Research Article

Cherry leafroll virus: Impact on olive fruit and virgin olive oil quality

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We performed a survey on the yield, quality, and chemical characteristics of virgin olive oils from two olive varieties in Croatian Istria: *Frantoio* and *Ascolana tenera*, on Cherry leafroll virus-infected and virus-noninfected trees and on two harvest dates. Free acidity, peroxide value, specific spectrophotometric absorptions at 232 and 270 nm, fatty acid composition, total phenols, *o*-diphenols, oil color, and pigments were determined. Infected olives had lower oil yield and maturity index versus healthy ones. Oils from infected fruits had significant lower value of K_{232} and K_{270} and very elevated total phenols content compared to those obtained from healthy olives. Infected *Frantoio* gave a lower content of *o*-diphenols than the healthy ones, which is in contrast to infected *Ascolana* that had higher values. The aim of this study is to determine the chemical changes in virgin olive oils from healthy and infected trees related to virus influence. According to our knowledge, this is the first survey on the possible influence of viruses on olive fruits, oil yield, and virgin olive oil quality.

Practical applications: There are only few papers which analyze the influence of viruses on crops (especially influence on wine quality) and their effects on yield or other agronomic parameters. This work evaluates for the first time the impact of Cherry leafroll virus on the quality of virgin olive oil obtained from *Frantoio* and *A. tenera* varieties in terms of basic parameters related to the hydrolitic and oxidative status, content in antioxidant compounds, and in pigments as well as in fatty acid composition.

Keywords: Istria / Oil quality / Olive viruses / Phenols / Pigments

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1 Introduction

Istria is a peninsula situated in the Northeast of the Adriatic Sea. It is the northernmost Croatian area of olive cultivation. There are many introduced olive varieties in Istria. *Ascolana tenera* is an early ripening variety originating from Italian Marche region, and mainly used as table fruit, but it is also used for oil production. In Istria, *Ascolana* is cultivated from 1940s and is present in older plantations (planted untill 1990) in a percentage of about 2.9%. *Frantoio* is also an Italian (Tuscan) variety and in Istria its presence in new plantations is about 7.4% [1].

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Abbreviations: ***A***, *Ascolana tenera*; **CGC,** capillary GC; **CIELab,** color model used conventionally to describe all the colors visible to the human eye; **CLRV,** *Cherry leafroll virus*; **F,** *Frantoio*; **GFLV,** *Grapevine fanleaf virus*; **GLRaV-1,** *Grapevine leafroll-associated virus 1*; **GLRaV-2,** *Grapevine leafroll-associated virus 2*; **GLRaV-3,** *Grapevine leafroll-associated virus 3*; **MI,** maturity index

Viruses are responsible for great economic losses. Some direct and indirect damages associated with viral infections are lower yield, the reduction in quality, or market value due to defects in visual attraction (changes in size, shape, and fruit color), reduced consumer appeal (grading, taste, texture, and composition) [2].

Until today, 15 viruses have been isolated from olive trees. Cherry leafroll virus (CLRV) as one of them is an isometric (+)RNA plant virus of the genus *Nepovirus* (Secoviridae) which consists of two single-stranded RNAs. CLRV has a wide natural host range which includes 17 genera of woody plants and a variety of herbaceous plants. It was first reported in 1933 in English walnut (*Juglans regia*) and sweet cherry (*Prunus avium*) [3]. This virus affects branches, buds, fruits, leaves, roots, shots and tillers, stems and trunks of a number of plants. It is an economically important virus and a quarantine pest in *Rubus* in the EPPO region [3]. CLRV causes losses of *Prunus cerasus* of up to 91–98% and is also associated with dieback and decline of *Fagus sylvatica*, death of walnut trees [4] and in *P. avium* it causes leaf rolling and death [5]. It is included in the list of plant viruses that should be closely monitored for walnut and olive trees [3]. Widespread and polyphagous, CLRV is transmitted on olive by pollen and seed [6]. On olive it causes latent infections, but it has been associated also with leaf-yellowing complex disease together with another four olive viruses causing leaf and fruit deformations, too [6, 7]. CLRV can be detected by methods as biological assay, DAS-ELISA, but the most sensitive and reliable methods are molecular methods such as several PCR-based protocols. The percentage of infection of olive with CLRV detected by several RT-PCR-based protocols in some countries were: in Lebanon 2%, in Spain 4.1%, in Italy 4.9%, in Tunisia 13.1%, in Syria 15%, and in Istria (Croatia) 24% [7–12]. In olive, CLRV was detectable in 90% of the seeds obtained from virus-infected trees and the rate of seedling infection was 41% [13].

