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Monovarietal Extra Virgin Olive Oils: Correlation Between Thermal Properties and Chemical Composition

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Thermal properties of monovarietal extra virgin olive oils were evaluated by means of differential scanning calorimetry (upon cooling) and related to their chemical composition (triacylglycerols, diacylglycerols, total and free fatty acids, oxidation status). The overall crystallization enthalpy did not significantly differ among samples and did not account for the differences observed in chemical compositions. On the contrary, a higher degree of unsaturation in the lipid profile induced a shift of the crystallization onset towards lower temperatures and narrowing of the crystallization temperature range. The presence of triacylglycerol lysis and lipid oxidation products shifted the crystallization towards higher temperatures and the phase transition developed over a larger temperature range. Differential scanning calorimetry thermograms were deconvoluted into three constituent exothermic peaks for all samples. The area of the two lower-temperature exotherms was found to be statistically correlated with the amount of triunsaturated and monosaturated triacylglycerols present in the oil. Thermal properties of extra virgin olive oil were found to be affected by oil chemical composition.

KEYWORDS: Extra virgin olive oil; olive cultivar; DSC; crystallization; thermal properties; chemical composition

INTRODUCTION

In the past few years, great attention has been devoted to the development of a market for high-quality extra virgin olive oil. The most recognized high-quality extra virgin olive oils are those carrying a protected mark (e.g., from the European Union) or the products obtained from a single olive cultivar (monovarietal) that display characteristic chemical composition and sensory profiles (*1*–*4*). *Biancolilla*, *Cerasuola*, and *Nocellara del Belice* are the most widespread Sicilian cultivars, and they exhibit different ripening and utilization characteristics. *Nocellara del Belice* is a late ripening variety (commonly early picked) that is used for the production of both table olives and olive oil; *Biancolilla* and *Cerasuola* are intermediate ripening cultivars that can be only utilized for olive oil production (*5*).

The application of thermoanalytical techniques, especially differential scanning calorimetry (DSC), to oils and fats is well-known for the determination of solid fat content, crystallization and melting profiles, enthalpy of transitions, and polymorphic forms (*6*, *7*). DSC evaluation of the thermal properties of vegetable oils of different botanical origins, was reported by several authors (*8*, *9*). It was found that the measurement of the thermal properties of oils undergoing first-order phase transition offered a reproducible method for oil characterization. DSC may have some advantages over the more classical characterization methods (i.e., triacylglycerol and fatty acid profiles), as it is rapid and does not require sample preparation and solvents (*10*, *11*). DSC was also used to assess the thermoxidative stability of oils (*12*).

Only a few studies reported DSC application to extra virgin olive oil. Jiménez Márquez and Beltrán Maza (*13*) analyzed thermal profiles of virgin olive oils from six different Spanish cultivars and correlated thermal properties with triacylglycerol and fatty acid compositions. A good correlation was found between temperature of transition (crystallization and melting) and the triacylglycerol composition for the different cultivars. In addition, the temperature range where crystallization and melting occurred was related to the differences in oleic and linoleic acid content. Oxidative stability (auto- and thermo-oxidation) of extra virgin olive oil has been evaluated by DSC (*14*) and modulated DSC (*15*), showing good correlations

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between thermal parameters (crystallization enthalpy and onset transition temperature) and the amount of oxidation products (volatile compounds). Angiuli and co-workers (*16*) verified the applicability of several calorimetric techniques (DSC, modulated DSC, and isothermal calorimetry) to evaluate quality and traceability of extra virgin olive oil. In particular, these authors reported that DSC may be used to efficiently discriminate between commercial extra virgin olive oil and other samples of known geographical origin. Detection of adulteration of extra virgin olive oil with other oils (sunflower oil, soybean oil, grapeseed oil, and corn-seed oil) by thermal analysis was also suggested. No correlations with chemical parameters were considered in this work.

Little information is available in the literature about how different chemical composition may affect thermal properties of extra virgin olive oils, especially minor components. Therefore, the aim of this study was to evaluate thermal properties (upon cooling) of extra virgin olive oil and to relate them to the chemical composition of the oil. Extra virgin olive oils considered were obtained from drupes of three cultivars collected at three different harvesting periods.

MATERIALS AND METHODS

Sampling. Three monovarietal (*Biancolilla*, *Cerasuola*, and *Nocellara del Belice*) extra virgin olive oils from Palermo (Sicily, Italy), were studied. The olives used for oil production were hand-picked at three different harvesting periods (October (A), November (B), and December (C)) in 2004. The extra virgin olive oil samples were produced using a continuous system, which comprised a hammer fixed crusher, a horizontal malaxation system (at a temperature of 27 °C), and two three-phases decanters (210–260 L water added per ton of olives) with a working capacity of 1.4 ton/h (Pieralisi, Jesi, Italy). Samples were stored in dark bottles without headspace at room temperature and analyzed after one-year storage at these conditions. One olive oil sample per harvesting period and olive cultivar was analyzed.

Reagents, Solvents, and Standards. All solvents used were analytical or HPLC grade (Merck, Darmstadt, Germany). Commercial standards of triacylglycerols (triolein (OOO), trilinolein (LLL)), diacylglycerols (dimyristin, dipalmitin, distearin, diolein), tridecanoic acid, and tridecanoic acid methyl ester were purchased from Sigma-Aldrich (St. Louis, MO). The standard mixture of fatty acid methyl esters (GLC 463) was supplied by Nu-Chek (Elysian, MN). Squalane standard was purchased from Carl Roth Gmbh + Co. KG (Karlsruhe, Germany). NH_2 and silica solid-phase extraction (SPE) cartridges (500-mg stationary phase/3-mL Strata cartridges) were purchased from Phenomenex (Torrance, CA).

