Effect of Talc Addition on the Extraction Yield and Quality of Extra Virgin Olive Oils from *Coratina* Cultivar after Production and during Storage

Francesco Caponio^{1*}, Julieta I. Monteleone², Giovanni Martellini³, Carmine Summo¹, Vito M. Paradiso¹ and Antonella Pasqualone¹

¹ University of Bari Aldo Moro, Department of Soil, Plant and Food Sciences, Food Science and Technology Unit, Via Amendola, 165/A, I-70126, Bari, Italy

² Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad Nacional de Cuyo, Mendoza, Argentina

³ SVINT – Cooperativa Sviluppo & Integrazione, Via Vaccaro, 33, I-70121 Bari, Italy

Abstract: An experimental investigation was carried out with the aim to evaluate the effect of talc on the extraction yield and quality of extra virgin olive oils from *Coratina* olives after production and during storage. A significant effect of talc, added in the malaxer, on both yield and oil quality was observed. The addition of 1% talc lead to a 15% decrease of the residual oil in the olive-pomace, while higher amounts of talc did not determine further significant variations. The use of talc caused also a significant decrease of the peroxide value and tocopherols and a significant increase of carotenoids, chlorophylls, phenols, antioxidant activity and K_{270} , while no influence was detected on free fatty acids and K_{232} . Finally, during storage the differences among the oils were maintained as immediately after their production, with the exception of chlorophylls.

Key words: coadjuvant, extra virgin olive oil, oil extraction, storage, talc, yield

1 INTRODUCTION

Extra virgin olive oil – a key element in the daily diet of a large part of the Mediterranean population – can be directly consumed after extraction without any further treatment since it is “obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions that do not lead to alteration in the oil, which have not undergone any treatment other than washing, decantation, centrifugation or filtration, to the exclusion of oils obtained using solvents or using adjuvants having a chemical or biochemical action, or by re-esterification process and any mixture with oils of other kinds.”¹⁾ Its consumption has been related to reduced incidence of coronary heart disease, low blood pressure levels and diabetes²⁻⁴⁾. Furthermore, olive oil is characterized by high amounts of monounsaturated fatty acids, as well as high content of antioxidant agents, able to scavenge free radicals and afford an adequate protection against peroxidation⁵⁾.

The productive process of extra virgin olive oil involves: i) olive crushing, aimed to tear the flesh cells and let the oil run out; ii) olive paste malaxation, to enable the merging of

oil droplets into larger drops (coalescence), until they form “pockets” that can be separated into a continuous liquid phase; iii) olive oil separation from the other phases (solid and water phase) by pressure, centrifugation or percolation. However, only 80-90% of the oil located into the vacuoles of olive pulp cells can be easily extracted: some oil remains inside the unsheltered cells, some is retained by the olive paste colloidal system as microgel, and some remains emulsified with the water phase⁶⁾. The difficulty of freeing this “bound” oil lies mainly in the fact that the droplets of dispersed or emulsified oil are surrounded by a lipoprotein membrane (phospholipids and proteins) that keeps them in that state⁷⁾. This phenomenon is more pronounced for the so-called “difficult olive pastes”⁸⁾, where for “difficult” is intended a low machinability usually due to low ripeness degree, that leads to low yields.

To improve oil yield, several researches have been evaluating the effects of both malaxation phase (time and temperature) and technological coadjuvants (enzymes, salt and talc) which, added at the malaxation step, help to break down water-oil emulsions and consequently make oil ex-

* Correspondence to: Francesco Caponio, Università degli Studi di Bari Aldo Moro, DiSSPA, Sezione di Scienze e Tecnologie Alimentari, Via Amendola 165/a, I-70126 Bari, Italy

E-mail: francesco.caponio@uniba.it

Accepted July 22, 2014 (received for review June 19, 2014)

Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online

<http://www.jstage.jst.go.jp/browse/jos/> <http://mc.manuscriptcentral.com/jjocs>

traction easier.

In particular, many authors observed that the increase of malaxation time up to 45–50 min raised the oil extraction yield, whereas longer time (60–75 min) determined a decrease of extraction yield^{6, 10}. Also increasing the malaxation temperature generally determines an increase of oil extraction yield^{6, 10}, due to a reduction of the oily phase viscosity¹¹, even if malaxing at temperature of 45°C caused lower yields, probably due to change in the paste rheology and increased interactions between lipids, proteins, and carbohydrates¹². Nevertheless, higher malaxation temperatures could accelerate the oxidative process and loss of both phenolics and volatile compounds, responsible for oil sensory properties, have been reported (although in some cases with controversial results)^{9, 13, 14}, compromising the nutritional value of extra virgin olive oil.

