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Study of the influence of triacylglycerol composition on DSC cooling curves of extra virgin olive oil by chemometric data processing

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Abstract In this work, a coupled high-pressure liquid chromatography-differential scanning calorimetry-partial least square (HPLC–DSC–PLS) procedure was applied to clarify the influence of triacylglycerol composition on the shape of cooling curves for extra virgin olive oil (EVOo), as chemometric processing of digitized DSC curves was previously reported to be an attractive alternative to the application of the common procedure of parameter extrapolation for the analysis of thermal transition. 69 samples of EVOo were analysed to obtain triacylglycerol (TAG) composition by means of HPLC and DSC cooling profiles. Results obtained by the application of PLS algorithm on TAG concentration (%) and digitized DSC curves showed that cooling transitions were markedly influenced by OOO, OLO and OOP + SLO that are the most representative TAG for EVOo. Other TAG as LLP + OLnO, LLL + LLPo and POPo developed good PLS models, appearing to influence EVOo cooling curve, although less markedly than the further. Otherwise, the other identified TAG did not render appropriate models. Finally, grouping TAG according to different unsaturation degree, high correlation coefficients (>0.80) and low relative standard deviations (<11 %) were found for sum of tri-unsaturated triacylglycerols in both calibration and validation sets. Starting from these encouraging results, this new and fast coupled approach may be applied to a wider set of EVOo samples to tentatively discriminate among oils according to different geographical and/or botanical origins taking into account relation established among TAG and cooling curves.

Keywords Extra virgin olive oil · DSC cooling curves · Partial least square regression · Triacylglycerols

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Introduction

Olive oils represent the main source of dietary fats in the countries of the Mediterranean area where their production is mainly concentrated [1]. Among them, extra virgin olive oil (EVOo) is highly appreciated as its consume has been associated with health benefits, such as a reduced risk of cardiovascular mortality, cancer frequency and incidence of Parkinson and Alzheimer disease, leading to an improved life quality [1]. EVOo is mainly composed (>98 %) of triacylglycerols (TAG), and other minor constituents of a wide range of chemical nature. In this high quality vegetable oil, TAG profiles are frequently employed for the search of the geographical and botanical origins as well as for the authenticity [2, 3].

Differential scanning calorimetry (DSC) is a thermoanalytical technique widely employed for the characterization of phase transitions of fats and vegetable oils. It is a very

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well-established method with several advantages for its application, as it does not require chemical treatments or time-consuming and hazardous manipulation practices before each measurement. Different vegetable oils showed complex thermal profiles that are mainly due to the great variety of TAG as their principal constituents, as well as to their natural polymorphism [4]. In fact, the positional distribution of the three fatty acids in TAG affects their physical properties such as crystal structure, solubility, viscosity and melting point [5].

The nature of the relation among TAG composition and thermal properties extracted by DSC profiles was studied in the past for vegetable oils [6, 7], and more recently also evaluated in EVOo [8], taking mainly into account transition upon cooling. In previous works, this relation was statistically evaluated by means of Pearson correlations, also applying deconvolution analysis on cooling curves to resolve overlapping transitions [8, 9]. The area of the three exothermic peaks obtained by deconvoluting overlapping cooling transition of EVOo was found to be correlated to TAG groups, showing high correlations mainly with triunsaturated (TUTAG) and also with monosaturated (MSTAG), while no correlation was established with disaturated (DSTAG) triacylglycerols [8]. More recently, Chiavaro et al. [9] completed this preliminary research evaluating correlations among main thermal parameters, extracted by both whole cooling profiles and the deconvoluted peaks, and single TAG species and groups in thirteen sample Italian EVOo samples. The authors found that cooling transition was influenced by OOO and some other TAG containing oleic acid moiety and quantified as pairs (LLP + OLnO and OLP + OOPo), as well as TUTAG, too [9].

Commonly, oil heating or cooling DSC curves were studied aiming at extrapolating from them several thermal properties (enthalpy as well as peak onset, offset temperatures along with the corresponding range of transition), but another interesting approach to the analysis of transition profiles consists in a chemometric processing of digitized DSC curves. This approach seems to be an attractive alternative to the application of a common DSC procedure, merging the advantages obtained from multivariate statistical calibration with higher procedure quickness, as no extrapolation of data by curves had to be applied [10].

