RESEARCH ARTICLE

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Simulation of Transport under Different Temperature Conditions: Effects on Extra Virgin Olive Oil Quality

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Extra virgin olive oil (EVOO) is imported/exported globally. However, little is known about the qualitative effects of the transport conditions, and, consequently, they are not usually controlled. This study simulates temperature fluctuations in summer (18–42.5 °C, 12 h–12 h, one week) and winter (4–16.5 °C, 12 h–12 h, one week), to examine their effect on EVOO quality. The EVOO samples undergo evaluation with chemical analyses before and immediately after treatment and after 24 weeks of storage (at room temperature in the dark). The simulated summer conditions cause oil oxidation. This situation bears a connection to an increased lipid oxidation rate, peroxide value, 1.2/1.3 diacylglycerol ratio, rancid score, and rancid-related volatile compounds. The simulated winter conditions also cause oil oxidation. In this case, the rancid score and the rancid-related volatile compound content show similarities to the samples exposed to the simulated summer conditions. In winter conditions, the temperature fluctuations seem to play a key role in the rancid defect appearance. Eleven of the 15 (summer) and 10 of the 15 (winter) samples are downgraded from the category of "extra virgin" to "virgin" after 24 weeks of bottle storage.
***Practical applications*: Transportation conditions constitute a critical factor in determining extra virgin olive oil quality. Transport is a critical control point in maintaining the quality of extra virgin olive oil, which can be transported without any specific controls. The obtained results improve the knowledge regarding the risks related to transport in hot and cold seasons, assessing the oxidative damages in both conditions on three different cultivars. A better understanding of the degradation phenomena during transportation helps develop specific technologies and practices (e.g., controlled-temperature transportation, thermal insulation materials, use of digi-sense nonreversible temperature labels, and so on) to counter the phenomena and evaluate the costs and limits of the existing protocols.**

1. Introduction

Extra virgin olive oil (EVOO) is a monounsaturated oil specific to the Mediterranean diet and recognized worldwide due to its health benefits and sensory properties. The biggest producers are in the Mediterranean area: Spain, Italy, Greece, Tunisia, Turkey, Morocco, and Portugal.[1] EVOO has a combined production of approximately three million metric tons and is sold globally, while the prime importers are Italy, the United States, Spain, France, Brazil, Portugal, Germany, and Japan.[2]

The above observations demonstrate that numerous commercial routes involve long land or sea journeys at all times. EVOO is transported as a liquid or general cargo (i.e., bottled oil) by ship, truck, or rail.[3] While in transit, conditions such as temperature, light exposure, and agitation are not usually controlled.[4,5]

Online cargo handbooks[6] indicate a lack of particular requirements for storing EVOO (the only recommendation is a temperature of 15 °C, which is often not applied). However, the scientific literature considers transport a critical control point (CCP) in the process management system,[7] and exported olive oils often lose the qualitative requirement to be classified as extra-virgin.[8] Transportation is often considered the weakest point in the olive oil chain as it does not fall under the producers' direct control. There are

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many ways it can affect EVOO quality, starting with oxidative stability.

Lipid oxidation (i.e., autoxidation) occurs naturally in oils, fats, and food matrices, and is consistent with exposure to light and air/oxygen, and high temperatures.[9–12] Although EVOO manufacturers have developed numerous technical solutions to reduce oxidation risks (e.g., reduced container headspace, oxygen-impermeable, and opaque packaging materials), the temperature during transport is often uncontrolled.[7,13–16]

In 2005, the Italian EVOO producer Monini S.p.A. carried out an internal trial that monitored the temperature inside trucks transporting bottles of oil. This study found that thermal conditions, especially in summer, risked degrading EVOO quality; when the external temperature increased to 38 °C, the temperature inside the truck stood at 55 °C. The replicated trial in 2006 confirmed an increase of 15–20 °C between the external environment and the internal truck temperature in summer. Similar studies of wine transport have shown that trucks are often parked for long periods in aprons, where the product can reach temperatures exceeding 60 °C.[17]

Inappropriate thermal conditions during EVOO storage have been widely studied. Researchers have examined the influence of storage temperature on legal parameters, phenol content, volatile compounds, and sensory profile in hot and cold treatments. The time and temperature conditions used in 27 articles about the effect of temperature during storage on olive oil quality are provided in Table S1 (Supporting Information). All of these articles kept the storage temperatures of the oils constant for the duration of the experiments. These studies highlighted that long storage periods (i.e., from two weeks to eight months) at a higher constant temperature (i.e., ≥40 °C) than room temperature (about 15–25 °C) degrade EVOO quality due to an increase in oxidative kinetics. Conversely, long storage periods in constant refrigerated, frozen, or cold operating conditions can preserve quality.