As regards the use of coadjuvants, the use of common salt (NaCl) as a physical-acting coadjuvant could represent a feasible mean for the improvement of oil extraction^{15, 16}. Moreover, different studies proved the efficacy of pectolytic enzymes addition during olive oil extraction to increase yield^{17, 18}. However, the oils obtained with the use of enzymes cannot be defined as “virgin”¹, because of the biochemical action causing cell wall rupture. The use of talc as coadjuvant during malaxation – not excluded by the same EC regulation due to its exclusively physical action¹⁹ – has been proved to increase oil extraction yield for difficult pastes^{8, 19–25}. However, almost all the researches until now carried out regard the use of talc on Spanish olive cultivars, whereas few studies have been made for Italian olive cultivars. In particular, Ranalli *et al.*²⁵ investigating on the effect of pectocellulolytic enzymes and/or talc addition in de-stoned and not-stoned olives from different Italian cultivars (*Gentile di Chieti*, *Caroleo* and *Coratina*) evinced higher nutraceutical value of the obtained oils. *Coratina*, that represents one of the most diffused Italian cultivars²⁶, gives a difficult paste because it has a rather low maturity index at the time of processing. Moreover, no studies are present in literature regarding the influence of talc addition on the evolution of oil quality indices during storage.

In this framework, the aim of the present research was to evaluate the effect of talc on extraction yield and quality of *Coratina* oil, both after production and during storage.

2 MATERIALS AND METHODS

2.1 Plant material

Olive fruits (*Olea europaea* L.) from *Coratina* cultivar, mechanically harvested in the first decade of December 2012, were transported immediately after harvesting in a local plant (Andria, Bari, Italy) where, unwashed and after leaf-removal, were milled within 24 h.

2.2 Oil extraction process

A lot of about 6,000 kg of olives was divided into six homogeneous batches: two batches were processed by adding micronized natural talc (hydrated magnesium silicate, Imerys Talc, Luzenac, France) at 1% level (Ta1); two were added of 2% talc (Ta2); the last two batches were processed without talc addition (C, control). Talc was added at the beginning of the malaxation phase.

In particular, each of the six batches was milled for 30 min by means of a 4-stone mill (F.lli Ferri, Andria, Bari, Italy), then the paste was hammer-crushed. The stone mill is constituted by a rough granite base (3.2 × 0.3 m) and 4-rough granite wheels (1.3 × 0.3 m), whereas all the other parts are in stainless steel. The hammer-crusher (model A40, power 30.75 kW, Amenduni Nicola S.p.A., Modugno, Bari, Italy) operated at 2,800 rpm and was equipped with a grid with holes of 5 mm of diameter, to break any olives not yet completely crushed. The crushed paste was then transferred into the malaxer (Amenduni Nicola S.p.A., Modugno, Bari, Italy), where it was mixed with talc only for the trials that provided its addition. After malaxation (50 min at 22 ± 1°C) the paste was pumped into a two-phase decanter (Taurus 902-XX, Amenduni Nicola S.p.A., Modugno, Bari, Italy) – operating at 2,800 rpm, with a processing capacity of 3,000 kg h⁻¹ – and diluted with 200 L h⁻¹ of water. Finally, the oily phase was separated from any aqueous residue by centrifugation at 6,400 rpm (vertical centrifuge, Alfa Laval, Tavarnelle Val di Pesa, Florence, Italy).

Then, the obtained oil was poured into 1-L clear glass bottles, with headspace of about 3 mL, that were hermetically sealed. Three bottles for each batch were sampled. One bottle for each batch was immediately analyzed (T0), whereas the others were placed in a carton box and stored in the dark to be analyzed after three (T3) and six (T6) months of storage. The average storage temperatures were 15°C during winter and 25°C in the spring.