Application examples for this procedure generally lacked in food literature [11, 12], although the combination of chemometric analysis with several chemical and physical techniques is nowadays fully recognized [13]. In particular, Cerretani et al. [14] proposed a chemometric approach based on partial least square (PLS) methodology coupled with unfolded DSC data for olive oil to quantify fatty acids, finding results statistically similar to traditional official procedures. In a more recent work, the same authors have explored the opportunity to adopt another chemometric procedure (principal component analysis, PCA) on entire DSC profiles to perform an accurate measurement of EVOo thermal stress degree [15]. Thus, starting from these previous researches, the goal of this work was to develop and validate a faster analytical method based on a HPLC–DSC–PLS procedure on a large set of EVOo samples from different Mediterranean area in order to add new information about the influence of TAG composition on cooling profiles for this vegetable oil with the final aim to enforce the application of DSC in the field of quality evaluation.

Experimental

Samples

A total of 69 EVOo samples was analysed and they differed in terms of cultivar, ripening degree, area of growth and extraction system (type, productive capacity and manufacturer). 43 of them came from seven Italian administrative regions (Emilia Romagna, Tuscany, Marche, Abruzzo, Apulia, Basilicata and Sicily from North to South of Italy, respectively) while the other 26 were from different Mediterranean areas of production (Iberian and Balkan Peninsulas, North Africa). Samples were chosen taking into account the difference existing in TAG composition among oils from different Mediterranean area of production. All data were collected through the years from samples obtained from olives hand-picked in different seasons from 2007–2008 to 2009–2010 and analysed within ten months from production. Samples were all stored in dark bottles without headspace at room temperature before analyses.

TAG analysis

TAG were analysed with an HPLC Agilent 1100 series (Agilent Technologies, Palo Alto, CA, USA), coupled to both diode-array (DAD) and mass spectrometer (MSD) detectors, as previously described [16], after dissolving the oil at 3 % in a mixture of 2-propanol:*n*-hexane:acetonitrile (2:1:2, v/v/v). MS detection was performed using an atmospheric pressure chemical ionization (APCI) interface in the positive mode. TAG were identified based on their UV–Vis and mass spectra, as well as on literature data [17]. The following TAG were identified: LLL, trilinolein; LLP, dilinoleoyl-palmitoyl-glycerol; LLPo, dilinoleoyl-palmitoleoyl-glycerol; OLL, dilinoleoyl-oleoyl-glycerol; OLLn, linolenoyl-oleoyl- linoleoyl-glycerol; OLO, dioleoyl-linoleoyl-glycerol; OLnO, dioleoyl-linolenoyl-glycerol; OLP, palmitoyl-oleoyl-linoleoyl-glycerol; OLPo, palmitoleoyl-oleoyl-linoleoyl-glycerol; OOO, triolein; OOP, dioleoyl-palmitoyl-glycerol; OOPo, dioleoyl-palmitoleoyl-glycerol; POP, dipalmitoyl-oleoyl-glycerol; POPo, palmitoyl-palmitoleoyl-oleoyl-glycerol; SLO, stearoyl-oleoyl-linoleoyl-glycerol; SOO,

dioleoyl-stearoyl-glycerol; SOP, palmitoyl-stearoyl-oleoyl-glycerol. TAG were also grouped according to the type of fatty acid (FA) bonded to the glycerol structure, as disaturated (DSTAG), monosaturated (MSTAG) and triunsaturated triacylglycerols (TUTAG). Triplicate analyses were carried out per sample.

DSC analysis

DSC analysis was performed as previously reported [18]. Samples of oil (8–10 mg) were weighed in aluminium pans, covers were sealed into place and analysed with a DSC Q100 (TA Instruments, New Castle, DE). The melting temperatures of indium and *n*-dodecane (156.6 and −9.65 °C, respectively) and their enthalpies of fusion (28.45 and 216.73 J g^{-1}, respectively) were used to calibrate the instrument, and an empty pan was used as reference. Oil samples were equilibrated at 30 °C for 5 min, cooled to −80 °C at a rate of 2 °C min^{-1}, equilibrated at −80 °C for 3 min and then re-heated to 30 °C at a rate of 2 °C min^{-1}. Dry nitrogen was purged in the DSC cell at 50 cm^3 min^{-1}. DSC cooling curves were analysed with Universal Analysis Software (Version 3.9A, TA Instruments) in order to be exported in an ASCII compatible format. Triplicate analyses were carried out per sample.