DSC. Samples of oil (8–10 mg) were weighed in closed aluminum pans and analyzed with a DSC Q100 (TA Instruments, New Castle, DE). Indium (melting temperature 156.6 °C, $\Delta H_f = 28.45$ J/g) and *n*-dodecane (melting temperature –9.65 °C, $\Delta H_f = 216.73$ J/g) were used to calibrate the instrument, and an empty pan was used as reference. Oil samples were equilibrated at 30 °C for 3 min and then cooled to –80 °C at a rate of 2 °C/min. Dry nitrogen was purged in the DSC cell at 50 cm^3/min. Thermograms were analyzed with a Universal Analysis Software, Version 3.9A (TA Instruments, New Castle, DE), and enthalpy (ΔH, J/g), onset (T_{on}), and offset (T_{off}) temperatures of the transitions were obtained. Range of the transitions was calculated as the temperature difference between T_{on} and T_{off}. At least triplicate analyses were carried out for each sample. Overlapping transitions of the cooling thermograms were deconvoluted into individual constituent peaks using PeakFit software (Jandel Scientific, San Rafael, CA). The following parameters were considered for each deconvoluted peak: onset (T_{on}), offset (T_{off}), and peak temperatures (T_p), % peak area (percentage area of the total peak area).

Gas Chromatographic Determination of Total Fatty Acids (FA). FA were determined according to Cercaci et al. (*17*). The limit of quantitation (LOQ) was 0.01 g/100 g of fatty acids. Three replicates were prepared and analyzed per sample.

Gas Chromatographic Determination of Free Fatty Acids (FFA). FFA were purified by NH_2 SPE and methylated with diazomethane, as reported by Bonoli et al. (*18*). Tridecanoic acid was used as internal standard. The methylated FFA were injected into a gas chromatograph under the same analytical conditions as used for analysis of total fatty acids (*17*). LOQ was 0.01 g/100 g of free fatty acids. Three replicates were prepared and analyzed per sample.

HPLC Determination of Triacylglycerols (TAG). TAG were analyzed according to a modified version of the method suggested by Holcăpek et al. (*19*). Different HPLC column dimensions, mobile phase gradient, and flow rate were employed. HPLC analysis were performed with a 5-μm Luna C18 (Phenomenex, Torrance, CA) column (25 cm × 3.0 mm i.d.). A C18 precolumn filter (Phenomenex) was used. The mobile phase flow rate was 0.7 mL/min. The gradient elution was performed by using 2-propanol and acetonitrile as mobile phases A and B, respectively, according to the following gradient: from 0 to 2 min 52% B, from 2 to 4 min increased to 58% B, from 4 to 25 min held at 58% B, from 25 to 30 min decreased to 10% B and from 30 to 37 min increased back to 52% B. Peak identification was carried out by comparing the peak retention times with those of the pure triacylglycerols standards (LLL and OOO), as well as with soybean oil for a correct assignment of trilinolenin (LnLnLn). Further confirmation of the identification was achieved by comparing the HPLC traces with those reported in the literature (*20*). The following TAG were identified: dilinoleyl-palmitoleyl-glycerol (PLL), dilinoleoyl-oleoyl-glycerol (OLL), palmitoyl-oleoyl-linolenoyl-glycerol (POLn), dioleoyl-linoleoyl-glycerol (OOL), palmitoyl-oleoyl-linoleoyl-glycerol (POL), OOO, dioleoyl-palmitoyl-glycerol (POO) dipalmitoyl-oleoyl-glycerol (PPO) and dioleoyl-stearoyl-glycerol (SOO). LOQ was 0.01 g/100 g of TAG.

Gas Chromatographic Determination of Total Diacylglycerols (DAG) and Major 1,2-DAG and 1,3-DAG. DAG were determined according to Bonoli et al. (*18*). Identification of DAG was carried out by comparing the peak retention times and the GC traces with those of the DAG standards and chromatograms reported in literature (*21*), respectively. The following DAG were identified: 1-palmitoyl-2-oleyl-*sn*-glycerol (1,2-PO), 1-palmitoyl-2-linoleoyl-*sn*-glycerol (1,2-PL), 1,2-diolein (1,2-OO), 1-oleoyl-2-linoleoyl-*sn*-glycerol (1,2-OL), and 1,3-diolein (1,3-OO). LOQ was 0.04 g/100 g of DAG. Three replicates were prepared and analyzed per sample.

OSI Time. The evaluation of the oxidative stability was performed by using an eight-channel oxidative stability instrument (Omnion, Decatur, IL), according to Jebe et al. (*22*). The conductibility was measured in polycarbonate tubes using twice distilled water. The air flow was set at 120 mL/min. The OSI were run at 110 ± 0.1 °C. Results were expressed as induction time (h). Three replicates were analyzed per sample.

Determination of Peroxide Value (POV). This determination was performed according to the NGD C-35 official method published by Norme Grassi e Derivati (*23*). Three replicates were analyzed per sample.

Spectrophotometric Determination of *p*-Anisidine (PAV). This determination was performed according to the NGD C-36 official method published by Norme Grassi e Derivati (*23*). Three replicates were analyzed per sample.

Statistical Analysis. Means and standard deviations were calculated with SPSS (Version 13.0, SPSS Inc., Chicago, IL) statistical software. SPSS was used to perform one-way analysis of variance (ANOVA) and Tukey's honest significant difference test (HSD) at a 95% confidence level ($p < 0.05$) to identify differences among groups. Pearson correlation coefficients were calculated among the measured variables at a 95% confidence level ($p < 0.05$).