2.3 Analytical determinations

The olive maturity index was determined by a visual evaluation of the color of the olive skin and flesh²⁷. The procedure consisted in distributing 100 olives in eight groups, according to the following characteristics: bright-green skin (group N = 0), green-yellowish skin (group N = 1), green skin with reddish spots (group N = 2), reddish-brown skin (group N = 3), black skin with white flesh (group N = 4), black skin with < 50% purple flesh (group N = 5), black skin with 50% < purple flesh < 100% (group N = 6), and black skin with 100% purple flesh (group N = 7). The index is given by $\sum (N_i n_i) / 100$, where N is the group number and *n* is the olive number in that group. Maturity index values range from 0 to 7.

The moisture content (% w/w) was determined by drying the milled olive at 105°C to constant weight²⁸ and the total oil content (% w/w) was determined by Soxhlet extrac-

tion²⁹⁾.

The determinations of the free fatty acids (FFA), peroxide value (PV) and spectrophotometric constants were carried out according to the official methods²⁹⁾.

The phenolic compounds of the oils were extracted and purified following the procedure reported by Gambacorta *et al.*³⁰⁾ with some modifications. About 1 g of oil was added with 1 mL of hexane and 5 mL of methanol/water (70:30 v/v). After 10 min vortexing, and centrifugation at 9,000 rpm for 10 min (Beckman Coulter, Fullerton, California, USA), the methanol/water phase was recovered and the extraction was repeated twice. The methanol/water phases were pooled, filtered through nylon filters (pore size 0.45 µm, Sigma, Ireland), and submitted to Folin-Ciocalteu reaction. In particular, an aliquot of the methanol/water solution of phenolic compounds (100 µL) was diluted in 6 mL of water, followed by the addition of 0.5 mL of Folin-Ciocalteu reagent; the mixture was incubated for 2 min at room temperature, and 2 mL of sodium carbonate (200 g L⁻¹) were added to the reaction mixture, which was finally mixed and diluted with water to 10 mL. The mixture was vortexed for 20 sec and incubated for 60 min at room temperature in the dark. The absorbance of the sample was read at 750 nm. Results were expressed as mg of gallic acid per kg⁻¹ of oil.

Chlorophylls and total carotenoids were determined as reported in Caponio *et al.*³¹⁾. In particular, the chlorophyll content was evaluated by the absorption spectrum of each oil sample, according to the AOCS method³²⁾, and expressed as mg of *pheophytin* a per kg of oil. The concentration of total carotenoids was calculated measuring the absorption of 0.25 g of oil dissolved in 5 mL UV-hexane at 449 nm. A calibration curve ($y = 0.0532x$; $R^2 = 0.9986$) was previously set up by measuring the absorption of solutions of β -carotene at known concentrations. For all the spectrophotometric determinations a Cary 60 spectrophotometer Agilent (Cernusco, Milan, Italy) (Cernusco, MI, Italy) was used.

Total tocopherols (α , β , γ , and δ) were determined by HPLC according to the method reported by Kamal-Eldin *et al.*³³⁾ with some modifications. Briefly, 950 µL of isooctane (containing 2 g L⁻¹ of BHT and 1 g L⁻¹ of ascorbic acid) were added to about 50 mg of oil. The samples were injected into a HPLC system consisting of a Waters 600E quaternary pump (Milford, MA, USA), a 7725i Rheodyne injector (20-µL sample loop) and a Waters 470 Spectrofluorimeter (excitation wavelength 294 nm, emission wavelength 330 nm). The stationary phase was a YMC-Pack SIL column (150 × 4.6 mm i.d., 5 µm particle size); the mobile phase was *n*-hexane-isopropyl alcohol (99.5 : 0.5 v/v) at a flow rate of 1 mL min⁻¹. Quantitative analysis was performed by external standard method on the basis of a previously set up calibration curve of α -tocopherol ($y = 3E-05x - 0.2823$; $R^2 = 0.9992$).

The antioxidant activity was determined by means of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) stable radical scavenging capacity test, according to the method proposed by Ramadan and Morset³⁴⁾. In particular, 50 µL of oil were dissolved in 500 µL of isooctane, the mixture was vortexed for 1 min and an aliquot of 200 µL was let to react in a 1-cm path cuvette at 22°C under continuous stirring with 1.8 mL of a 10⁻⁴ M isooctane solution of DPPH. The decrease of absorbance at 515 nm was recorded after 60 minutes against a blank of isooctane using a Cary 60 spectrophotometer Agilent (Cernusco, Milan, Italy). The antioxidant activity was considered as the difference of absorbance between each sample and an isooctane DPPH solution without the sample (control) and was calculated as percentage of inhibition according to the following equation:

$$\% \text{ of inhibition} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100$$

2.4 Statistical analysis

Analysis of variance (one-way and two-way ANOVA) was carried out on the experimental data by the XLStat software (Addinsoft SARL, New York, NY, USA). Two-way ANOVA was performed considering the amount of talc added at the beginning of the malaxation phase (*talc*) and storage time of the extra virgin olive oils (*time*), as well as their first order interaction, as independent variables; Tukey's HSD test was applied for multiple comparisons.

