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Headspace Solid-Phase Microextraction−Gas Chromatography−Mass Spectrometry Quantification of the Volatile Profile of More than 1200 Virgin Olive Oils for Supporting the Panel Test in Their Classification: Comparison of Different Chemometric Approaches

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Supporting Information

ABSTRACT: A reliable and robust tool for supporting the panel test in virgin olive oil classification is still required. We propose four chemometric approaches based on *t* test, principal component analysis (PCA) and linear discriminant analysis (LDA), applied for combining sensorial data, and chemical measurements. The former was from the panel test, and the latter was from headspace solid-phase microextraction−gas chromatography−mass spectrometry quantitation of 73 volatile organic compounds (VOCs) of 1223 typical commercial virgin olive oils, with most of them recognized as difficult to classify with accuracy by the panel test. The approaches were developed and validated, and the best results, with 83.5% correct classification, were using the PCA−LDA approach. Among the other methods, developed for proposing simplified procedures based on a smaller number of VOCs, the best method gave 80.1% correct classification only using 10 VOCs. All of the approaches suggested that octane, heptanal, pent-1-en-3-ol, *Z*-3-hexenal, nonanal, and 4-ethylphenol should be considered as a basis of volatiles for classification of olive oil samples.

KEYWORDS: *PCA, LDA, oxidative indices, microbiological indices, extra virgin olive oil, volatile compounds*

INTRODUCTION

There are several reasons that lead to the consideration of extra virgin olive oil (EVOO) as the highest quality product among edible oils. It is only obtained by physical−mechanical methods, is rich in oleic acid, shows pleasant taste and smell, and contains the highest amount of bioactive phenols responsible for several biological properties.[1−5]

The composition of the volatile fraction of a virgin olive oil is crucial to define the sensorial notes, which, in turn, are responsible for sample commercial classification. According to European legislation[6] and International Olive Council (IOC) trade standards, the official method to classify VOOs is the panel test, carried out by a group of at least eight trained panelists and a panel leader.[7]

The panel test classifies virgin olive oils taking into account the presence of a defined group of sensory defects as rancid, winey, musty, earthy, and fusty, and only those oils showing no defects are classified as EVOO. This latter category is also characterized by the presence of positive attributes, particularly the green and fruity sensations. These sensations are mainly related to the activation of the lipoxygenase cascade (LOX pathway) during the olive crushing and malaxation steps of the milling process.[8−11] On the contrary, samples characterized by the presence of defects with a median below 3.5 are classified as virgin olive oil (VOO), while those with a median of defects greater than 3.5 are classified as lampante virgin olive oil (LVOO). This latter category is not edible as such but, after a refining process, is mixed to VOO or EVOO to obtain olive oil (OO).

Overall, the composition of VOCs is affected by not only pedoclimatic conditions, cultivar, ripening stage, drupes harvesting, and storage conditions of the fruit[12−16] but also the technological parameters applied during milling.[17,18]

Over the years, the possibility to support the sensorial evaluation with reliable chemical data related to the high number of volatile organic compounds (VOCs) of virgin olive oil has been recognized as crucial. Morales, Luna, and Aparicio[19] proposed the use of dynamic headspace gas chromatography−mass spectrometry (DHS−GC−MS) to detect the molecules responsible for the main sensorial defects of virgin olive oil, specifically of fusty, mustiness−humidity, winey−vinegary, and rancid. To improve the capability of detecting and quantifying also the VOCs present in very low concentrations, an eligible method to concentrate the volatile compounds of the headspace before the gas chromatography (GC) analysis is required and the use of solid-phase microextraction (SPME) was introduced for this goal. Thus, nowadays, one of the more appropriate tools is certainly the headspace solid-phase microextraction−gas chromatography−mass spectrometry (HS−SPME−GC−MS) analysis, widely

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applied to study the VOCs of virgin olive oils.[7,20−22] This technique has shown great potential for olive oil quality control, being suitable for targeted but also untargeted investigations.[23] To improve the power of HS−SPME−GC−MS analysis, the application of validated procedures suitable to guarantee reliability and consistency of the results and the possibility to routinely apply the method to check the presence of defects and evaluate the quality of EVOOs are crucial. Romero et al.[20] validated a HS−SPME−GC−MS method for quantification of 29 VOCs in virgin olive oils. More recently, Fortini et al.[7] proposed a validated method for quantification of 71 VOCs, including not only analytes responsible for the negative attributes but also the molecules associated with positive sensorial notes. One of the key steps of such a method is the use of 11 internal standards for area normalization, selecting the more suitable one for each of the analyzed VOCs.

The main goal of this work was to propose a reliable and objective tool for supporting the panel test in virgin olive oil classification, helping the work of the expert panelists. The steps of the work are summarized as follows: (i) quantitation of 73 VOCs in more than 1200 virgin olive oil samples using the method proposed by Fortini et al.,[7] (ii) sensory evaluation of the same samples by the panel test, (iii) development and validation of several predictive models for sample classification based on correlation between chemical and sensory data, (iv) definition of chemical indices based on selected VOCs responsible for oxidative and microbiological defects and green and fruity notes of virgin olive oils, and (v) comparison of the predictive results obtained from the different approaches.

MATERIALS AND METHODS

Chemicals. All chemicals and standards of analytical reagent grade were from Sigma-Aldrich (Steinheim, Germany). A refined olive oil (ROO) free from VOCs was used for preparing solutions for external calibration curves of the 73 VOCs (ExtStd). Internal standard solution was prepared weighing acetic acid-d_3, 6-chloro-2-hexanone, butanol-d_{10}, 4-methyl-2-pentanol, 3-octanone, ethyl acetate-d_8, 3,4-dimethylphenol, toluene-d_8, and trimethylacetaldehyde in ROO (IntStd). Six diluted solutions were then prepared and used for building a six-point linear least squares calibration line for each analyte: each diluted solution was constituted by the same amount of IstStd and different amounts of ExtStd, chosen according to previous works,[7] to cover their typical contents in olive oils. The diluted standard solutions were stored in the dark at −20 °C until chromatographic analyses.