Nevertheless, to the best of the authors' knowledge, little is known—only from Pagliarini et al.[18]—about the effect of real-life transport and storage conditions on EVOO quality. Therefore, this work aimed to simulate realistic operating conditions (inappropriate transport followed by optimal storage) and assess their impact on bottled EVOO quality. Specifically, short-term (one week) inappropriate conditions, with a daily temperature cycle, were coupled with subsequent storage in appropriate operating conditions. We examined the effect of hot and cold (i.e., summer and winter) conditions before a long period of storage in optimal conditions (in the dark at room temperature) on EVOO quality.

2. Experimental Section

2.1. Samples and Experimental Conditions

During the trial 15 monovarietal olive oil samples were used—five replicates for three cultivars (i.e., *Coratina*, *Nocellara del Belice*, and *Peranzana*)—provided by Monini S.p.A. (Spoleto, Perugia, Italy). The samples underwent processing in two different industrial mills in southern Italy in November and December 2018. The *Coratina* and *Peranzana* olive oil samples were processed at Frantoio Muraglia (Barletta, Puglia, Italy), whereas the *Nocellara del Belice* olive oil samples were processed in Palermo (Sicily, Italy). The oils were cold extracted (malaxation temperature < 27 °C, according to the EU implementing regulation 29/2012), with malaxing times of between 20 and 40 min. The olive oil samples were immediately transferred to the laboratory and packaged in 500 mL green glass bottles, with a headspace of air of ≈8% of the bottle volume and closed with a plastic cap. The choice to study the quality changes in bottled olive oil samples is because this is the packaging in which it is sold (and transported) to large-scale distribution. Fifteen bottles (five replicates for each cultivar) underwent immediate analysis after bottling (t_0) and classified as "extra virgin" as defined in IOC / T.15 / NC No. 3 / Rev. 14[19] (**Table 1**). Then, a total of 90 bottles, 45 for the analyses after one week/immediately after thermal treatment (t_1) and 45 for the analyses after one week of thermal treatment +23 weeks of storage (t_{24}), were subjected to the following three thermal treatments for one week. Hence, this experiment used a total of 105 olive oil samples. For the sake of clarity, the experimental plan was schematized in Figure S1 (Supporting Information).

Each treatment examined 30 samples (i.e., five bottles for each cultivar (three)), for two storage times. The hot treatment (*HT*) simulated severe transport conditions in summer. The bottles were subjected to a daily temperature cycle ranging between 18 °C (minimum) and 42.5 °C (maximum) in the dark for one week. The cold treatment (*CT*) simulated severe transport conditions in winter. Here, the bottled oil was subjected to a daily temperature cycle ranging from 4 °C (minimum) to 16.5 °C (maximum) in the dark for one week. In the control treatment (*CON*), the bottles were kept in the dark at room temperature (20–22 °C) for one week.

A thermostated cell (Heraeus, Thermo, Waltham, MA) and a refrigerator (Electrolux, Stockholm, Sweden) equipped with a timer obtained the temperature cycles (Figure S2, Supporting Information). For the HT treatment, the cell was set at 40 °C for 12 h a day. When the cell turned on, the maximum temperature was reached after 1 h, with a heating rate of ≈20 °C h^{-1}, maintained for the following 11 h. At the end of the 12-h period, the cell turned off, and the interior temperature of the refrigerator fell to room temperature (≈15–20 °C) over 12 h, decreasing at a rate of roughly 1.5 °C h^{-1}. For the *CT* treatment, the refrigerator temperature was set at 5.5 ± 0.5 °C for 12 h a day. When the refrigerator turned on, the temperature fell at a rate of ≈2 °C h^{-1}. When it fell below 5.5 °C, cooling stopped, only resuming when the registered temperature rose above 6.0 °C. At the end of the 12-h period, it turned off, and the temperature returned to roughly 15–20 °C (room temperature) over 12 h, decreasing at a rate of about 2 °C h^{-1}. After these treatments, the EVOO samples were stored in protected conditions, in the dark, at room temperature (20–22 °C) for 23 weeks. Bottles were stored all on the same shelf, in randomized positions. The temperature was monitored during the treatments and the storage with temperature probes and a data-logger (WatchDog DataLogger, Spectrum, USA).

2.2. Chemical Analyses and Sensory Evaluations

Free fatty acids (FFA), peroxide value (PV), UV indices (K_{232}, K_{270}, and ΔK), and 1.2/1.3 diacylglycerol (1.2/1.3 DAG) content

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Table 1. Means for olive oil quality characteristics immediately after production (t_0), after one week (t_1), and 24 weeks of storage (t_{24}), and after hot (HT) and cold (CT) temperature cycles; CON refers to the control samples. a, b, and c indicate significant differences ($p < 0.05$) as a function of the temperature cycle, while x, y, and z indicate significant differences ($p < 0.05$) as a function of storage time. The interaction between treatment and storage time was not significant for all characteristics, except PV ($p = 0.0228$). The last column reports the residual standard error (RSE).