3 RESULTS AND DISCUSSION

Compositional characteristics and maturity index of the olive lot used for the experiment are given in **Table 1**. Moisture and oil content were those normally detected in olives, whose low ripeness degree was evinced by the maturity index.

Table 2 reports moisture level and oil content of the olive-pomace remaining from the trials, as well as the results of one-way ANOVA followed by Tukey HSD test. The addition of talc lead to an increase of the oil extraction yield, as pointed out by the significantly lower residual oil content in the olive-pomaces deriving from the trials involving the use of talc than in control. These results agree with previous studies carried out on other cultivars (such as *Picual*, *Arbequina*, *Hojiblanca*, and *Carrasqueña*) with different ripening status and composition^{6, 19–23, 28, 35)}. When adding talc, the residual oil content of the olive-pomace was significantly lower than in case of control, with a decrease of about 15%. However, no significant difference was observed in the amount of oil in olive-pomaces when varying the amount of talc, confirming the findings of other authors^{20, 35)}. Finally, no significant differences were found in moisture content of olive-pomace.

Table 3 reports the results of two-way ANOVA with first

Table 1 Compositional characteristics and ripening index of the processed olives^a.

Maturity index	Moisture (%)	Oil (%)	Solids (%)
1.2	51.54	20.01	28.45

^a Means of five replicates: coefficient of variance in all cases <5%**Table 2** Moisture and oil content of olive-pomace (mean value and standard deviation) and results of one-way ANOVA followed by Tukey HSD test.

Olive lot	Moisture (%)	Oil (% dry matter)
C	67.56 ± 1.60	9.77 ± 0.26 A
Ta1	66.49 ± 0.50	8.35 ± 0.35 B
Ta2	64.77 ± 0.42	8.49 ± 0.18 B
<i>p</i> -value	0.144	0.024

Olive processed without (C) and with 1% (Ta1) or 2% (Ta2) of talc added. Different letters indicate significant differences at *p* < 0.05**Table 3** Results of the analysis of variance (two-way ANOVA with first-order interaction) performed on the analytical data.

Determinations	<i>Model</i>		<i>Talc</i>		<i>Time</i>		<i>Talc*time</i>	
	F	<i>p</i> -value	F	<i>p</i> -value	F	<i>p</i> -value	F	<i>p</i> -value
FFA	2.25	0.125	1.044	0.391	7.190	0.014	0.379	0.818
PV	2698.14	<0.001	535.04	<0.001	10209.97	<0.001	23.78	<0.001
K ₂₃₂	17.37	<0.001	2.79	0.114	62.67	<0.001	2.01	0.177
K ₂₇₀	4.98	0.014	13.55	0.002	2.25	0.161	2.09	0.168
Phenols	8.03	0.003	15.54	0.001	15.94	0.001	0.315	0.861
Tocopherols	32.60	<0.001	5.12	0.033	124.19	<0.001	0.55	0.706
Carotenoids	31.91	<0.001	126.88	<0.001	0.64	0.549	0.05	0.994
Chlorophylls	24.30	<0.001	35.30	<0.001	47.40	<0.001	7.24	0.007
Antioxidant activity	9.75	<0.001	37.76	<0.001	0.83	0.469	0.20	0.993

FFA, free fatty acids; PV, peroxide value; K₂₃₂, specific absorption at 232; K₂₇₀, specific absorption at 270 nm

order interaction performed on all the chemical and physical analytical data obtained. The ANOVA models resulted to be significant in all cases; *talc* variable proved to have a significant effect on all the indices except free fatty acids and K₂₃₂, whereas *time* variable had not a significant effect only on K₂₇₀, carotenoids and antioxidant activity. The first-order interaction *talc*time* resulted significant only for peroxide value and chlorophylls.

Table 4 reports the results of the analytical parameters (mean values and standard deviations) of the oil obtained both immediately after production and during storage, and the results of two-way ANOVA followed by Tukey HSD test for multiple comparisons. As shown, all the oils obtained belonged to the “extra virgin olive oil” category³⁶⁾.