RESULTS AND DISCUSSION

Chemical Composition. Chemical composition and oxidative parameters of the extra virgin olive oil from *Biancolilla*, *Cerasuola*, and *Nocellara del Belice* are reported in **Table 1**. TAG represent about 96% of the extra virgin olive oil composition. Nine TAG were identified, and their content (expressed as percentages of total TAG) exhibited some variations among

Table 1. TAG, DAG, Total FA Compositions, FFA Content, OSI Time, POV, and PAV of the Monovarietal Extra Virgin Olive Oils at Three Harvesting Periods[a]

harvesting period	A			B			C		
cultivar	*Biancolilla*	*Cerasuola*	*Nocellara del Belice*	*Biancolilla*	*Cerasuola*	*Nocellara del Belice*	*Biancolilla*	*Cerasuola*	*Nocellara del Belice*
TAG (% of total TAG)									
PLL	n.d.[b]	0.1	n.d.	0.2	n.d.	n.d.	0.7 a	0.5 b	0.3 c
OLL	0.8 b	0.3 c	1.2 a	1.1 a	0.6 b	0.4 b	0.9 a	0.8 ab	0.8 b
POLn	0.6 b	0.4 c	0.7 a	0.9 a	0.4 b	n.d.	0.4 a	0.4 a	0.1 b
OOL	12.2 a	12.9 a	7.1 b	9.5 b	13.9 a	11.2 ab	11.7 a	12.5 a	11.8 a
POL	3.7 a	2.9 b	2.7 b	3.0 a	2.5 a	3.5 a	2.9 a	2.0 b	3.0 a
OOO	48.1 a	53.4 a	53.1 a	52.9 a	57.1 a	53.7 a	51.7 c	60.5 a	55.7 b
POO	32.8 a	27.6 b	33.2 a	30.9 a	24.8 b	29.9 a	30.2 a	22.2 c	27.0 b
PPO	1.0 a	0.4 b	0.7 a	0.8 a	0.4 c	0.7 ab	0.7 a	0.4 b	0.4 b
SOO	0.5 b	1.2 a	1.3 a	0.8 a	0.7 a	0.7 a	0.9 a	0.7 b	1.0 a
total DAG (g/100 g oil)	1.3 c	1.7 a	1.5 b	1.3 b	2.1 a	1.4 b	3.1 b	3.5 a	2.3 c
DAG (% of total DAG)									
1,2-PO	9.0 b	8.3 c	10.3 a	8.1 b	5.7 c	9.8 a	5.1 a	4.0 c	4.7 b
1,2-PL	7.7 a	6.6 b	7.9 a	8.9 a	8.7 a	7.6 b	11.9 a	10.2 b	10.6 b
1,2-OO	29.2 b	34.3 a	29.2 b	25.2 b	24.6 b	27.5 a	16.2 a	16.4 a	18.4 a
1,2-OL	29.5 b	31.0 a	27.7 c	32.6 b	38.5 a	28.0 c	42.3 a	43.1 a	42.0 a
1,3-OO	9.2 a	8.4 b	7.2 c	10.8 a	10.3 b	9.7 c	10.1 b	10.8 a	9.9 c
FA (%)									
palmitic acid	14.0 a	12.4 b	14.5 a	14.8 a	11.9 c	13.9 b	12.9 a	10.9 c	12.3 b
stearic acid	2.0 b	2.1 b	3.0 a	2.1 c	2.3 a	2.2 b	2.5 b	2.3 c	2.8 a
oleic acid	71.4 b	74.2 a	70.5 b	69.7 b	73.6 a	69.1 c	72.0 c	75.2 a	73.0 b
linoleic acid	7.86 b	8.2 a	6.4 c	8.1 c	8.7 a	8.4 b	7.5 b	8.0 a	7.5 b
linolenic acid	0.50 b	0.5 b	0.6 a	0.5 b	0.5 b	0.6 a	0.5 a	0.5 b	0.5 b
SFA	16.5 b	15.0 c	18.1 a	17.4 a	14.6 b	16.6 c	15.9 a	13.7 b	15.6 a
MUFA	75.1 b	76.3 a	74.8 b	73.6 b	76.1 a	74.4 b	76.0 c	77.7 a	76.4 b
PUFA	8.4 b	8.6 a	7.0 c	8.6 c	9.2 a	9.0 b	8.1 b	8.5 a	8.0 b
total FFA (g/100 g oil)	0.1 b	0.1 a	0.1 a	0.2 b	0.2 a	0.1 c	1.0 b	1.1 a	0.7 c
OSI (h)	5.1 c	12.4 b	25.4 a	2.8 c	5.9 a	4.2 b	1.7 c	2.4 b	3.4 a
POV (meq O_2/kg oil)	46.4 a	21.6 b	4.7 c	53.2 a	33.1 c	40.5 b	52.8 a	41.1 b	29.8 c
PAV	6.1 b	4.8 c	6.5 a	8.0 a	3.9 c	6.1 b	3.0 b	3.5 a	2.1 c

[a] Data are expressed as the mean of three determinations. RSD of TAG, DAG, FA, FFA, OSI, POV, and PAV are ≤8.0%, ≤4.5%, ≤2.5%, ≤3.0%, ≤3.5%, ≤4.5%, and ≤3.5%, respectively. Same letters in the same row at each harvesting period do not significantly differ ($p < 0.05$). [b] n.d., not detected.

the different monovarietal oils and harvesting periods. OOL, POL, OOO, and POO accounted for more than 96% of the total TAG, whereas PLL, OLL, POLn, PPO, and SOO were present in low percentages (**Table 1**). The highest percentages of OOL and OOO were detected in the *Cerasuola* cultivar, whereas POO was more present in *Nocellara del Belice*. OOO increased with increasing harvesting time in all cultivars, whereas OOL only rose in *Nocellara del Belice*. POO registered a decrease in all cultivars with increasing harvesting time and were significantly relevant in both *Cerasuola* and *Nocellara del Belice*.