		Coratina			Nocellara			Peranzana			RSE
		CT	CON	HT	CT	CON	HT	CT	CON	HT	
FFA (% oleic acid)	t_0	0.20 ^{ax}	0.20 ^{ax}	0.20 ^{ax}	0.13 ^{ay}	0.13 ^{ay}	0.13 ^{ay}	0.41 ^{ax}	0.41 ^{ax}	0.41 ^{ay}	0.02
	t_1	0.20 ^{ax}	0.20 ^{ax}	0.20 ^{ax}	0.13 ^{ay}	0.13 ^{ay}	0.13 ^{ay}	0.40 ^{ax}	0.41 ^{ax}	0.41 ^{ay}	
	t_{24}	0.21 ^{ax}	0.18 ^{ax}	0.21 ^{ax}	0.17 ^{ax}	0.16 ^{ax}	0.16 ^{ax}	0.41 ^{bx}	0.42 ^{abx}	0.44 ^{ax}	
PV (meq O_2 kg^{-1})	t_0	5.2 ^{ay}	5.2 ^{ay}	5.2 ^{ay}	6.3 ^{ay}	6.3 ^{ay}	6.3 ^{ay}	8.7 ^{ay}	8.7 ^{az}	8.7 ^{az}	0.8
	t_1	5.9 ^{cx}	6.4 ^{bx}	7.6 ^{ax}	6.4 ^{aby}	5.4 ^{bz}	7.2 ^{ax}	10.7 ^{ax}	10.1 ^{ay}	10.4 ^{ay}	
	t_{24}	6.0 ^{cx}	7.0 ^{bx}	8.4 ^{ax}	8.3 ^{ax}	8.3 ^{ax}	7.8 ^{ax}	10.4 ^{bx}	11.4 ^{ax}	11.5 ^{ax}	
K232	t_0	1.69 ^{az}	1.69 ^{az}	1.69 ^{az}	1.82 ^{ay}	1.82 ^{az}	1.82 ^{ay}	1.97 ^{az}	1.97 ^{az}	1.97 ^{ay}	0.06
	t_1	1.75 ^{by}	1.82 ^{ay}	1.81 ^{aby}	1.82 ^{ay}	1.88 ^{ay}	1.86 ^{ay}	2.19 ^{ay}	2.11 ^{ay}	2.18 ^{ax}	
	t_{24}	1.87 ^{ax}	1.89 ^{ax}	1.87 ^{ax}	2.01 ^{ax}	2.05 ^{ax}	2.10 ^{ax}	2.25 ^{ax}	2.18 ^{ax}	2.19 ^{ax}	
K270	t_0	0.134 ^{ax}	0.134 ^{ax}	0.134 ^{ax}	0.112 ^{ax}	0.112 ^{ay}	0.112 ^{axy}	0.132 ^{axy}	0.132 ^{ax}	0.132 ^{ax}	0.005
	t_1	0.129 ^{ay}	0.131 ^{ax}	0.129 ^{ax}	0.117 ^{ax}	0.113 ^{axy}	0.109 ^{ay}	0.131 ^{ay}	0.133 ^{ax}	0.134 ^{ax}	
	t_{24}	0.135 ^{ax}	0.125 ^{ay}	0.129 ^{ax}	0.113 ^{ax}	0.116 ^{ax}	0.116 ^{ax}	0.137 ^{ax}	0.133 ^{ax}	0.135 ^{ax}	
ΔK	t_0	−0.002 ^{ax}	−0.002 ^{ax}	−0.002 ^{ax}	−0.005 ^{ay}	−0.005 ^{ay}	−0.005 ^{ay}	−0.002 ^{ay}	−0.002 ^{ay}	−0.002 ^{ay}	0.001
	t_1	−0.002 ^{ax}	−0.002 ^{ax}	−0.002 ^{ax}	−0.004 ^{axy}	−0.004 ^{axy}	−0.004 ^{axy}	−0.001 ^{ax}	−0.001 ^{ax}	−0.002 ^{ay}	
	t_{24}	−0.002 ^{ax}	−0.003 ^{ax}	−0.002 ^{ax}	−0.003 ^{ax}	−0.003 ^{ax}	−0.003 ^{ax}	−0.001 ^{ax}	−0.001 ^{ax}	−0.001 ^{ax}	
1.2/1.3 DAG	t_0	94.2 ^{ax}	94.2 ^{ax}	94.2 ^{ax}	97.7 ^{ax}	97.7 ^{ax}	97.7 ^{ax}	94.4 ^{ax}	94.4 ^{ax}	94.4 ^{ax}	3.4
	t_1	95.9 ^{ax}	93.7 ^{abx}	90.5 ^{by}	95.8 ^{ax}	97.7 ^{ax}	91.4 ^{by}	88.3 ^{ay}	86.6 ^{ay}	81.2 ^{by}	
	t_{24}	85.4 ^{ay}	83.7 ^{aby}	81.2 ^{bz}	79.4 ^{abz}	81.3 ^{ay}	77.1 ^{bz}	65.8 ^{az}	66.4 ^{az}	58.5 ^{bz}	
Total phenolic compounds ($mg_{tyrosol}$ kg^{-1})	t_0	489 ^{ax}	489 ^{ax}	489 ^{ax}	156 ^{ax}	156 ^{ax}	156 ^{ay}	306 ^{ax}	306 ^{ax}	306 ^{ay}	8
	t_1	490 ^{ax}	492 ^{ax}	417 ^{bz}	140 ^{by}	147 ^{by}	170 ^{ax}	296 ^{by}	285 ^{cy}	321 ^{ax}	
	t_{24}	483 ^{ax}	472 ^{by}	473 ^{by}	136 ^{ay}	139 ^{az}	136 ^{az}	276 ^{az}	281 ^{ay}	282 ^{az}	