Statistical data analysis

TAG and TAG groups were individually analysed by PLS algorithm in order to find an analytical correlation between DSC data and its HPLC concentration (%). PLS models were computed by MVC1 routines written for MATLAB (MathWorks Inc., Natick, MA, USA) [19]. Optimal PLS-factor number for each model was established by Haaland and Thomas criteria [20]. The best temperature range for PLS calibration was reached using mobile window of variable size strategy [21], also implemented by MVC1 toolbox. Calibration and validation sets of samples were selected from the original set of samples for each TAG, using Kennard & Stone algorithm for each PLS model. PLS was run on digitally pretreated data by mean centre (MC), minimum–maximum normalization (MM), smoothing (SM), derivative (D′), multiplicative scatter correction (MSC), as needed (Tables 2, 3). Statistical analyses and data plots were performed with OrigingPro8 (OriginLab, Northampton, MA, USA).

Results and discussion

TAG and DSC analyses of samples

Table 1 reports TAG data for the set of EVOo samples used in the PLS calibration. Six different fatty acid moieties were presented in the measured TAG chain (P, palmitic, 16:0; Po, palmitoleic, 18:1; S, stearic, 18:0; O, oleic, 18:1; L, linoleic, 18:2; Ln, linolenic, 18:3). Twelve TAG were identified in all samples; seven of them were separately quantified (OLLn, OLO, POPo, OOO, POP, SOO and SOP) and the others were quantified as pairs (LLL + LLPo, OLL + OLPo, LLP + OLnO, OLP + OOPo and OOP + SLO). For all samples, about 80 % of the total TAG was represented by OLL + OLPo, OLO, OLP + OOPo, OOO and OOP + SLO (Table 1), whereas SOP was present in the lowest percentage. If TAG are grouped according to the type of FA bonded to the glycerol structure, it can be observed that TUTAG represents the most important fraction in EVOo, as a consequence of the high content of OOO and OLO. MSTAG and DSTAG are present in lower percentage, particularly the latter.

TAG data are in an agreement with those reported in literature for samples of the same geographical provenience [22, 23], but they revealed that their profiles were quite dissimilar among samples. In the same way, large data ranges were exhibited not only by single TAG (for example OOO) but also by their sum. As it is well-known, these differences are related to several factors as botanical origin, geographical provenience as well as to agronomical practices, harvesting periods and processing technologies [1], making it difficult to find a mean TAG composition for this vegetable oil in literature. In this sense, data presented in this study could be considered as one of the first efforts to give ranges of TAG composition for samples from the different Mediterranean areas.

Only cooling curves were considered in the present study and they were all shown in Fig. 1 (insert a) for the analysed samples. This choice was made considering that

Table 1 Main statistical parameters obtained for TAG in all EVOo samples

TAG/ %	Minimum	Maximum	Median	Mean	SD
LLL + LLPo	0.90	4.01	2.10	2.31	0.77
OLLn	0.39	1.32	0.93	0.89	0.26
OLL + OLPo	8.60	15.26	11.70	11.70	1.54
LLP + OLnO	2.31	12.78	4.70	6.05	3.19
OLO	14.37	25.20	20.04	19.54	2.69
OLP + OOPo	10.40	18.05	13.93	14.10	2.04
POPo	0.79	7.65	2.73	3.31	1.84
OOO	11.92	30.59	22.87	21.75	5.04
OOP + SLO	9.70	19.35	14.10	14.11	2.21
POP	0.77	3.85	2.30	2.44	0.71
SOO	0.34	5.10	2.85	2.79	1.20
SOP	0.30	2.60	0.80	1.02	0.60
DSTAG	1.66	6.67	3.30	3.71	1.33
MSTAG	28.55	54.29	37.86	40.09	6.54
TUTAG	39.69	69.49	58.42	56.19	7.39