There are only few papers dealing with the influence of viruses on crops. These works show that virus infections induce decrease in sugar content in a heat-treated clone due to differences in yield between infected and sanitized grapevine [14]. In 78% of studies, fruit from infected vines had significantly lower sugar levels, which ranged from 0.3 to 5.1 °Brix, than that recorded in healthy vines [15]. Chlorophyll and carotenoid content in some infected grape cultivar (GLRaV-3 alone or in combination with other viruses) was reduced in comparison with the healthy one (Chl about 33.5% and Car 22%). In some papers authors demonstrated that berry anthocyanin levels were significantly reduced in leafroll infected vines, and conversely, anthocyanin levels accumulated at a faster rate in healthy ones [15]. Anthocyanin content decrease was directly related to GFLV infection in 4-year survey [16].

Leafroll infection also delays fruit maturity by more than 1 month. Several studies on grape yield reported a statistically significant decline in the yield of about 14–80% of leafroll infected vines compared with the healthy ones [15]. Yield reduction was over 20% in plants infected by GFLV, but almost 40% because of GLRaV-1 [16]. Infected vines were found to have significantly higher levels of titratable acidity (range from 0.2 to 1.3 g/L) than healthy vines [15].

Seed weight, pH, titratable acidity, total phenolics and tannins, total polyphenolics, and anthocyanin were significantly different in healthy versus vines infected with GLRaV-2 and GLRaV-3. Infected vines also had reduced concentration of soluble solids, decreased individual and total anthocyanins, and increased skin and pulp weight for all three rootstock/scion combinations examined [17].

Total polyphenols index was 21.2% higher and total tannins were 55% higher in GLRaV-1 positive samples compared to GLRaV-1 negative samples [16]. However, according to literature available, chemical content of virus-infected plants is usually quite unpredictable.

The aim of this experimental study was to examine the influence of CLRV infection on chemical composition of virgin olive oil derived from infected *Frantoio* and *A. tenera* varieties. According to our knowledge, this is the first survey focused on the influence of viruses on the yield of virgin olive oil and its quality.

2 Materials and methods

2.1 Detection of olive viruses

The detection of CLRV was performed by molecular analysis through one-step RT-PCR [10] in spring 2009 using scrapings of olive shoot tissue of nine varieties including varieties *Frantoio* and *A. tenera* (as described earlier, Ref. [7]). Total RNA extraction was performed using Rneasy Plant Mini Kit (Qiagen Gmbh, Germany) according to protocol supplied by the manufacturer.

2.2 Olive fruit samples for oil production

The samples were collected in olive plantation of the Institute of Agriculture and Tourism Poreč (Istria, Croatia) (45°13′N; 13°36′E). About 5 kg of fruits of each variety (*Frantoio* (*F*) and *A. tenera* (*A*)) were handpicked at two harvest dates: first on October 24th and second on November 14th, 2010. There were eight samples in total—two varieties, two harvest dates (1st and 2nd) and two category of infestation: infected (i) and healthy tree. Oil yield, maturity index (MI) and average fruit weight of both varieties have been determined. MI was determined according to Uceda and Frias [18]. The processing has been done on October 25th and on November 15th, 2010 by using the laboratory oil mill which consisted of hammer mill, malaxator (working at temperature below 26°C), and centrifugal machine. Untill further analyses, oil samples were stored in a dark and cool place.

2.3 Detemination of the quality characteristics of virgin olive oil

2.3.1 Qualitative basic parameters

Free acidity, peroxide value, and ultraviolet spectrophometric indices (K_{232} and K_{270}) were evaluated according to the official methods described in the EEC regulation no. 2568/91 [19].