were measured as specified in IOC / T.15 / NC No. 3 / Rev. 14.[19] At t_0 (i.e., immediately after the production) oils were further characterized for turbidity, using a Hach 2100AN turbidimeter (Loveland, CO) and for water content, using the 37858 Hydranal – Moisture Test Kit (Honeywell Fluka, Bucharest, Romania).

Phenolic compounds were measured in line with the International Olive Council (IOC) method[20] using an HPLC 200 LC series (Perkin Elmer Inc., Waltham, MA) coupled with a UV–vis detector (Varian 9050 UV-Vis variable-wavelength detector; Varian Inc., Palo Alto, CA) and a LiChroCART column (250-4.6 Purospher STAR RP-18E 5 μm; Merck KGaA; Darmstadt, Germany). The phenol results are expressed as mg kg^{-1} of tyrosol equivalents. Volatile compounds were measured using the headspace solid-phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS) method described in Fortini, Migliorini, Cherubini, Cecchi, and Calamai.[21] A Thermo Scientific TRACE GC-MS and a ZB-FFAP capillary column (Zebron) 30 m, 0.25 mm ID, with 0.25 mm film thickness, were used.

The sensory evaluation followed the procedure described in IOC / T.20 / Doc. No 15 / Rev. 10.[22] The panel comprised five men and three women with training in the official IOC procedure.[23]

2.3. Experimental Plan and Data Processing

The experimental plan adopted a randomized block design, as described in Pinheiro and Bates.[24] The thermal treatments and storage times were deemed experimental factors, while the cultivars acted as a blocking factor. Five replicates were performed. A mixed-effect ANOVA model (Statgraphics Centurion software ver. XV, Statpoint Technologies, Warrenton, VA) assessed the main effects of time, treatment, and their interaction. The main cultivar effect was also considered in the statistical analysis. The significance threshold was set at $p < 0.05$. For the experimental plan, three cultivars with different characteristics in terms of phenolic content and sensory aspects were chosen. Hence, the experiment featured a high degree of variability. In line with this choice, the chemical and sensorial changes simultaneously in the three cultivars were first discussed. Then, the differences among the cultivars were discussed. Applying the Chi-squared test ($p < 0.05$) to the sensory attribute values compared the frequency of EVOO and virgin olive oil (VOO) samples at the end of the storage period. The relationships between water content and rancidity and between turbidity and rancidity were tested in two different ways: i) with a linear regression between the rancid score of the panel and the turbidity/water content and ii) with a logit regression between the

Table 2. Means of median values of olive oil quality sensory characteristics immediately after production (t_0), after one week (t_1), and 24 weeks of storage (t_{24}), and after hot (HT) and cold (CT) temperature cycles; CON refers to the control samples. a, b, and c indicate significant differences ($p < 0.05$) as a function of the temperature cycle, while x, y, and z indicate significant differences ($p < 0.05$) as a function of storage time. The last column reports the residual standard error (RSE).

		Coratina			Nocellara			Peranzana			RSE
		CT	CON	HT	CT	CON	HT	CT	CON	HT	
Positive attributes											
Fruity	t_0	3.7	3.7	3.7	4.1	4.1	4.1	3.0	3.0	3.0	0.6
	t_1	3.2	3.1	3.2	4.2	3.8	3.6	2.7	3.0	2.8	
	t_{24}	4.9	4.3	5.0	4.3	4.6	4.4	4.1	3.8	3.8	
Bitter	t_0	5.1	5.1	5.1	1.8	1.8	1.8	1.3	1.3	1.3	0.7
	t_1	2.9	3.4	3.0	1.2	1.1	1.1	1.0	1.1	1.1	
	t_{24}	4.5	4.1	4.9	1.1	1.1	1.4	1.0	1.3	1.0	
Pungent	t_0	4.7	4.7	4.7	4.0	4.0	4.0	3.2	3.2	3.2	0.9
	t_1	3.8	4.4	3.5	4.5	4.4	4.2	1.8	2.5	2.8	
	t_{24}	5.8	4.4	5.6	3.9	4.2	3.9	3.0	2.7	2.5	
Negative attributes											
Rancid	t_0	0.0 ^{az}	0.0 ^{az}	0.0 ^{az}	0.0 ^{ay}	0.0 ^{ax}	0.0 ^{ay}	0.0 ^{az}	0.0 ^{az}	0.0 ^{az}	0.4
	t_1	0.8 ^{ax}	0.0 ^{ay}	0.9 ^{ax}	0.2 ^{ay}	0.0 ^{ax}	0.0 ^{ay}	0.8 ^{ay}	0.0 ^{ay}	1.0 ^{ay}	
	t_{24}	1.1 ^{ax}	1.0 ^{ax}	1.1 ^{ax}	1.2 ^{bx}	0.0 ^{cx}	1.7 ^{ax}	1.3 ^{bx}	1.2 ^{bx}	1.8 ^{ax}	
Fusty/Muddy sediments	t_0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	–
	t_1	0.6	n.d.	1.0	1.2	n.d.	0.7	1.0	n.d.	0.8	
	t_{24}	n.d.	0.6	n.d.	n.d.	1.1	n.d.	0.6	n.d.	n.d.	
Musty/Humidity/Earthy	t_0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	–
	t_1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
	t_{24}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
Winey/Vinegary	t_0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	–
	t_1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
	t_{24}	n.d.	1.1	n.d.	1.1	n.d.	1.9	1.0	n.d.	1.0	