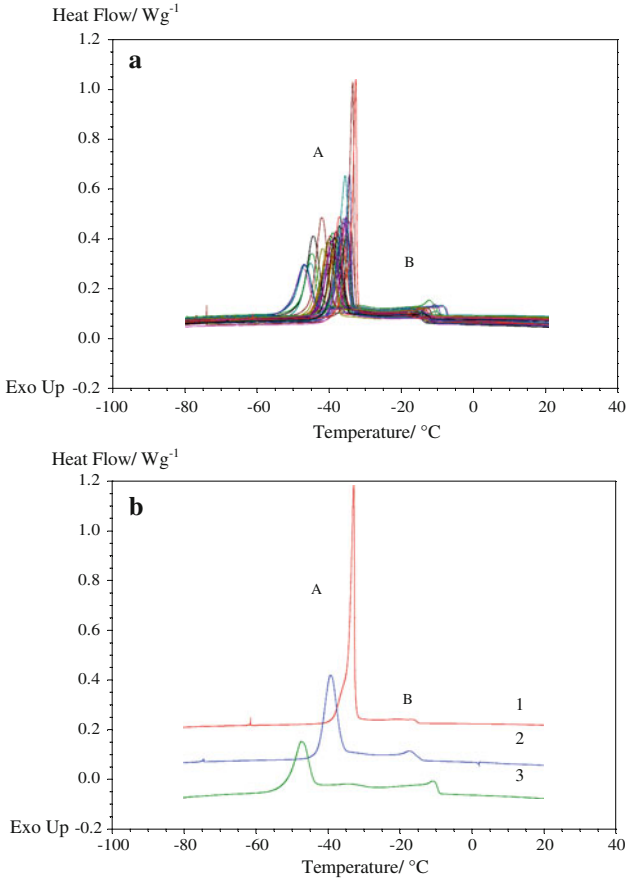

Fig. 1 **a** DSC cooling curve obtained from overall set of oil samples. **b** Representative curves for samples from Apulia (Italy), (*red colour*, number 1), Balkan Peninsula, (*blue colour*, number 2) and North Africa, (*green colour*, number 3) were also shown in (**b**). (Color figure online)

crystallization transitions were well-known to be more interpretable than those obtained upon heating, where melting-re-crystallisation phenomena, named polymorphism, could occur for the original oil crystals [4].

All oil samples showed a distinctive cooling profile for this type of vegetable oil, as previously reported in literature [8, 9, 15, 22], with two well-defined exothermic events, the major (A of Fig. 1a) peaking at approximately −40 °C and the minor (B) with maximum at higher temperature (about −13 °C). Differences about peak maximum and shapes were previously attributed to the difference in fatty acid composition and/or initial oxidative status for this oil [9, 15], as shown if Fig. 1b where three quite different cooling curves were reported. These differences were also related to TAG composition, as the Italian sample, indicated as number 1 in Fig. 1b, showed the highest TUTAG amounts (about 66.3 %) in comparison with samples from Balkan peninsula (number 2, Fig. 1b) and North Africa (number 3, Fig. 1b) that presented intermediate (59.5 %) and the lowest (43.5 %) TUTAG values, respectively. The opposite was for DSTAG amounts (2.5, 3.3 and 5.2 % for Italian, Balkan Peninsula and North Africa oils, respectively).

Chemometric analysis results

Starting from preliminary encouraging results [8, 9], we have applied a more powerful multivariate statistical procedure, as PLS, on the whole DSC cooling curve to deepen the evaluation and add new analytical information on the influence of TAG on EVOo crystallization profiles. To achieve this goal, several multivariate calibration models were built using the PLS regression.

Table 2 Calibration and validation data obtained for single TAG

	LLP + OLnO	OOP + SLO	OOO	OLO	LLL + LLPo	POPo
Statistical summary						
PLS factors	4	11	12	8	6	12
Temperature range/°C	−54.6 to 30.0	−67.0 to 30.0	−48.4 to −6.2	−49.6 to 30.0	−54.6 to 30.0	−48.4 to −6.21
Optimal pretreatment	MC	MC	MC	MC	MC	MC
RMSD/% in EVOo	0.99	1.22	1.30	1.59	0.36	0.47
REC/%	21.29	8.73	5.42	7.72	17.42	20.51
R^2	0.85	0.83	0.83	0.75	0.75	0.70
Figures of merit						
Sensitivity	0.48	0.08	0.03	0.11	0.59	0.11
Analytical sensitivity	3.30	2.20	2.00	1.80	5.60	7.90
Selectivity based on total signal	0.72	0.27	0.05	1.00	0.36	0.10
Mean signal residue/°C	0.09	0.03	0.02	0.07	0.09	0.01
External validation						
Mean recovery/%	102.12	101.38	100.29	100.86	99.32	107.71
SD/%	18.76	10.31	7.46	7.84	18.63	21.62

R*MSD* root mean square deviation, *REC* percentage of relative error in calibration, *SD* standard deviation

First, single TAG evaluation was attempted. However, some TAG were paired for the calculus due to its no complete HPLC resolution (LLL + LLPo, OLL + OLPo, LLP + OLnO, OLP + OOPo and SLO + POO) while the rest (OLLn, OLO, POPo, OOO, POP, SOO and SOP) was individually utilized.