2.3.2 Spectrophotometric estimation of total pigments

The chlorophyll fraction at 670 nm and the carotenoid fraction at 470 nm were evaluated from the absorption spectrum of each virgin olive oil sample (7.5 g) dissolved in cyclohexane (25 mL) [20, 21]. All the absorbance measurements were performed in a UV–Vis 1800 spectrophotometer (Shimadzu Co., Kyoto, Japan), which had a six slot shuttle and a system for temperature control.

The chlorophyll and carotenoid contents were expressed as mg of pheophytin *a* and lutein per kg of oil, respectively; they were calculated using the specific extinction values [21, 22]: $E_0 = 613$ for pheophytin *a* and $E_0 = 2000$ for lutein. Thus, pigment contents were calculated as follows:

$$[\text{chlorophyll}]\,(\text{mg/kg}) = \frac{A_{670} \times 10^6}{613 \times 100 \times d}$$

$$[\text{carotenoid}]\,(\text{mg/kg}) = \frac{A_{470} \times 10^6}{2000 \times 100 \times d}$$

where A is the absorbance and d is the spectrophotometer cell thickness (1 cm).

2.3.3 Oil color

A color measuring spectrophotometer (mod. Colorflex, HunterLab, USA), with illuminant D65 and a transparent glass sample cup specific for the liquids, was used to assess the oil color with the Optiview 1.1 computer software. We applied the CIELAB colorimetric system [20]. The oil samples were examined without dilution to avoid color variation and the tristimulus values X, Y, and Z were calculated for illuminant D65 from the absorption spectrum. The oil color was expressed as chromatic coordinates L^*(lightness), a^*(redness), and b^*(yellowness).

2.3.4 Liquid–liquid extraction of phenolic compounds from olive oils

The phenolic compounds extraction followed the protocol described by Pirisi [23], modified according to Rotondi et al. [24]. Extractions were performed in three replicates and extracts were stored at −18°C before analysis.

2.3.5 Spectrophotometric assays: Total phenolic compounds and *o*-diphenols

Total phenol and *o*-diphenol contents of the virgin olive oil extracts were determined using a UV–Vis 1800 spectrophotometer (Shimadzu Co., Kyoto, Japan). They were evaluated according to Singleton and Rossi [25], Mateos et al. [26], and Cerretani et al. [27] and detected at 750 and 370 nm, respectively. Specific calibration curves ($r^2 = 0.9908$ and 0.9897, respectively) were built for the quantification and data were expressed as mg gallic acid/kg of oil.

2.3.6 Fatty acid methyl ester analysis by CGC

The fatty acid composition of oil samples was determined as FAMEs by capillary GC analysis after alkaline treatment, according to Christie [28]. Gas chromatographic analyses were carried out according to Rotondi et al. [24]. A GC AutoSystem XL Perkin-Elmer (Wellesley, MA), was used which was equipped with a FID. We mixed 0.05 g of oil dissolved in 2 mL of *n*-hexane with 1 mL of 2 N potassium hydroxide in methanol; the upper phase was injected into a split (split ratio 1:30) GC port set at 250°C; a fused silica capillary column RTX-2330 (30 m length, 0.25 mm i.d., 0.2 μm film thickness) by Restek (Bellefonte, PA, USA) was utilized. A flow rate of 0.8 mL/min of helium as a carrier gas was used. The FID detector was at 250°C, the initial oven temperature was kept at 140°C for 5 min, then it raised to 240°C at a rate of 4°C/min, and it was maintained at 240°C for 5 min.

2.4 Statistical analysis

One-way ANOVA at $p \leq 0.05$ significance level were used and Fisher's LSD for rank sums were calculated. Multiple comparison procedure was performed to determine the significance of differences between samples using the software package Statistica 7.1 (StatSoft Inc., Tulsa, USA).

3 Results and discussion

3.1 Presence of CLRV in olive trees and sampling of fruits for oil production

Molecular analysis of total RNA isolated from olive shoot tissue revealed that two trees of varieties *Frantoio* and *A. tenera* were infected by CLRV (data not shown). Total of eight fruit samples were handpicked for processing and olive oil production (these were virotic and nonvirotic, of both varieties *Frantoio* and *A. tenera* and picked on two harvest dates), weighting about 5 kg of fruits from each variety.

