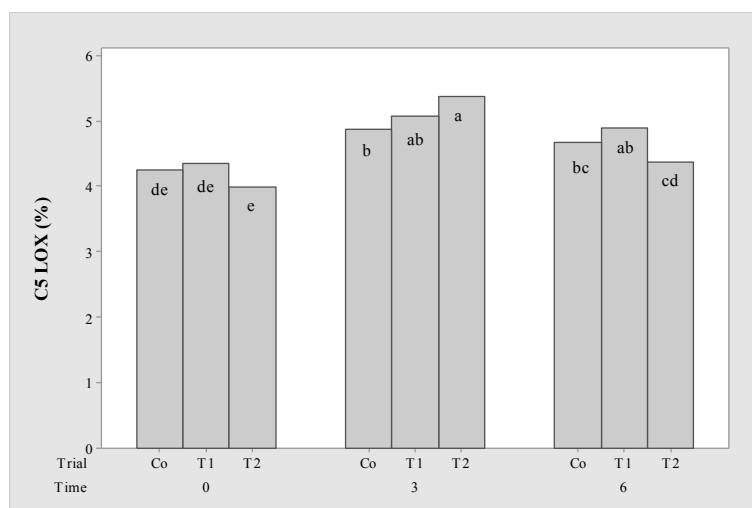


Figure 2: Percent mean value and results of two-way ANOVA ($p < 0.05$) of C5 volatile compounds deriving from the lipoxygenase (LOX) pathway of the oils obtained without (Co) and with addition of 1% and 2% of talc (T1 and T2, respectively) during storage. 0, fresh oils; 3, oil stored for three months; 6, oil stored for six months.

The C6-LOX compounds (Fig. 1A), which represent about 90% of the volatile compounds, showed a significant decrease during storage, as observed also by other authors (BENDINI *et al.*, 2009), more evident in the trials involving the use of talc. The observed trend was mainly attributable to the evolution of the C6-LOX aldehydes (Fig. 1B), which significantly decrease during storage, while C6-LOX alcohols (Fig. 1C), that representing about 5% of the volatile fraction, did not show a significant variation among trials. The significant increase of the latter during time, as reported by CAVALLI *et al.* (2004), could be attributed to the formation of alcohols by decomposition of methyl linolenate hydroperoxides (FRANKEL, 1980).

The C5-LOX compounds (Fig. 2) showed a significant increase during storage, more evident after 3 months. The increase of C5-LOX compounds was mainly due to the increase of *trans*-2-pentenal (Fig. 4B) and of 2-penten-1-ol (data not shown), that could arise from the decomposition of methyl linolenate hydroperoxides (FRANKEL, 1980). In fact, BENDINI *et al.* (2009) reported that *trans*-2-pentenal increases significantly during the oxidation of virgin olive oils in presence of metals. Ketones (Fig. 3) did not show significant variation up to 3 months of storage whereas a significant increase after six months of storage was observed, in agreement with the findings of CAVALLI *et al.* (2004). Talc addition did not show to significantly influence the variations of ketones (Fig. 3) and of C5-LOX compounds (Fig. 2).

Several authors report that the hexanal/nonanal ratio is an appropriate index to detect the beginning of olive oil oxidation and to follow its evolution (MORALES *et al.*, 1997; KIRITSAKIS, 1998; ANGEROSA *et al.*, 2004; KANAVOURAS *et al.*, 2004; BENDINI *et al.*, 2009). Moreover, also *trans*-2-pentenal, an unsaturated aldehyde originated by secondary reactions of the primary auto-oxidation products (13-LnOOH, 9-LOOH and 9-OOOH, respectively), could effectively monitor the oxidative process evolution of extra virgin olive oil (LUNA *et al.*, 2006). Finally, propanoic and hexanoic acids, the latter due to the oxidation of hexanal, and 2,4-decadienal, derived from fatty acid oxidation (REINERS and GROSCH, 1998), have also been detected during the oxidation (GUTIERREZ *et al.*, 2002; VICHI *et al.*, 2003).

Fig. 4 shows the variations of the above reported indices during storage.