DAG content of extra virgin olive oil ranged from 1.3 to 3.5% (**Table 1**), which is typical of extra virgin olive oils (*24, 25*). The highest total DAG content was found in *Cerasuola*, followed by *Nocellara del Belice* and *Biancolilla*. The DAG content of the three monovarietal extra virgin olive oils increased in oils obtained from more ripened olives, due to lysis of TAG. The lysis effect was more evident in all varieties at the last harvesting period, where the DAG content was twice as much as the one detected at the A harvesting period.

The concentrations of specific DAG varied among the monovarietal extra virgin olive oils as a function of olive harvesting time. DAG were mostly present as 1,2-DAG (mainly 1,2-OO and 1,2-OL). 1,2-PL, 1,2-OL, and 1,3-OO increased, whereas 1,2-PO and 1,2-OO decreased with increasing harvesting time in all cultivars. 1,2-DAG are naturally present in oils, due to the incomplete biosynthesis of triacylglycerols (*21, 24*), while 1,3-DAG derive mainly from TAG hydrolysis, with the consequent rise of FFA (*21*). The initial content of 1,3-DAG was below 10% of total DAG. 1,2-DAG present in fresh oil can isomerize to the more stable 1,3-DAG during storage (*25, 26*), as was observed in the three monovarietals oils tested in this study.

Oleic, palmitic, and linoleic acids represented 91–95% of the total FA (**Table 1**). *Nocellara del Belice* contained the largest amount of oleic and linoleic acids, whereas *Cerasuola* and *Biancolilla* had the highest content of palmitic acid. Upon ripening, palmitic acid decreased in all cultivars, oleic acid rose in *Cerasuola*, and linoleic acid significantly increased only in *Nocellara del Belice*. The percentage of the different fatty acid classes (according to their unsaturation degree) also varied among the monovarietal extra virgin olive oils and upon olive ripening.

The total FFA content of the monovarietal extra virgin olive oils ranged from 0.1 to 1.1% (**Table 1**). The initial content of FFA in the three cultivars was quite similar (<0.15%) and was below the limit set by the EC Regulation (*27*), but it tended to rise with increasing time to harvest (mainly in the *Cerasuola* cultivar). FFA increased in oils obtained from more ripened olives, due to the enzymatic lysis of TAG (*21*). The lysis effect in all varieties was more evident at the last harvesting period, where the FFA content was 6–10 times higher as compared with samples tested at the A harvesting period. FFA composition of the three monovarietal oils (data not shown) was slightly different from that of total FA and also varied depending on the olive cultivar and the harvesting time.

The oxidative status of the oils was assessed by OSI time, peroxide (POV), and *p*-anisidine (PAV) values. The oils

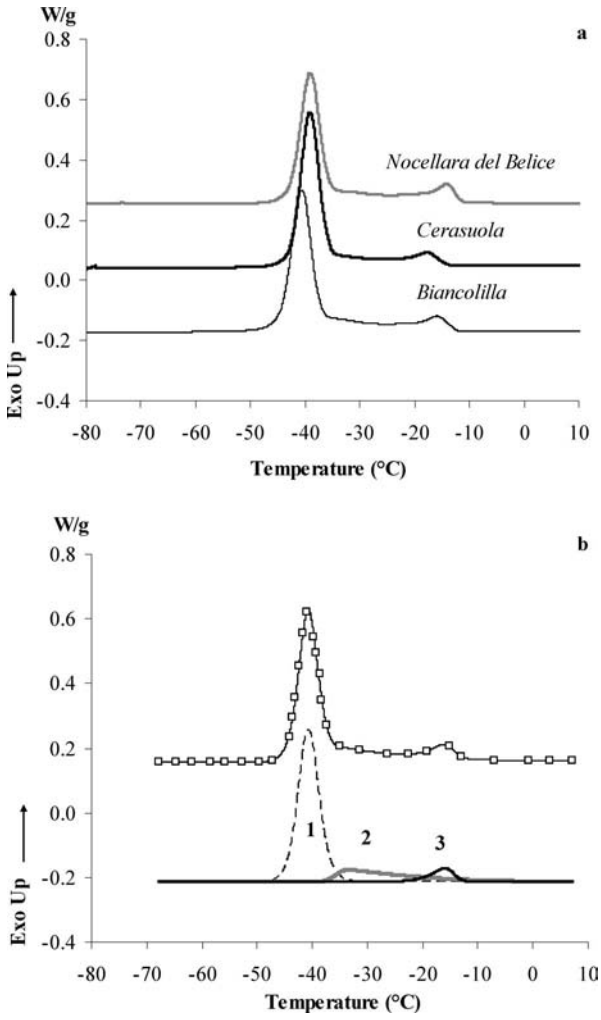

Figure 1. (**a**) Representative DSC cooling thermograms of the three monovarietal extra virgin olive oils; (**b**) deconvolution of the cooling thermogram of a *Biancolilla* sample at the C harvesting period; experimental data (□), fitted curve (solid line), and the constituent peaks are shown.

obtained with the *Nocellara del Belice* cultivars presented the highest OSI time (25 h) at the beginning of ripening, whereas the *Cerasuola* and the *Biancolilla* cultivars exhibited an OSI time that was 2 and 4 times lower, respectively. All the Sicilian olive oils displayed a decrease in OSI time upon olive ripening, which is related to a drop in olive phenol content and, consequently, to their overall antioxidant capacity (*3, 28*). This effect was more evident in the *Nocellara del Belice* cultivar, followed by *Cerasuola* and *Biancolilla* cultivars, which are known to have different phenol contents (*29*).