3.2 Weight per 100 olives, maturity index, and oil yield

As it is possible to observe in Fig. 1 the average fruit weight for healthy and infected *Ascolana* was 8.4 and 8.9 g, respectively,

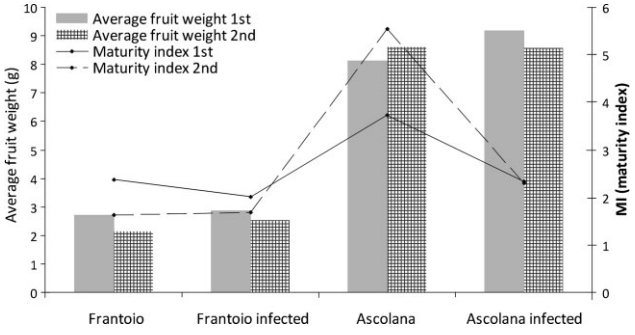

Figure 1. Average fruit weight and maturity index of healthy and infected olive fruits (1st and 2nd harvest).

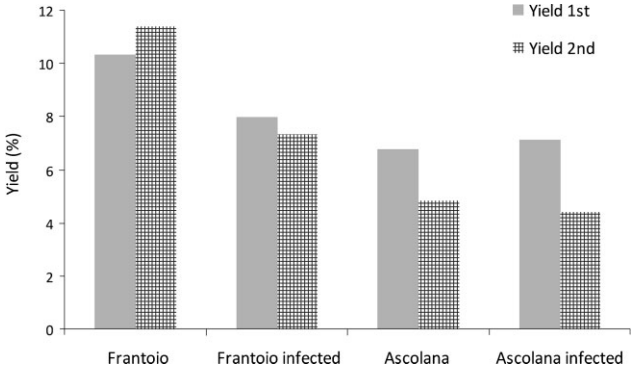

Figure 2. Oil yield of healthy and infected olive fruits (1st and 2nd harvest).

whereas for healthy and infected *Frantoio* the registered data were 2.4 and 2.7 g. The fresh weights of these two health olive varieties were in agreement with data reported by other authors [29]. Average MI was higher for healthy olive fruits than for infected ones (for *Ascolana* 4.6 vs. 2.3, for *Frantoio* 2 vs. 1.8). The average oil yield (Fig. 2) was quite similar for healthy and infected *Ascolana* (about 5.8%) instead of for healty *Frantoio* was higher (10.9%) than for infected *Frantoio* (7.6%).

3.3 Free acidity

There were no significant differences between oils obtained from healthy or infected olives (Table 1). In all cases, the value of free acidity in virgin olive oils was well below the legal limit for the category extravirgin ($\leq$0.8%). However, *Ascolana* tended to have lower acidity than *Frantoio*.

3.4 Peroxide number

The oil samples obtained from infected *Ascolana* fruits (1st and 2nd harvest) were no significantly different from healthy ones in terms of peroxide values. The extravirgin olive oils produced by infected *Frantoio* 1st and 2nd harvest had a peroxide number significantly higher and lower than the healthy ones. In all cases, the peroxide number values were well below the legal limit for the extravirgin category ($\leq$20 meq O_2/kg). *Ascolana* gave oils with peroxide number significantly lower than *Frantoio*.

Table 1. Parameters/variables measured for virgin olive oil samples produced by virotic (CLRV) and healthy olives (mean value ± SD; *A* = *A. tenera*; *F* = *Frantoio*; 1st = first harvest; 2nd = second harvest; i = infected tree)