MC, MM, SM, D′ and MSC were tried as pre-treatments for each developed PLS model, establishing that MC was the best one for all models. PLS models parameters for LLP + OLnO, OOP + SLO, OOO, OLO, LLL + LLPo and POPo are listed Table 2. Other identified TAG did not render appropriate models. Optimal numbers of PLS factor were individually found for each model applying the Haaland and Thomas statistical criterion ($\alpha = 0.75$) [20].

PLS models for internal validation of single TAG were carried out by evaluation of figure of merits (Table 2), including the RMSD (root mean square deviation) and REC (percentage of relative error in calibration), whose values were into acceptable limits (below 2 and 20 %). Additionally, coefficients of correlation (R^2) were above 0.70 for all models. As a next step, functional model external validation was performed, using an independent set of samples (selected prior to calibration from the initial set using Kennard-Stone algorithm). The results obtained

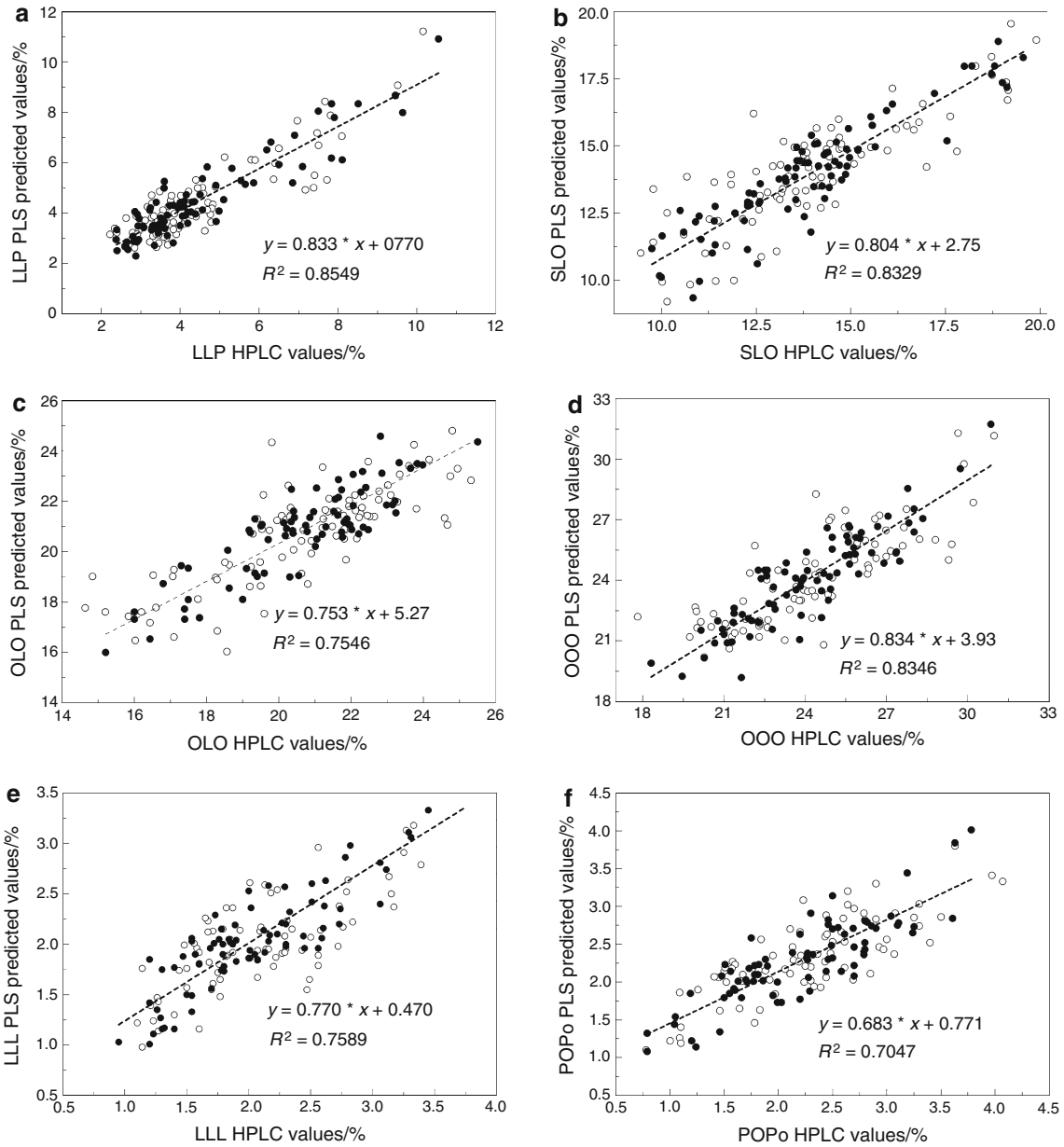

Fig. 2 Percentage values calculated by HPLC–DAD-MS vs. PLS calculated in calibration (*black circle*) and validation (*white circle*) sets for individual TAG (LLP + OLnO, a; OOP + SLO, b; OLO, c; OOO, d; LLL + LLPo, e and POPo, f)

Table 3 Calibration and validation data obtained for DSTAG, MSTAG and TUTAG

	TUTAG	MSTAG	DSTAG
Statistical summary			
PLS factor	7	8	10
Temperature range/°C	−49.7 to 30.0	−60.8 to 30.0	−67.0 to 30.0
Optimal pretreatment	MC	MC	MM