panel rancid detection (yes/no) and the turbidity/water content.

At t_{24}, linear discriminant analysis (LDA) was used on the measured variables in the samples to establish the possibility of classifying the samples into three groups corresponding to the three treatments (*CON, HT*, and *CT*).

3. Results

3.1. Quality Characteristics of the Oil Samples

Table 1 shows the quality characteristics of the samples. The experimental data indicate the mean for the five replicates of each cultivar as analyzed immediately after production, after one week of treatment, and after 24 weeks of storage.

Although the olive oil samples remained within the IOC / T.15 / NC No. 3 / Rev. 14[19] legal chemical limits for the extra virgin category (FFA $\leq$ 0.08; PV $\leq$ 20; K232 $\leq$ 2.50; K270 $\leq$ 0.22; $\Delta K \leq 0.01$), storage revealed statistically significant differences. FFA increased slightly in all the olive oil samples, but the effect of storage time was only statistically significant in the Nocellara and Peranzana *HT* samples. In the thermal-treated olive oil samples, *PV* and K_{232} increased significantly, while the 1.2/1.3 DAG ratio and total phenolic compound content decreased significantly, indicating slow EVOO degradation. After six months, statistically significant differences in *PV* and the 1.2/1.3 DAG ratio were observed as a function of the treatment. *PV* was higher in the *HT* samples than in the *CT* and *CON* samples; the 1.2/1.3 DAG ratio was lower in the *HT* samples than in the *CT* and *CON* samples, starting from t_1. No statistically significant differences due to treatment were identified for any other chemical characteristics.

No significant differences were found for the fruity, bitter, or pungent positive sensory attributes as a function of storage time or treatment (**Table 2**). The findings showed that the negative rancid attribute was a function of storage time and treatment. During storage time, the rancid attribute increased in a statistically significant manner in all the olive oil samples. After six months, the *HT* samples had higher rancid values than the *CON* and *CT* samples, while the *CT* values lay between the *HT* and *CON* values.

The Chi-squared test, applied to the sensory defect values (**Table 3**), showed that, at t_1, all of the *CON* samples remained within the EVOO category, while five *CT* samples (two of cv. Nocellara and three of cv. Peranzana) and nine *HT* samples (three of each cv.) were downgraded from EVOO to VOO. After t_{24}, more significant differences were found between the treated and control samples (Tables 2 and 3). Eleven (five of cv. Coratina, four of cv. Nocellara, and two of cv. Peranzana) of the 15 *CON* samples

Table 3. Frequency of EVOO and VOO samples after one week (t_1) and 24 weeks of storage (t_{24}), and after hot (HT) and cold (CT) temperature cycles measured by the Chi-squared test; CON refers to the control samples. The "*p*-value versus CON" row shows the outcome of the Chi-squared test.

	t_1						t_{24}					
	CT		CON		HT		CT		CON		HT	
	EVOO	VOO	EVOO	VOO	EVOO	VOO	EVOO	VOO	EVOO	VOO	EVOO	VOO
Coratina	5	0	5	0	2	3	5	0	5	0	2	3
Nocellara	3	2	5	0	2	3	0	5	4	1	0	5
Peranzana	2	3	5	0	2	3	0	5	2	3	2	3
p-value vs CON	**				***		***		–		***	

** and *** indicate statistically significant differences at $p < 0.01$ and $p < 0.001$, respectively.

Table 4. Mean volatile compound content with a statistically significant correlation to the treatment after 24 weeks of storage (t_{24}); HT and CT refer to hot and cold temperature cycles, respectively; CON refers to the control samples.