	A 1st	*A* 2nd	i*A* 1st	i*A* 2nd	*F* 1st	*F* 2nd	i*F* 1st	i*F* 2nd
FA (%)	0.15 ± 0.03 d	0.19 ± 0.01 cd	0.19 ± 0.02 bcd	0.17 ± 0.001 d	0.24 ± 0.03 ab	0.24 ± 0.04 ab	0.25 ± 0.02 a	0.22 ± 0.02 abc
PV (meq O_2/kg)	5.96 ± 0.29 e	4.66 ± 0.02 f	5.88 ± 0.01 e	4.35 ± 0.13 f	7.74 ± 0.28 b	7.01 ± 0.40 c	10.6 ± 0.18 a	6.49 ± 0.09 d
K_{232}	1.20 ± 0.07 a	1.02 ± 0.05 bc	1.04 ± 0.08 bc	1.13 ± 0.20 abc	1.20 ± 0.10 a	1.15 ± 0.09 ab	1.21 ± 0.16 a	0.99 ± 0.10 c
K_{270}	0.12 ± 0.01 b	0.07 ± 0.00 e	0.14 ± 0.01 a	0.09 ± 0.00 d	0.10 ± 0.01 c	0.10 ± 0.00 c	0.10 ± 0.00 c	0.08 ± 0.01 d
OA (%)	73.4 ± 0.2 f	75.1 ± 0.2 c	73.0 ± 0.2 g	75.8 ± 0.2 b	74.4 ± 0.2 d	76.3 ± 0.2 a	73.9 ± 0.3 e	76.1 ± 0.08 ab
LA (%)	6.7 ± 0.0 a	6.4 ± 0.02 c	6.4 ± 0.02 c	5.6 ± 0.02 f	6.4 ± 0.02 b	5.7 ± 0.03 e	6.7 ± 0.01 a	5.8 ± 0.02 d
SFA (%)	14.6 ± 0.2 b	13.6 ± 0.14 d	15.1 ± 0.1 a	13.6 ± 0.1 cd	14.3 ± 0.1 b	13.8 ± 0.2 c	14.4 ± 0.2 b	13.6 ± 0.1 d
MUFA (%)	78.1 ± 0.2 ef	79.5 ± 0.1 c	78.0 ± 0.1 f	80.2 ± 0.1 a	78.6 ± 0.1 d	79.9 ± 0.2 b	78.3 ± 0.2 e	80.1 ± 0.1 ab
PUFA (%)	7.4 ± 0.01 a	7.0 ± 0.04 c	7.0 ± 0.02 c	6.3 ± 0.01 e	7.1 ± 0.02 b	6.3 ± 0.02 e	7.3 ± 0.01 a	6.4 ± 0.00 d
OA/LA	11.0 ± 0.04 g	11.8 ± 0.04 c	11.4 ± 0.05 e	13.5 ± 0.03 a	11.5 ± 0.03 d	13.5 ± 0.07 a	11.0 ± 0.03 f	13.1 ± 0.03 b
L^*	52.6 ± 0.07 c	57.5 ± 0.01 a	48.8 ± 0.01 d	54.5 ± 0.01 b	47.4 ± 0.03 e	33.9 ± 0.02 h	43.6 ± 0.96 f	39.4 ± 0.02 g
a^*	3.7 ± 0.02 d	−0.4 ± 0.02 g	5.3 ± 0.04 b	0.5 ± 0.02 f	6.2 ± 0.035 a	4.2 ± 0.10 c	6.0 ± 0.16 a	2.6 ± 0.06 e
b^*	77.9 ± 0.2 ab	54.0 ± 0.1 f	76.3 ± 0.3 bc	76.0 ± 0.6 c	78.3 ± 0.1 a	52.5 ± 0.4 f	70.0 ± 1.5 d	63.1 ± 0.5 e
CHLs (mg/kg)	2.1 ± 0.09 ef	1.0 ± 0.01 f	3.3 ± 0.3 de	1.5 ± 0.2 f	4.6 ± 0.4 d	11.4 ± 1.0 a	6.7 ± 0.7 c	8.3 ± 0.3 b
CARs (mg/kg)	1.4 ± 0.05 e	0.9 ± 0.1 f	2.0 ± 0.02 d	1.2 ± 0.1 ef	3.2 ± 0.1 c	6.7 ± 0.2 a	3.4 ± 0.2 c	5.2 ± 0.2 b

FA, free fatty acidity; PV, peroxide value; OA, oleic acid; LA, linoleic acid; SFA, saturated fatty acids; OA/LA, oleic acid/linoleic acid; CHLs, chlorophylls; CARs, carotenoids.
Different letters (a–g) indicate significant differences among mean values (Fisher LSD, $p \leq 0.05$).