OSI data were further confirmed by POV and PAV results, which showed high concentrations of both primary and secondary lipid oxidation in the olive oil samples, probably as a consequence of one-year storage at room temperature. In fact, most POV data were noticeably higher than 20 meq O_2/kg of lipids, which is the regulation limit for extra virgin and virgin categories (*27*). Upon ripening and storage of the samples, POV concentrations decreased due to their decomposition into both volatile and nonvolatile secondary oxidation products. However, this POV decrease did not lead to a net PAV increase, since the latter does not measure volatile oxidation products.

Thermal Properties. Representative DSC cooling thermograms of the three monovarietal extra virgin olive oils selected in this study are shown in **Figure 1a**. The cooling curves of all samples had a similar line shape and exhibited exothermic transitions as the lipids crystallized. Lipid crystallization started around −11 °C and developed over a 35–40 degree range. Two well-defined events, peaking at −16 and −40 °C, were distinguishable, as previously observed in samples of olive oil (*8*). The characterizing thermal properties (enthalpy, T_{on} and range of the transition) were obtained for each sample and summarized in **Table 2**. Crystallization enthalpy was comparable in all samples considered within each cultivar at different harvesting periods or among cultivars at each crop season period. Differences were found in the crystallization temperature range as lipids in the *Cerasuola* samples began to crystallize at significantly lower temperatures than the *Biancolilla* and *Nocellara del Belice* varieties at all harvesting periods (**Table 2**). Moreover, *Cerasuola* samples crystallized in a significantly narrower range of temperatures in comparison to the other cultivars at harvesting periods A and B. At harvesting period C, lipid crystallization occurred over the same temperature range in *Cerasuola* and *Nocellara del Belice*, while the phase transition developed over a larger range of temperature in *Biancolilla*. The lower T_{on} of crystallization in *Cerasuola* samples may be related to the presence of molecules that either had lower melting points (i.e., unsaturated lipids) or retarded/hindered alignment of crystallizing molecules (e.g., free fatty acids and other polar molecules). This was indeed found in *Cerasuola* samples, whose compositional analysis (**Table 1**) indicated significantly higher quantities of oleic acid, linoleic acid, monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA) with respect to the other two cultivars. OOO and OOL (**Table 1**) were also detected in higher amounts in *Cerasuola*. Lower crystallization temperature onset (as analyzed by DSC) was previously reported for oils with higher degree of unsaturation (*11*), and a narrower range of crystallization was found in high oleic virgin olive oils (*13*).

Crystallization significantly shifted towards higher temperatures (T_{on}) and occurred over a larger temperature range as consequence of ripening (from A to C); this was more pronounced in *Biancolilla* and *Cerasuola* cultivars (**Table 2**), which suggested an increasing complexity of oil composition. A significant increase in FFA (8–9 times) and DAG (3–4 times), resulting from TAG lysis, were observed for all cultivars from A to C harvesting periods, particularly in *Biancolilla* and *Cerasuola* samples (**Table 1**). Moreover, lipid oxidation products (POV and PAV) were expected to be present in oils obtained from more ripened olives, as FFA markedly increased, leading to lower oil oxidative stability upon ripening (**Table 1**). The ability of the crystallizing molecules to get together and form a crystal may have been obstructed by the presence of FFA, DAG, and lipid oxidation products (POV and PAV) that, having polar and "irregular" conformational structure, may

Table 2. DSC Data Obtained from the Cooling Thermograms of the Monovarietal Extra Virgin Olive Oils at Three Harvesting Periods[a]

harvesting period	cultivar	ΔH (J/g)	T_{on} (°C)	range[b] (°C)
A	*Biancolilla*	66.7 a	−12.1 b	36.3 a
	Cerasuola	67.3 a	−13.6 b	32.5 b
	Nocellara del Belice	67.4 a	−10.9 a	35.5 a
B	*Biancolilla*	65.9 x	−11.2 x	37.6 x
	Cerasuola	66.3 x	−13.1 y	33.8 y
	Nocellara del Belice	67.6 x	−11.9 x	36.3 x
C	*Biancolilla*	68.1 A	−10.2 A	39.4 A
	Cerasuola	69.3 A	−10.9 B	35.0 B
	Nocellara del Belice	68.8 A	−10.1 A	35.6 B

[a] Data are expressed as mean of three determinations. RSD ≤ 6%. Same letters within each column at each harvesting period are not significantly different ($p < 0.05$). [b] Temperature difference between T_{on} and T_{off}.