	Coratina			Nocellara			Peranzana			RSE
	CT	CON	HT	CT	CON	HT	CT	CON	HT	
Acids (mg kg^{-1}):										
Propanoic acid	0.10 ^{b}	0.03 ^{c}	0.17 ^{a}	0.03 ^{a}	0.00 ^{a}	0.04 ^{a}	0.08 ^{a}	0.08 ^{a}	0.12 ^{a}	0.06
Butanoic acid	0.19 ^{ab}	0.10 ^{b}	0.27 ^{a}	0.09 ^{a}	0.05 ^{a}	0.09 ^{a}	0.16 ^{a}	0.16 ^{a}	0.20 ^{a}	0.08
Pentanoic acid	0.11 ^{b}	0.04 ^{c}	0.18 ^{a}	0.03 ^{a}	0.00 ^{a}	0.03 ^{a}	0.07 ^{a}	0.06 ^{a}	0.10 ^{a}	0.06
Hexanoic acid	0.39 ^{b}	0.12 ^{c}	0.53 ^{a}	0.10 ^{a}	0.01 ^{b}	0.06 ^{ab}	0.14 ^{ab}	0.12 ^{b}	0.22 ^{a}	0.10
Unsaturated aldehydes (mg kg^{-1}):										
E,E-2,4-Heptadienal	0.37 ^{b}	0.12 ^{c}	0.51 ^{a}	0.16 ^{b}	0.08 ^{c}	0.23 ^{a}	0.10 ^{b}	0.15 ^{ab}	0.22 ^{a}	0.07
Hexanal	2.06 ^{b}	1.57 ^{c}	2.33 ^{a}	1.46 ^{a}	1.03 ^{b}	1.36 ^{a}	0.92 ^{b}	1.15 ^{a}	0.90 ^{b}	0.22
E-2-decenal	17.57 ^{c}	49.90 ^{a}	30.93 ^{b}	11.18 ^{a}	3.55 ^{b}	2.91 ^{b}	6.57 ^{a}	6.10 ^{a}	4.24 ^{a}	5.78
Ketones (mg kg^{-1}):										
3-Pentanone	0.42 ^{a}	0.47 ^{a}	0.19 ^{b}	0.19 ^{a}	0.06 ^{b}	0.04 ^{b}	0.21 ^{ab}	0.28 ^{a}	0.18 ^{b}	0.07
2-Nonanone	0.08 ^{ab}	0.04 ^{b}	0.11 ^{a}	0.04 ^{a}	0.03 ^{a}	0.04 ^{a}	0.05 ^{a}	0.05 ^{a}	0.06 ^{a}	0.04
Alcohols (mg kg^{-1}):										
1-Penten-3-ol	0.61 ^{b}	0.55 ^{c}	0.66 ^{a}	0.62 ^{c}	0.67 ^{b}	0.71 ^{a}	0.57 ^{a}	0.58 ^{a}	0.59 ^{a}	0.03
1-Hexanol	4.14 ^{a}	4.34 ^{a}	1.70 ^{b}	1.74 ^{a}	0.28 ^{b}	0.25 ^{b}	0.89 ^{a}	1.01 ^{a}	0.79 ^{a}	0.66
Z-2-Hexen-1-ol	0.03 ^{b}	0.02 ^{b}	0.05 ^{a}	0.02 ^{a}	0.03 ^{a}	0.03 ^{a}	0.03 ^{a}	0.03 ^{a}	0.03 ^{a}	0.01
2-Heptanol	0.034 ^{a}	0.019 ^{b}	0.024 ^{b}	0.024 ^{a}	0.018 ^{b}	0.018 ^{b}	0.032 ^{a}	0.031 ^{a}	0.028 ^{a}	0.005
Acetate esters (mg kg^{-1}):										
Z-3-hexenyl-acetate	0.93 ^{a}	0.99 ^{a}	0.41 ^{b}	0.36 ^{a}	0.05 ^{b}	0.04 ^{b}	0.25 ^{a}	0.29 ^{a}	0.22 ^{a}	0.18

a, b, and c indicate significant differences ($p < 0.05$) as a function of the temperature cycle. The last column reports the residual standard error (RSE).

remained classified as EVOO, compared to 4 out of the 15 *HT* samples (two of cv. Coratina and two of cv. Peranzana), and 5 out of the 15 *CT* samples (only the cv. Coratina oil).

The used EVOO had different degrees of turbidity. Particularly, turbidity was 99 ± 39 NTU for Nocellara, 266 ± 121 NTU for Coratina, and 637 ± 319 NTU for Peranzana. Water content was 0.13 ± 0.04%, 0.19 ± 0.06%, and 0.27 ± 0.08% in Nocellara, Coratina, and Peranzana, respectively. Water content during the storage has been related to enzymatic hydrolysis, and thus, water is considered a factor favoring the autoxidation.[11] Hence, taking advantage from the wide range of water contents measured, the relationship between rancidity and water content has been tested. However, in our data, neither water nor turbidity was found to have a significant effect on the panel evaluation of rancidity.