RMSD/% in EVOo	2.29	2.32	0.57
REC/%	5.08	6.18	19.13
R^2	0.92	0.72	0.49
Figures of merit			
Sensitivity	0.01	0.04	0.17
Analytical sensitivity	0.78	0.93	9.60
Selectivity based on total signal	0.31	0.26	0.10
Mean signal residue/ W g^{-1}	0.01	0.04	0.02
External validation			
Mean recovery/%	101.22	100.96	105.20
SD/%	6.53	7.16	21.05

RMSD root mean square deviation, *REC* percentage of relative error in calibration, *SD* standard deviation

exhibited two different ranges of errors. The first group (OOP + SLO, OOO and OLO) exhibited an error below 15 %, while in the second one (LLP + OLnO, LLL + LLPo and POPo) stands at about 20 %. Supporting these results, some of the TAG of both groups (OOO, LLP + OLnO, LLL + LLPo and POPo) were previously found to be correlated to thermal properties of cooling and deconvoluted peaks extracted [9].

Figure 2 shows plots of HPLC values of TAG versus PLS predicted values for LLP + OLnO, OOP + SLO, OOO, OLO, LLL + LLPo and POPo. The developed PLS models showed good performance, being slope and intercept of actual vs predicted regression line close to 1 and 0, respectively.

As second step, TAG grouped according to the type of fatty acid (FA) bonded to the glycerol structure (DSTAG, MSTAG and TUTAG) were evaluated by PLS models. In Table 3, statistical parameters obtained for DSTAG, MSTAG and TUTAG are reported. As for single TAG, PLS model parameters were analysed to carry out the internal validation. Low RMSD and REC values were found for TUTAG showing the goodness of fit of the