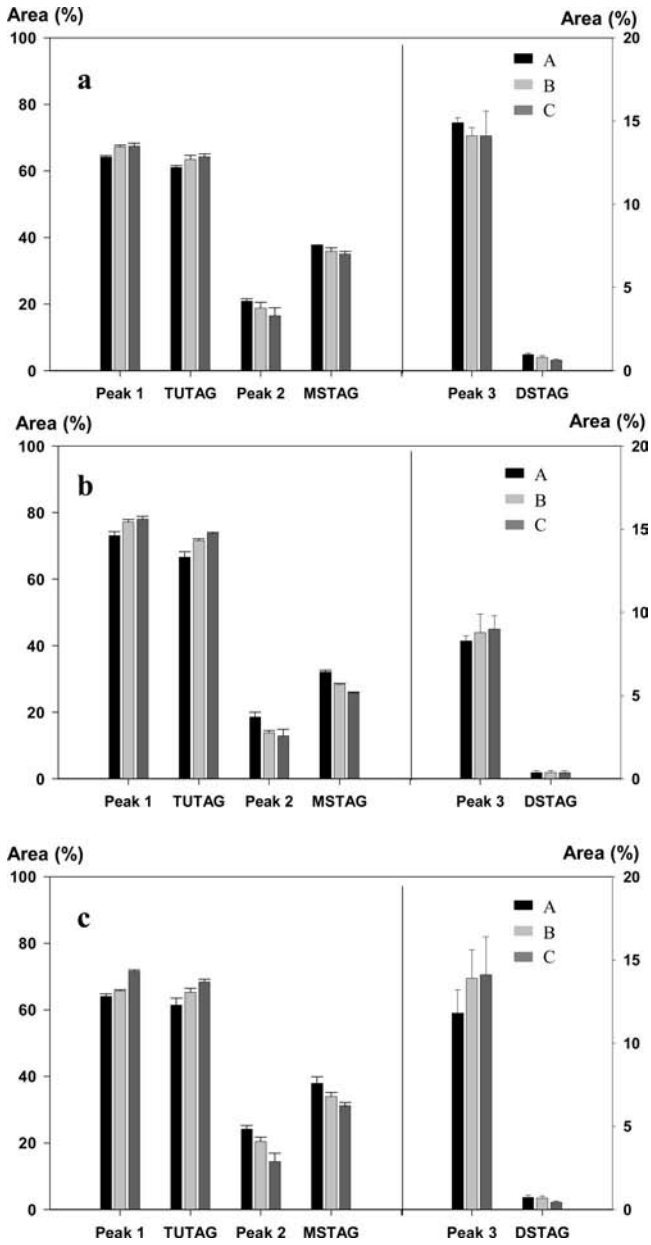

Figure 2. Comparison between percentage area of deconvoluted cooling peaks and TAG composition (DSTAG (PPO); MSTAG (PLL + POLn + POL + POO + SOO); TUTAG (OLL + OOL + OOO)) at A (black histogram), B (light grey histogram), and C (dark grey histogram) harvesting period for *Biancolilla* (**a**), *Cerasuola* (**b**) and *Nocellara del Belice* (**c**) cultivars. The *y*-axis for peak 3 and DSTAG comparison was expanded to indicate differences (right *y*-axis). Error bars represent +/−1 standard deviation, ($n = 3$).

have complicated and hindered the TAG crystallization process (*30*, *31*). The intermolecular bonding between TAG crystals may be weakened by other minor components not determined in this study, such as monoacylglycerols (MAG), which may have also hindered crystallization process due to their polarity and small dimensions. Shea nut oil with high amounts of hydrolyzed lipids was reported to crystallize at higher temperature, which was measured with an adapted Shukoff flask (*32*). Moreover, incorporation of DAG into the crystal lattice of TAG was reported to retard and alter the orientation of molecules at the crystal surfaces, resulting in more irregular crystals (*33*, *34*). DAG were previously reported to influence the TAG crystallization by either accelerating or delaying crystallization in palm, coconut oils, and other

Table 3. Deconvolution Parameters of Cooling Thermograms of the Monovarietal Extra Virgin Olive Oils at Three Harvesting Periods[a]

peak[b]	cultivar	T_{on} (°C)	T_p (°C)	T_{off} (°C)	range[c] (°C)
		Harvesting Period A			
1	*Biancolilla*	−30.3 a	−40.7 b	−48.3 b	18.0 a
	Cerasuola	−31.4 b	−39.3 ab	−46.9 a	15.2 b
	Nocellara del Belice	−30.9 ab	−37.4 a	−48.7 b	17.8 ab
2	*Biancolilla*	−0.9 a	−32.8 a	−40.1 a	38.2 a
	Cerasuola	−1.2 a	−33.9 b	−40.5 a	39.1 a
	Nocellara del Belice	−1.1 a	−33.2 a	−40.8 a	40.2 a
3	*Biancolilla*	−9.2 a	−13.4 a	−35.6 b	25.5 a
	Cerasuola	−11.6 b	−17.3 b	−38.3 ab	26.7 a
	Nocellara del Belice	−10.4 ab	−15.3 ab	−32.7 a	22.4 b
		Harvesting Period B			
1	*Biancolilla*	−31.4 x	−41.1 y	−49.7 y	18.3 x
	Cerasuola	−33.6 y	−39.5 x	−47.6 x	14.6 y
	Nocellara del Belice	−31.9 x	−40.6 xy	−49.6 y	18.4 x
2	*Biancolilla*	−2.7 y	−33.9 x	−43.3 y	39.8 x
	Cerasuola	−2.0 x	−32.9 x	−38.3 x	36.9 y
	Nocellara del Belice	−1.9 x	−33.3 x	−40.1 xy	38.2 xy
3	*Biancolilla*	−9.8 x	−13.3 x	−33.5 x	23.9 x
	Cerasuola	−11.7 y	−16.4 y	−34.9 x	24.4 x
	Nocellara del Belice	−9.8 x	−14.8 xy	−33.6 x	25.5 x
		Harvesting Period C			
1	*Biancolilla*	−31.8 A	−41.1 C	−52.1 B	20.5 A
	Cerasuola	−33.4 B	−38.9 B	−47.3 A	13.9 B
	Nocellara del Belice	−32.5 AB	−38.4 A	−50.5 B	18.3 AB
2	*Biancolilla*	−2.3 A	−34.9 B	−43.3 B	40.4 A
	Cerasuola	−1.9 A	−32.3 A	−36.7 A	34.9 B
	Nocellara del Belice	−2.3 A	−33.7 A	−41.9 B	39.4 A
3	*Biancolilla*	−8.5 A	−11.9 A	−42.4 A	34.6 B
	Cerasuola	−8.5 A	−14.0 B	−47.8 C	39.3 A
	Nocellara del Belice	−8.0 A	−12.9 AB	−44.6 B	35.6 B

[a] Data are expressed as mean of three determinations. RSD ≤ 6%. Same letters within each column for single peak at harvesting period stage are not significantly different ($p < 0.05$). [b] See **Figure 1** for peak number assignation. [c] Temperature difference between T_{on} and T_{off}.

fats (*34*–*36*). The nature of FA present in glycerol molecule was reported to also influence crystallization of palm oil (*34*, *35*). Among DAG, 1,2-PL, 1,2-OL, and 1,3-OO underwent the largest increase during ripening in all the three cultivars. These DAG may have largely influenced the crystallization process of *Biancolilla* and *Cerasuola*, by inducing a marked shift of the crystallization process towards higher temperatures during ripening (**Table 2**). However, no data were found in the literature on the DAG influence on the crystallization of olive oil to support this hypothesis.