The samples were measured for volatile compounds at t_{24} to confirm the above observations. **Table 4** shows that the concentrations of the volatile compounds were significantly affected by the thermal treatments. Treatment aligned with a significant difference in volatile compound content in general, and volatile compounds related to the rancid defect in particular, as reported in Table 4.[25–28]

For most of the volatile compounds shown in Table 4, the *HT* samples had a higher volatile compound content than the *CON* samples, while the *CT* samples fell somewhere between. Treatment aligned with an increase in hexanal and hexanoic acid content during storage. Hexanal often acts as an index of olive oil aging,[29–31] as it increases during oxidation and relates to the "unpleasant, rancid, penetrating" sensory perception when present

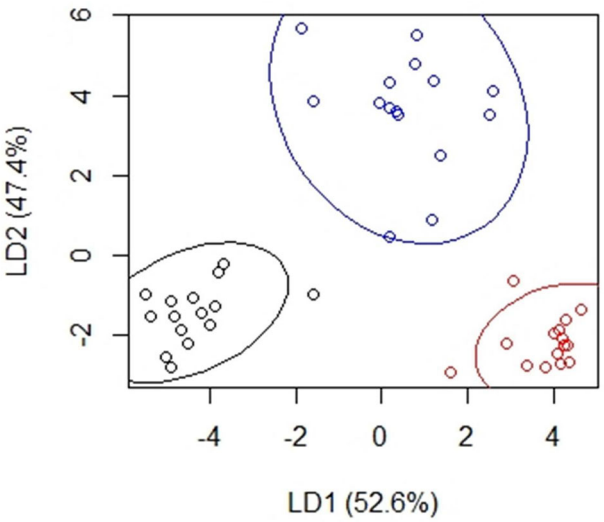

Figure 1. Linear discriminant analysis (LDA). The CON samples are shown in black, CT samples in blue, and HT samples in red. The figure also reported the 95% confidence ellipses.

at a high concentration.[32–34] Hexanoic acid forms from hexanal oxidation, which contributes to the rancid perception.[25,32–36]

Both the *HT* and *CT* treatments were related to an increase in E,E-2,4-heptadienal and E-2-decenal content during storage. E,E-2,4-heptadienal is considered a crucial indicator of rancidity, although it does not contribute to the rancid sensory perception due to its odor threshold.[25,33,37,38] E-2-decenal is considered one of the primary compounds linked to the rancid defect.[25,34,37]

Finally, butanoic acid content increased during storage as a function of treatment. The compound is always present in the chromatograms of standard rancid VOO and contributes to the rancid perception.[25,26,33,34]

3.2. LDA Analysis

The LDA analysis tested the global effect of all variables to determine whether samples could be grouped into classes as a function of the thermal treatment.

Figure 1 shows the samples classified using all 75 measured variables. Table S2 (Supporting Information) reports the composition of the discrimination factors. For the LD1 component (52.6%), the *HT* samples (on the right) could be separated from the *CON* samples (on the left). The *CT* samples lay between the *HT* and *CON* samples; they were further to the right and more dispersed. For the LD2 component (47.4%), the *HT* and *CON* samples (on the bottom) could be separated from the *CT* samples (on the top). These results clearly show that hot and cold thermal conditions changed the olive oil quality during storage.

4. Discussion

Our experimental findings underline that transportation conditions are a critical factor in determining EVOO quality. They highlight the importance of temperature fluctuations that may occur during winter and summer transportation for detrimental oxidative phenomena.

Short-term temperature fluctuations between 18 and 42.5 °C (comparable to severe summer conditions and/or transport to equatorial countries—*HT*) have an association with lipid oxidation, which can degrade oil quality from EVOO to VOO. Quality characteristics, such as *PV*, the 1.2/1.3 DAG ratio, the rancid sensory defect, and some volatile compounds related to rancidity, were significantly related to the above behavior.[9–12,39] In our experiment, lipid oxidation occurred during storage due to the incremental effect on the oxidation kinetics of the high temperatures suffered during the simulation of transport. The relationship between high temperature and lipid oxidation was well documented in the olive oil literature for long storages. During the last 20 years, several papers focused on the harmful effects of prolonged exposure to high temperature.[35,40–42] Furthermore, compositional changes of olive oil in packaging (i.e., bottled) due to high temperature are well documented. Technological improvements and mathematical models for shelf-life prediction are available. Also in this case, long-term storages are considered.[14,16,29,30,43] However, during olive oil transportation, an in-bottle short-term storage is done, and few studies documented the damage of temperature to the product.[4,44,45] The storage time considered in these studies ranged from 2 to 3 weeks, while the temperature ranged from 35 to 65 °C. Our results remark that bottled olive oil is particularly prone to oxidation. The development of a perceivable sensory defect could be found immediately after a shorter heat exposure (i.e., at t_1 9 out of the 15 *HT* oils had a sensory defect) even if the temperature fluctuated between a harmful (i.e., 42.5 °C) and a harmless (i.e., 18 °C) temperature.