The use of talc determined a significant decrease of the PV, confirming the findings both of Ben-David *et al.*³⁷⁾, in

oil from cv. *Barnea* obtained by adding 5.7% of talc, and Carrapiso *et al.*²³⁾. Other authors^{20–22, 28)} reported that talc produces only a physical action on oil, with no influence on the quality indexes prescribed by current Regulations. Fernández Valdivia *et al.*²²⁾, considering cv. *Hojiblanca*, reported that talc addition protects the oil from oxidation during extraction, while Cruz *et al.*¹⁵⁾ observed in several cultivars that talc addition tended to increase oil stability. Moreover, talc addition did not significantly influence FFA and K₂₃₂, as found by Carrapiso *et al.*²³⁾ and Moya *et al.*²⁸⁾, whereas K₂₇₀ significantly increased, although Cert *et al.*¹⁹⁾ and Carrapiso *et al.*²³⁾ did not found significant variation. These results show that talc does not exert any alteration on the oil quality parameters prescribed by the EEC official method n. 2568/91²⁹⁾ and successive amendments.

As regards the other analytical parameters, the use of

Table 4 Results of the analytical parameters (mean values and standard deviations) of the oil obtained both immediately after production and during storage, and the results of Tukey HSD test for multiple comparisons.

Parameter	Talc		T0		T3		T6	
FFA (g 100 g ⁻¹)	C	A	0.18 ± 0.02	a	0.21 ± 0.02	a	0.20 ± 0.02	a
	Ta1	A	0.18 ± 0.02	a	0.23 ± 0.01	a	0.22 ± 0.01	a
	Ta2	A	0.18 ± 0.02	a	0.23 ± 0.01	a	0.23 ± 0.03	a
PV (meq O ₂ kg ⁻¹)	C	A	2.33 ± 0.01	e	6.63 ± 0.01	a	6.62 ± 0.01	a
	Ta1	C	1.66 ± 0.01	g	5.32 ± 0.01	d	5.55 ± 0.35	c
	Ta2	B	1.99 ± 0.01	f	5.63 ± 0.04	bc	5.82 ± 0.02	b
K ₂₃₂	C	A	1.579 ± 0.036	b	1.886 ± 0.040	a	1.914 ± 0.069	a
	Ta1	A	1.643 ± 0.098	b	1.941 ± 0.007	a	1.893 ± 0.077	a
	Ta2	A	1.638 ± 0.056	b	1.906 ± 0.004	a	2.065 ± 0.049	a
K ₂₇₀	C	B	0.145 ± 0.013	b	0.146 ± 0.010	b	0.135 ± 0.007	b
	Ta1	A	0.158 ± 0.001	ab	0.160 ± 0.007	ab	0.179 ± 0.010	ab
	Ta2	A	0.169 ± 0.003	ab	0.165 ± 0.001	ab	0.194 ± 0.027	a
Phenols (mg kg ⁻¹)	C	B	606 ± 42	abc	488 ± 44	c	504 ± 49	c
	Ta1	A	764 ± 39	a	633 ± 54	abc	604 ± 19	abc
	Ta2	A	710 ± 54	ab	612 ± 42	abc	583 ± 41	bc
Tocopherols (mg kg ⁻¹)	C	A	226.72 ± 15.76	a	211.20 ± 2.69	a	128.29 ± 16.52	b
	Ta1	AB	221.37 ± 20.00	a	205.86 ± 1.77	a	114.85 ± 9.18	b
	Ta2	B	195.99 ± 10.54	a	194.49 ± 1.62	a	112.11 ± 8.90	b
Carotenoids (mg kg ⁻¹)	C	B	2.94 ± 0.08	b	3.30 ± 0.34	b	3.21 ± 0.48	b
	Ta1	A	6.21 ± 0.22	a	5.99 ± 0.32	a	5.84 ± 0.40	a
	Ta2	A	6.33 ± 0.09	a	6.26 ± 0.36	a	6.15 ± 0.42	a
Chlorophylls (mg kg ⁻¹)	C	B	12.33 ± 0.11	b	11.95 ± 0.08	b	11.88 ± 0.05	b
	Ta1	A	14.72 ± 0.61	a	12.89 ± 0.15	b	13.05 ± 0.41	b
	Ta2	A	14.64 ± 0.09	a	12.39 ± 0.22	b	12.07 ± 0.49	b
Antioxidant activity *	C	C	56.29 ± 0.67	b	53.87 ± 1.95	b	53.24 ± 3.39	b
	Ta1	A	73.86 ± 2.35	a	70.90 ± 5.31	a	69.81 ± 4.70	a
	Ta2	B	63.20 ± 1.83	ab	63.49 ± 3.58	ab	62.90 ± 4.02	ab