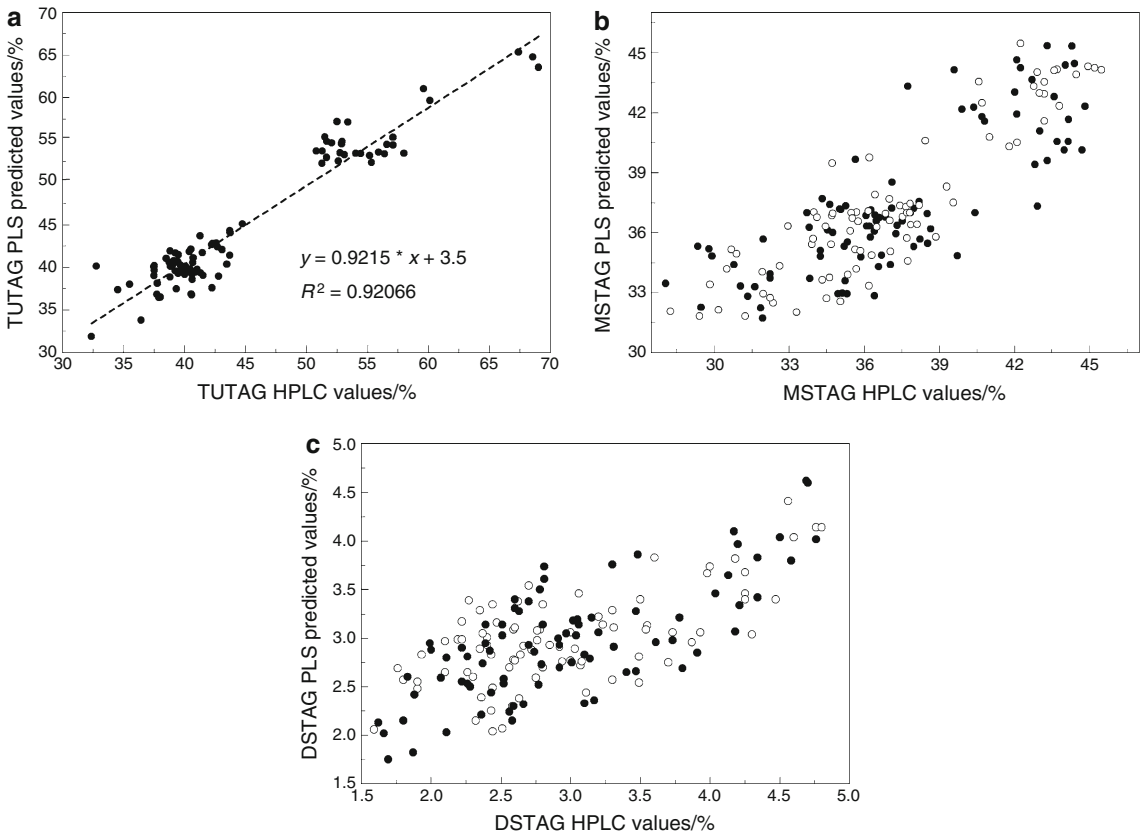

Fig. 3 Percentage values calculated by HPLC–DAD-MS vs. PLS predicted values in the calibration (*black circle*) and validation (*white circle*) sets for **a** TUTAG, **b** MSTAG and **c** DSTAG

calibration data. In addition, R^2 value of 0.92 obtained for TUTAG showed the goodness of fit between PLS predicted concentrations and their actual values.

During the external validation, TUTAG exhibited nearly quantitative recoveries (101 %) and relative standard deviation below 10 %. The yields obtained are illustrated in Fig. 3, part a. The slope and intercept of the regression line depicted in this plot are close to 1 and 0, respectively, indicating low bias and the absence of systematic regression errors. As observed in Fig. 3, PLS predictions for TUTAG were into acceptable parameters. This is related to the large influence of OOO on DSC profile, which is the main TAG in EVOo. In agreement with this observation, OOO and TUTAG were previously found to influence thermal properties of the main exothermic peak (peak A, Fig. 1) of cooling profiles also after deconvolution analysis [9].

On the other hand, MSTAG and DSTAG show a big dispersion for its predicted values (Fig. 3, part b and c). The slope and intercept of the actual vs predicted regression line in these plots are no close to 1 and 0, respectively. Additionally, MSTAG exhibited a relative standard deviation on prediction above 20 %. This dispersion could be attributed to a poorer influence of DSTAG and MSTAG components on DSC profile, confirming results previously obtained with a more complex analytical approach [9].

Conclusions

In conclusion, cooling transition profile of EVOo appeared to be more influenced by OOO, OLO and OOP + SLO, among TAG, exhibiting high correlation coefficients and low RMSD values in both calibration and validation sets, as well as TUTAG percentages. Other TAG, as LLP + OLnO, LLL + LLPo and POPo, also showed to influence cooling profiles, although less markedly. Thus, the findings of this study showed which among TAG exhibited a larger influence on EVOo crystallization profiles, further clarifying the nature of the TAG influence on DSC cooling curves for this vegetable oil.

The further step may be the application of this new and fast approach to DSC cooling curves obtained by a wider set of EVOo samples to discriminate among them according to different geographical and/or botanical origins, taking into account TAG compositional differences.

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