3.5 K_{232} and K_{270}

i*A* 1st and i*F* 2nd had significantly lower K_{232} than the healthy ones. i*A* 2nd harvest and i*F* 1st did not show different K_{232} values compared to the healthy one. i*A* 1st and i*A* 2nd harvest had different K_{270} than the healthy ones, in particular the samples produced by infected olives showed higher K_{270}; on the other hand, i*F* 1st did not show difference in K_{270} values from the healthy one whereas i*F* 2nd had significantly lower K_{270}. In all the cases the values of both K_{232} and K_{270} were below the legal limit for the extravirgin category ($\leq$2.50 and $\leq$0.22, respectively).

3.6 Total phenols

The mean values for virgin olive oil from *Frantoio* ranged from about 260 (*F* 1st) to about 1046 (i*F* 2nd) mg gallic acid/kg of oil (Fig. 3). i*A* 1st had significantly lower content of total phenols regard to the healthy *A* (488 vs. 864 mg/kg). On the contrary i*A* 2nd showed significantly higher content of total phenols compared to the healthy one (1029 vs. 766 mg/kg). For i*F* (1st and 2nd harvest), higher content of total phenols than the healthy one was evidenced. Healthy *F* 1st gave a minor content of phenols than the one of the 2nd harvest and i*F* of both harvests gave an oil of much higher phenols content as compared to the healthy ones. Contrary to *Frantoio*, the healthy *Ascolana* 1st had higher phenols content in comparison to one of the 2nd harvest. In general, *Frantoio* tended to gave an oil with lower phenols content compared to *Ascolana* and i*F* 2nd and i*A* 2nd harvest gave oils with very elevated total phenols content (about 1046 and 1029 mg/kg).

3.7 *o*-Diphenols

The mean value ranged from 78 (i*F* 2nd) to 151 (i*A* 1st) mg gallic acid/kg of oil (Fig. 4). i*A* 1st harvest had significantly higher content of total *o*-diphenols in contrast to the healthy

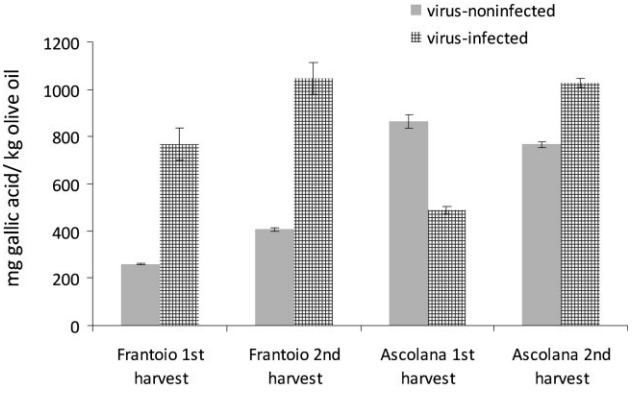

Figure 3. Total phenols in four virgin olive oil samples: two healthy and two infected with CLRV.

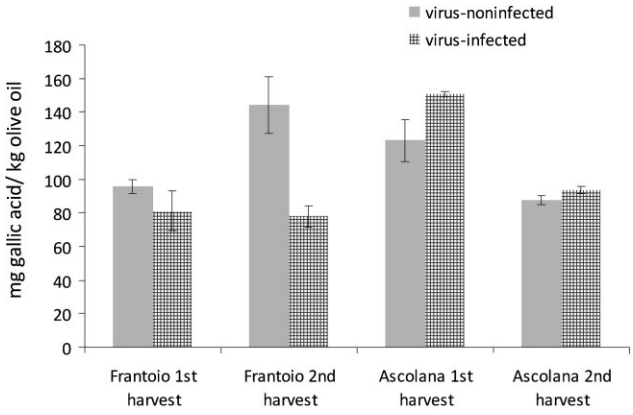

Figure 4. *o*-Diphenols in four virgin olive oil samples: two healthy and two infected with CLRV.

one instead of i*A* 2nd harvest did not show different *o*-diphenols content compared to healthy *Ascolana*. On the other hand, i*F* 1st did not show differences in terms of *o*-diphenols content compared to the healthy one while i*F* 2nd harvest showed a significantly lower content of these molecules. In general, *F* 1st gave an oil with a minor content of *o*-diphenols than the one of the 2nd harvest (about 96 vs. 144) and i*F* gave a lower content of *o*-diphenols when compared to the healthy one for both harvests. *A* 1st produced an oil with increased content of *o*-diphenols in comparison to the fruits of the 2nd harvest.