Short-term temperature fluctuations between 4 and 16.5 °C (comparable to severe winter conditions—*CT*) resulted in behavior between the *CON* and *HT* samples. After six months of storage, the *PV* values and the 1.2/1.3 DAG ratio were not significantly different from the *CON* samples; however, the rancid score and volatile compound related to rancidity content bore similarities with the *HT* samples. The LDA analysis found that the *CT* and *CON* samples did not overlap, which confirms that *CT* modified the oil quality. However, differences between the *CT* and *HT* samples suggest that specific phenomena could drive oil degradation under *CT*. Two contrasting phenomena, leading to two contrasting effects, could be involved in the *CT* samples. First, cold conditions slow down reaction kinetics and, for this reason, low temperatures could help preserve EVOO quality. On the other hand, oxygen solubility could increase in cold conditions.[46–48] The olive oil samples solubilized oxygen during the "cold temperature hours" and later (during the "mild temperature hours") the improved oxygen concentration increased the rate of the oil oxidation reaction kinetics. In addition, at a low temperature, polyunsaturated fatty acids and triacylglycerols are more mobile than saturated fatty acids and, consequently, more likely to undergo an oxidation reaction.[49] Hence, during the cold period, the conditions became optimal for lipid oxidative reactions; then, when the temperature got milder, the energetic requirements for these reactions were fulfilled, increasing the reaction rate. Previous works focusing on the olive oil storage at cold and constant temperature observed a delay of detrimental reactions.[15,16,31,50–52] These works well describe the benefit provided by storage in tanks or bottles in rooms at a controlled constant temperature. However, during transportation, the

temperature fluctuations to which the oil was subjected played a vital role in the oxidation, and temperature should remain as constant as possible to avoid detrimental oxidative reactions. Even low temperatures can be harmful to quality if followed by higher temperatures. Finally, we must consider the different total phenol content of the three tested cultivars. The protective action of phenols against lipid oxidation during storage is well known in literature. Moreover, since 2000, the phenolic content of olive oil was introduced in shelf-life prediction model.[18,53] Consistently, the protective effect of olive oil phenols is confirmed during our simulation of transportation phase. Coratina showed the highest phenolic content and was the cultivar least prone to lipid oxidation; two oils were downgraded at t_1, as effect of the *HT*, and at t_{24} the same two oils were recognized as defective. Conversely, Nocellara was the cultivar with the lower phenolic content and the most prone to lipid oxidation. At t_1 five oils were downgraded from EVOO to VOO, while at t_{24} 11 oils were VOO. Particularly, all the *HT* and all the *CT* oils were downgraded, while only four out of five *CON* oils remained EVOO. This strongly remarks the importance of protecting adequately the EVOO during the transportation, especially if they show a low phenolic content.

5. Conclusions

EVOO producers find it tricky to control transport and storage conditions; transportation is usually out of their hands, and they have to place their trust in haulage companies. These companies, however, often lack operational protocols regarding factors such as temperature, which can fluctuate greatly during long trips, notably due to seasonal change. Therefore, a risk exists that the oil leaving the producer's premises as EVOO will arrive on the large distribution companies' shelves and, consequently, on the consumer's table as VOO, damaging the producer's reputation.

Our study underlined the importance of temperature control during transport and measured the effect of temperature fluctuation (notably, severe summer and winter transport conditions) on EVOO quality. Although our olive oil samples were stored in optimal conditions (in the dark, at room temperature), 11 of the 15 (summer) and 10 of the 15 (winter) samples were downgraded from EVOO to VOO due to an increase in lipid oxidation.

Transport is a CCP in the maintenance of EVOO quality. Currently, EVOO can be transported without any specific controls. Therefore, it is necessary to develop and apply good practices and techniques to prevent a decline in EVOO quality characteristics. Hence, EVOO requires protection from heat and temperature variations. During transportation, it can be recommended to implement all practices to contrast temperature variations in the olive oil, for example, controlled-temperature transportation, thermal insulation materials, use of temperature labels, and so on. Furthermore, it is advisable to avoid storage in outdoor areas, where the olive oil could be exposed to high and low temperatures and daily temperature variations. However, further studies are still required to better understand the degradation phenomena during transportation, develop specific technologies to counter the phenomena, and evaluate the costs and limits of the existing protocols. Finally, it is crucial to highlight that the results obtained in this study relate to the transportation of bottled EVOO samples. Therefore, further studies on the quality changes of olive oils transported in large tanks are still required.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Carlotta Breschi: data curation, investigation, methodology, writing – original draft; writing – review and editing. Lorenzo Guerrini: conceptualization, data curation, formal analysis, investigation, validation, writing – original draft, writing – review and editing. Bruno Zanoni: funding acquisition, project administration, supervision, writing – review and editing. Piernicola Masella: conceptualization, methodology. Lorenzo Lunetti: funding acquisition, investigation, supervision. Alessandro Parenti: conceptualization, funding acquisition, project administration.

Data Availability Statement

Data are available on request from the authors.

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