Deconvolution of Cooling Thermograms. The cooling transitions were further characterized by deconvolution into their constituent peaks to better describe the complex nature of the crystallization process in an attempt to separate and characterize overlapping transitions. To the authors' knowledge, deconvolution of thermograms has not previously been applied to DSC oil and fat analysis.

The thermograms of all samples were best deconvoluted using three peaks, as shown in **Figure 1b**, where experimental data, fitted curves, and constituent peaks are shown for a representative curve ($R^2 \geq 0.98$ for all fitted curves). The three peaks identified were consecutively numbered, starting from the lowest

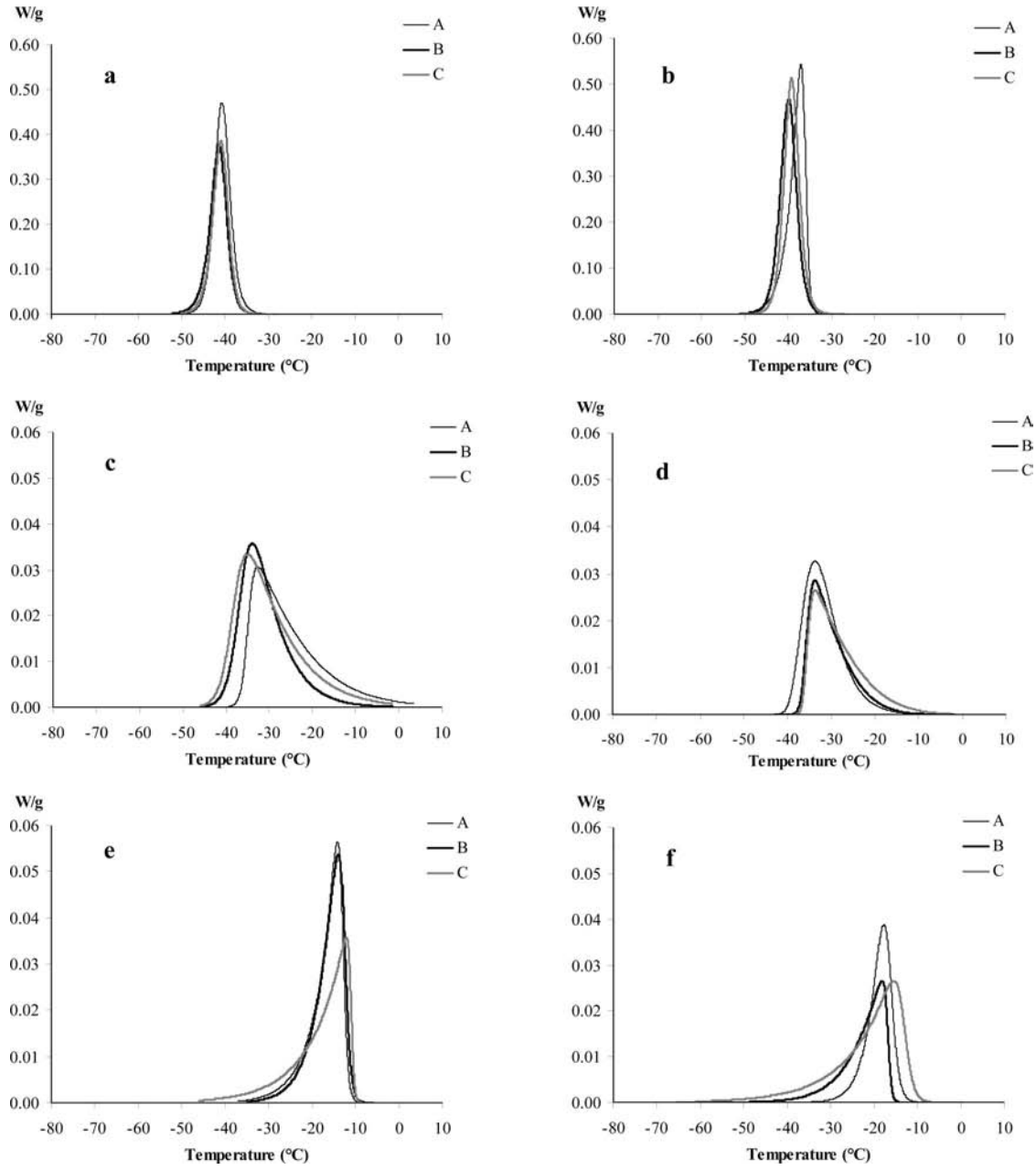

Figure 3. Deconvoluted peak profiles of cooling thermograms at the A (dark solid line), B (dark bold line), and C (grey bold line) harvesting period: (**a**) peak 1 *Biancolilla*; (**b**) peak 1 *Cerasuola*; (**c**) peak 2 *Biancolilla*; (**d**) peak 2 *Cerasuola*; (**e**) peak 3 *Biancolilla*; (**f**) peak 3 *Cerasuola*.

to the highest temperature and named as peaks 1, 2, and 3, respectively. Peak 1 was an asymmetric double Gaussian, while peaks 2 and 3 were asymmetric double sigmoid functions. The predominant peak (peak 1) was characterized by a quite symmetrical line shape, and a narrow profile as the transition developed over a limited temperature range, indicating a highly cooperative transition and homogeneity of crystallizing molecules. Peaks 2 and 3 had more complex, asymmetrical line shape, suggesting a more heterogeneous crystallization process. In particular, the peak 2 maximum was skewed towards higher and peak 3 maximum towards lower temperatures than mid-range temperature.