3.8 Fatty acid composition

The values of the major monounsaturated fatty acid, the oleic acid, tended to increase from the 1st to the 2nd harvest for both *Ascolana* and *Frantoio* (Table 1). Considering the most important polyunsaturated fatty acid, the linoleic acid, both for *Ascolana* and *Frantoio* its content tended to decrease from the 1st to the 2nd harvest. The values of saturated fatty acids, primarily represented by palmitic and stearic acids, decreased from the 1st to the 2nd harvest for both *Ascolana* and *Frantoio*. Concerning the monounsaturated fatty acid category, there was a significant increase for both *Ascolana* and *Frantoio* from the 1st to the 2nd harvest. i*A* (1st and 2nd harvest) had lower value of polyunsatured fatty acids than healthy ones (6.6 vs. 7.2%) whereas i*F* (1st and 2nd harvest) had higher value than healthy ones. For both *Ascolana* and *Frantoio* the values of polyunsatured fatty acids tended to decrease from the 1st to the 2nd harvest. The oleic/linoleic ratio is an important parameter to value the possible stability of the lipid matrix to the oxidation. In all the cases the ratio oleic/linoleic was superior of 7, indicating a generally good stability of oil. i*A* (1st and 2nd) olives gave an oil with higher oleic/linoleic ratio than the healthy ones whereas the opposite trend was registered for i*F* (1st and 2nd harvest). Generally, for both *Ascolana* and *Frantoio* the values of

the ratio oleic/linoleic tended to increase from the 1st to the 2nd harvest.

3.9 Colorimeter

The values for lightness (L^*) are all statistically different. In general the values for infected samples for L^* were lower respect to healthy except for i*F* 2nd. Sample *F* 1st had no significant difference between infected and healthy and reached the highest value of redness (a^*). All samples were statistically different and *A* 2nd gave a negative value. The healthy samples were statistically different in yellowness (b^*) from the infected ones. In general, the samples from the 1st harvest had higher values for b^* except for i *F*1st.

3.10 Spectrophotometric estimation of total pigments

Frantoio olive oil showed significantly higher chlorophyll content than the oil from *Ascolana*. Olive oil of *A* 2nd tended to give minor chlorophyll content in contrast to the first harvest. There was a significant decrease of chlorophyll from the first to the second harvest when infected *Ascolana* was taken into consideration. *Frantoio* evidenced a higher chlorophyll content in the second harvest than the first and there was a significant difference for healthy compared to infected *Frantoio* between the first and the second harvest. Carotenoid content followed the trend of chlorophyll content being richer in the *Frantoio* derived olive oil than from *Ascolana*. In particular, a statistically significant decrease in carotenoid content was evidenced from the first to the second harvest of infected *Ascolana*. *Frantoio* olive oil at the second harvest showed a higher carotenoid content than in the first harvest and there were significant differences for both healthy and infected *Frantoio* from the first to the second harvest.

To summarize, infected samples, especially from *Frantoio*, had lower yield versus healthy ones and, in particular from *Ascolana*, lower MI. On the other hand, the infected samples of both varieties showed higher average fruit weight in contrast to healthy fruits. We have also found that virgin olive oils from infected olives had very elevated total phenols content compared to healthy fruits. Concerning the specific phenolic group of *o*-diphenols, infected *Frantoio* was less rich than its healthy specimens for both harvests, in contrast to infected *Ascolana* which was characterized by higher *o*-diphenols values for both harvests. The infected *Ascolana* olives gave an oil with higher oleic/linoleic ratio than the healthy ones whereas the opposite trend was registered for the infected *Frantoio*. Concerning the carotenoid contents, virgin olive oils obtained from the infected *Ascolana* fruits showed, for both harvests, tendencially higher values than healthy ones.

4 Conclusions

To conclude, the CLRV infection seems, above all, to negatively influence the *Frantoio* variety; in fact, a lower oil yield was registered for the infected olives versus healthy ones. Moreover, the quality of the virgin olive oils obtained from the infected fruits resulted depauperated in terms of richness in *o*-diphenols (especially in the 2nd harvest) as well as the decrease in the oleic/linoleic ratio may suggest a possible lowering of the oxidative stability during the oil storage.

The authors have declared no conflict of interest.

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