Olive processed without (C) and with 1% (Ta1) or 2% (Ta2) of talc added. Oils analyzed after production (T0) and during storage after three (T3) and six (T6) months. FFA, free fatty acids; PV, peroxide value; K₂₃₂, specific absorption at 232; K₂₇₀, specific absorption at 270 nm.

* Calculated as percentage of inhibition. Uppercase letters were used to compare the samples considering the effect of talc, lowercase letters were used to compare the samples considering the combined effect of talc, storage time (months) and talc x time interaction: different letters indicate significant differences at $p < 0.05$

talc caused a significant increase of total phenols, chlorophylls and carotenoids, probably due to the lower amount of water in the olive paste subjected to extraction, ascribable to the hygroscopic effect of talc. In any case, Moya *et al.*²⁸⁾ reported that the use of talc does not produce any significant variation on the amount of photosynthetic pigments (chlorophylls and carotenoids) – as found also by Fernández Valdivia *et al.*²²⁾ and Ranalli *et al.*²⁵⁾ – and polyphenols. Also Carrapiso *et al.*²³⁾ and Ranalli *et al.*²⁵⁾ did not

observe any significant influence of talc addition on total phenol content; on the contrary Uceda *et al.*²¹⁾ reported higher phenol contents in oils when talc was used. Moreover, the talc addition caused a significant decrease of tocopherols only when 2% talc was used. Also Carrapiso *et al.*²³⁾ found a significant decrease of α -tocopherol whereas Ranalli *et al.*^{24, 25)} did not find any substantial variation in the amounts of tocopherols in oils obtained from destoned olives by addition of talc. Up to now, the effect of talc addi-

tion on tocopherol content has been scarcely studied, and it seems to depend on the cultivar³⁷⁾. Finally, the addition of talc determined a significant increase of the antioxidant activity, measured by the DPPH method, more evident when 1% talc was used. This result is correlated with the content of total phenols obtained in the different trials, and agrees with previous findings³⁸⁾ that highlighted a significant correlation between total phenols and induction time, being the latter related to the total antioxidant compounds of the oil.

As regards the evolution of analytical data during storage, the differences among the oils (Ta1, Ta2 and C) were maintained as immediately after their production. The only exceptions were represented by chlorophylls – that at the beginning were significantly lower in control than in trials with talc, but from 3 months onwards did not show statistical difference anymore – and by PV that increased significantly at T3, due to the oxidation of triglycerides provoked by oxygen dissolved in the oil.

As the storage proceeded, primary oxidation increased in the oils (as shown by the trend PV and K_{232}), while antioxidant compounds decreased. On the whole, the evolution of the oils during storage confirmed the findings reported by us and by other authors^{31, 39, 40)}. The significant decrease of chlorophylls after three months of storage in oils obtained adding talc during malaxation needs further investigation, involving also the analysis of chlorophylls degradation products. About the significant decrease of tocopherols after six months of storage – confirmed by other findings^{40, 41)} – it could be due to the protective effect of α -tocopherol, the most representative isomer, against oil oxidation, when oxidation takes place under non-accelerated conditions⁴²⁾.

4 CONCLUSIONS

The obtained data pointed out a significant effect of talc on the extraction yield and on the quality of the oils obtained from olives of cv. *Coratina*, that represent one of the most influential Mediterranean olive oil varieties. In particular, the amount of residual oil in the olive-pomace decreased of about 15%, while the oils obtained presented lower oxidation levels and higher amounts of chlorophylls and antioxidants than the corresponding oils obtained without talc addition. Finally, no effect of talc was observed during oil storage, with the exception of chlorophylls and PV.

Further studies are needed in order to evaluate the addition of technological coadjuvants on olives at different ripeness degree.

ACKNOWLEDGMENTS

Authors want to express their gratitude and acknowledgment to “Oleificio Cooperativo della Riforma Fondiaria di Andria” for technical assistance and for its contribution to the development of this research.

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