Peak 1 was probably associated with the crystallization of the triunsaturated triacylglycerols (TUTAG), as they are known to represent the majority of the oil (**Table 1**) and to crystallize at lower temperatures. The oil fractions crystallizing at higher temperatures should be likely associated with more saturated TAG, and, therefore, peaks 2 and 3 were attributed to monosaturated (MSTAG) and disaturated triacylglycerols (DSTAG), respectively. Similarly, Tan and Che Man (*11*) had previously associated the major exothermic event observed in cooling thermograms of vegetable oils to the crystallization of highly unsaturated TAG and the others thermal events to the crystallization of more saturated TAG fractions. In particular, they attributed the major exothermic event in olive oil to the cocrystallization of OOO, POO, and OOL (*8*). Other minor components present in the oils (FFA, DAG, and lipid oxidation products) are expected to influence the crystallization process of the more abundant TAG, resulting in both shifted transition temperatures and more complex and asymmetrical lineshapes. Although a limited number of samples was considered, good agreement was found between the percent peak area (from deconvolution analysis) and percent TAG during ripening, as shown in **Figure 2** for *Biancolilla* (**Figure 2a**), *Cerasuola* (**Figure 2b**), and *Nocellara del Belice* (**Figure 2c**). In particular, peaks 1 and 2 correlated well with TUTAG ($R = 0.96$; $p \leq$

0.01) and MSTAG ($R = 0.87$; $p \leq 0.01$), respectively. However, the correlation between percent peak 3 area and DSTAG was not completely established ($R = 0.73$; $p \leq 0.05$). To confirm these correlations, a larger number of samples should be evaluated. TAG area percentages obtained by means of either DSC or HPLC techniques were slightly different, as expected, because of the different analytical approaches of the two techniques. DSC was carried out on the whole samples, and areas were quantified after mathematical deconvolution of the peak, while HPLC analysis leads to the separation and quantification of the different chemical species. The presence of other minor chemical components, such as DAG and FFA and lipid oxidation products which can interfere with the crystallization process of TAG, may have influenced the transition by affecting the size of the deconvoluted peaks.

Cerasuola samples showed the largest area (%) of peak 1 and the lowest of peak 2, which seems to be related to TUTAG and MSTAG distribution. In particular, area (%) of peaks 1 and 2 reflected OOO and POO percentages, respectively, which were the two most representative TAG in all samples. During ripening of the sample, a significant increase in peak 1 area was observed in all cultivars, which may reflect the TUTAG rise. This trend was more evident from A to B harvesting periods in *Biancolilla* and *Cerasuola* and from A to C in *Nocellara del Belice*, following the increase in OOO (**Table 1**). Peak 2 areas showed a sharp decrease as observed for MSTAG, more markedly in *Nocellara del Belice* upon ripening, reflecting the POO profile (**Table 1**).

Deconvoluted peaks were more extensively studied by means of other parameters, such as initial (T_{on}) and final (T_{off}) peak temperatures, as well as the peak temperature (T_p) and the temperature transition range (**Table 3**). Deconvoluted peaks of *Cerasuola* and *Biancolilla* samples at different harvesting periods were shown in **Figure 3** to evaluate differences in peak profiles. The deconvoluted peaks for the *Nocellara del Belice* sample were not shown because they closely resembled the *Biancolilla* results.

Peak 1 of *Cerasuola* showed significantly lower T_{on} and higher T_{off} than the other two cultivars at all harvesting periods, corresponding to a narrower peak profile (significantly lower peak temperature range). This may be ascribable to the presence of a higher amount of unsaturated components with lower crystallization temperatures (larger presence of OOO, OOL, and PUFA) for *Cerasuola*, as compared to *Biancolilla* and *Nocellara del Belice* (**Table 1**). Peak 1 also shifted toward lower temperatures for all cultivars during ripening. Such a shift was more marked from A to B in *Biancolilla* and *Cerasuola* (**Table 3**) and from A to C harvesting periods in *Nocellara del Belice*, following the OOO increase (**Table 1**).

Peak 2 was similar in the three oils at harvesting periods A with T_{on} at about -1.5 /-2.0 °C and T_{off} at about -40 °C. Upon ripening, peak 2 shifted towards lower temperatures at harvesting periods B and C in *Biancolilla* and *Nocellara del Belice*, respectively (**Table 3**). Peak 2 of *Cerasuola* did not significantly shift in onset temperature upon ripening, but it narrowed, particularly at harvesting period C (**Table 3**). Peak 2 was tentatively attributed to MSTAG, but multiple events may have contributed to such crystallization behavior, including the presence of a variety of crystallizing species and process kinetics.

Peak 3, characteristic of the higher temperature crystallizing molecules, developed over a larger temperature range (lower T_{off}) in *Cerasuola*, as compared to the other cultivars and became wider in all samples upon ripening. A role may be played by the presence of DAG, MAG, FFA originated from TAG lyses and lipid oxidation products (**Table 1**).

The results of this study showed that DSC cooling thermograms are affected by chemical composition of extra virgin olive oil. Some thermal properties (crystallization peak onset and temperature range) were influenced not only by major (TAG and FA) but, probably, also by minor components (FFA, DAG, and primary and secondary oxidation products). On the contrary, the total enthalpy of crystallization did not discriminate among oils with dissimilar chemical composition (e.g., from drupes of different cultivars and/or harvesting period). The presence of minor components in extra virgin olive oil may have also influenced the lineshape of the deconvoluted peaks.

These first findings must be confirmed by the analysis of a larger number of extra virgin olive oils accounting for the variability of chemical composition originated by olive cultivar, geographical origin, seasonality, and agronomical and technological oil production conditions.

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