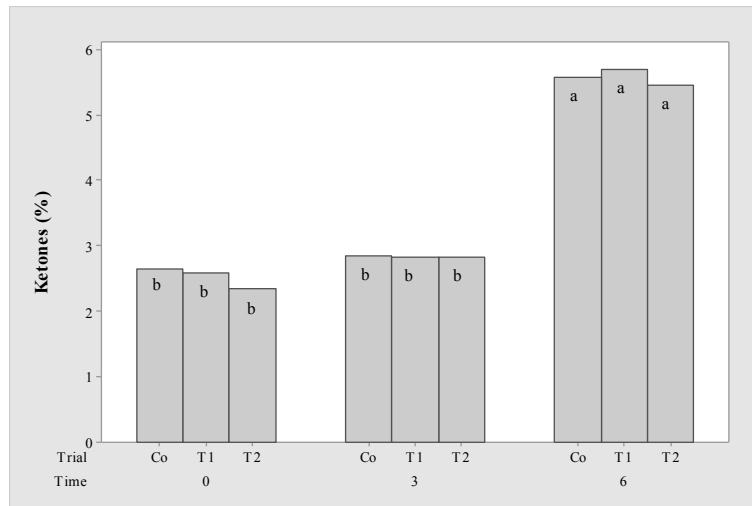
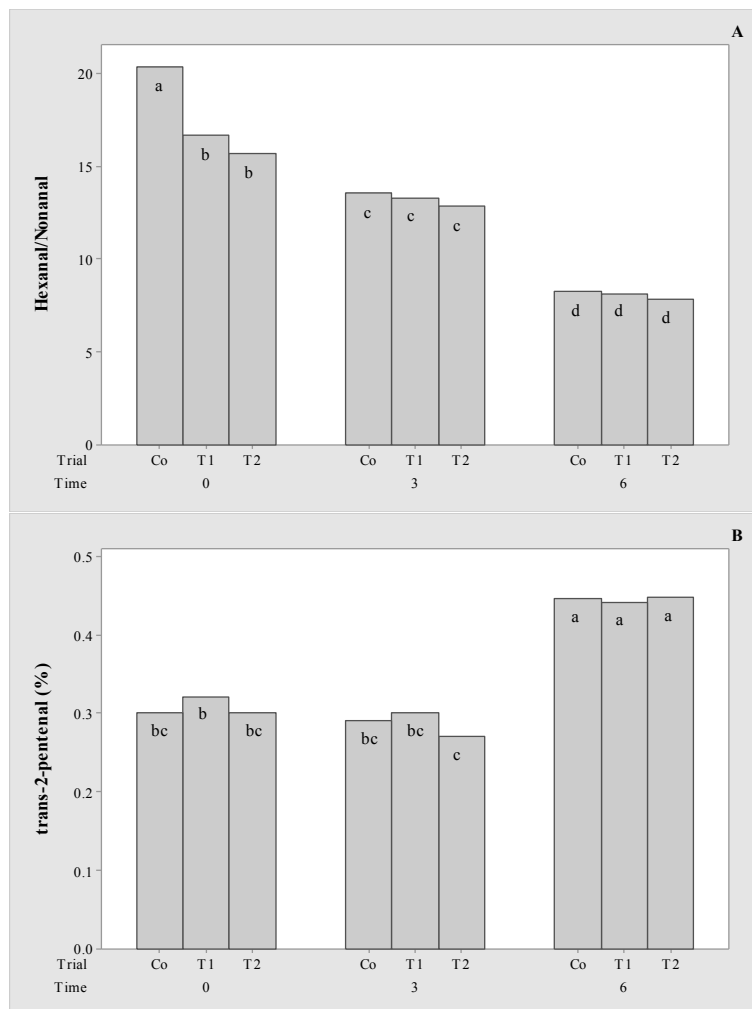


Figure 3: Percent mean value and results of two-way ANOVA ($p < 0.05$) of sum of ketone volatile compounds of the oils obtained without (Co) and with addition of 1% and 2% of talc (T1 and T2, respectively) during storage. 0, fresh oils; 3, oil stored for three months; 6, oil stored for six months.



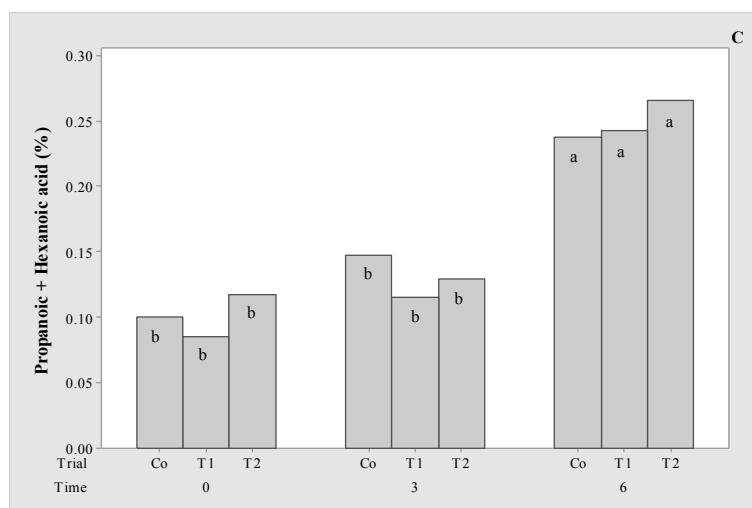


Figure 4: Percent mean value and results of two-way ANOVA ($p < 0.05$) of hexanal/nonanal ratio (A), *trans*-2-pentalen (B), and sum of propanoic and hexanoic acids (C) of the oils obtained without (Co) and with addition of 1% and 2% of talc (T1 and T2, respectively) during storage. 0, fresh oils; 3, oil stored for three months; 6, oil stored for six months.

An increase of auto-oxidation phenomena occurred during 6 months, irrespective of the addition of talc, with the only exception of the hexanal/nonanal ratio of fresh oil that indicated a stronger oxidation of talc-added oils than in the control.

4. CONCLUSIONS

The obtained results confirm that talc addition during olive processing determines only slight differences in the volatile compounds profile of the corresponding extra virgin olive oil compared to control obtained without talc addition. In the majority of cases, these differences were devoid of statistical significance. During storage a significant increase of the volatiles deriving from the oxidative process was observed, as shown by the hexanal/nonanal ratio and the sum of propanoic and hexanoic acids, and a concomitant significant decrease of the C6-LOX aldehydes responsible for bitter, almond, and green notes of extra virgin olive oil. The evolution of the volatile compounds of extra virgin olive oils during storage was not significantly influenced by talc addition, with the exception of the sum of C6-LOX aldehydes which showed a significant higher decrease.

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