Virgin Olive Oil Samples. A total of 1223 virgin olive oil samples were collected from the Carapelli laboratory (Carapelli S.p.A., Tavarnelle Val di Pesa, Florence, Italy) during three harvesting years, i.e., 2016/2017, 2017/2018, and 2018/2019. Samples were from several geographic origins (Spain, 34.5%; Italy, 26.7%; Greece, 23.6%; Portugal, 6.9%; Tunisia, 6.7%; and other, 1.6%) and were classified after chemical and sensorial analysis, as described in the next paragraph.

Chemical and Sensorial Analysis for Oil Classification. Samples were classified according to chemical and sensorial analysis: legal quality indices were determined according to the analytical methods reported in the European Regulation EEC 2568/91,[6] and results used for confirming the classification of samples are not reported in the paper.

Sensorial characteristics were then assessed according to EEC 2568/91 by a panel of 8−12 trained tasters coordinated by a panel leader, acknowledged by the Italian Ministry of Agricultural Policies (MIPAAF). Each taster smelled and tasted the sample and marked the intensity of negative (rancid, fusty/muddy, musty/humid, winey/vinegary, and other) and positive (fruity, bitter, and pungent) attributes on a 0−10 cm unstructured scale. Samples with a median of defects of 0 and median of fruity notes greater than 0 were classified as EVOO; those with a median of defects between 0 and 3.5 and median of fruity notes greater than 0 were classified as VOO; and finally those with a median of defects greater than 3.5 and/or median of fruity notes of 0 were classified as LVOO. Except for five samples, classified as LVOO and considered as outliers, all of them were classified as EVOO (562) and VOO (656) and were with a median of defects lower than 1.5.

Samples were first labeled as EV (those classified as EVOO) or DE (defective, those classified as VOO). The defective samples were further labeled as OX (defective for oxidative defect, if the major defect was rancid) or MI (defective for microbiological defect, if the major defect was mustiness−humidity, fusty, or winey−vinegary). When the two types of defects were at a similar extent (i.e., difference of medians below 0.5), the sample was considered defective for both of the types of defects.

HS−SPME−GC−MS Analysis. The volatile fraction of all samples was analyzed by HS−SPME−GC−MS, with the method proposed by Fortini et al.,[7] slightly modified by the addition of acetic acid, methanol, and ethanol to the set of quantified molecules. Briefly, 4.3 g of sample and 0.1 g of internal standard mix solution were weighed in 20 mL screw cap vials. A SPME fiber 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) by Agilent (Palo Alto, CA, U.S.A.) was exposed for 20 min in the vial headspace under orbital shaking at 400 rpm, after equilibration for 5 min at 45 °C. The absorbed molecules were then desorbed for 1.7 min in the injection port of a 6890N GC system equipped with a MS detector, model 5975 (Agilent, Palo Alto, CA, U.S.A.). After desorption, a fiber backout was carried out in a backout unit for 20 min at 260 °C to avoid carryover phenomena among subsequent samples. A HP-Innowax capillary column (50 m × 0.2 mm inner diameter, 0.4 μm film thickness) was employed. The initial oven temperature was kept at 40 °C for 2 min, raised to 156 °C with a 4 °C min^{-1} gradient, and then raised to 260 °C with a 10 °C min^{-1} gradient. Helium was used as the carrier gas at 1.2 mL min^{-1}. The temperatures of the ion source and transfer line were 230 and 250 °C, respectively. Mass detector conditions were scan mode within the range of 30−350 Th, 1500 Th/s, and ionization energy (IE) of 70 eV. Mass spectra and retention times of injected authentic standards were compared to those of each peak for identification of the 73 VOCs.

The two VOCs 2-methylbutan-1-ol and 3-methylbutan-1-ol co-eluted; thus, they were evaluated together, and the output of the analysis was given by 72 quantitative data for each oil sample. Each of the 72 VOCs was quantified on the basis of a six-point linear least squares calibration line, where the area ratio [analyte peak area over the peak area of the selected internal standard (Table S1 of the Supporting Information)] was plotted versus the amount ratio (analyte amount over internal standard amount). For each analytical sequence, carried out in different working days, the 72 calibration lines were rebuilt using the same stored standard solutions.

The method was validated again in the Carapelli chemical laboratory, following the same approach used by Fortini et al.[7] The parameters of validation are reported in Table S1 of the Supporting Information for all of the quantified VOCs: limit of quantification (LOQ), limit of detection (LOD), linearity (in terms of R^2_{adj} and range of linear calibration), accuracy (in terms of trueness and precision), sensitivity, and selectivity.[24,25]

Statistics. Typical statistical tools were used working on a data set of 1218 virgin olive oil samples, to propose reliable and robust approaches to support the panel test in virgin olive oil classification. The t test was applied to assess the capability of each VOC in discriminating between different categories of samples, through the evaluation of calculated p values (Microsoft Office Professional Plus 2016). Principal component analysis (PCA) was used as the non-supervised technique for reducing the dimensionality of the data, using the concentrations of each VOC as variables. Finally, linear discriminant analysis (LDA) was run using reduced sets of variables [the scores on the selected principal components (PCs) or the more significant VOCs according to the t test] to find linear combinations

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Table 1. Accuracy of the Classification of Samples during the 10-Fold Cross-Validation by the PCA−LDA Model Based on the First 20 PCs[a]

			among the classified samples (%)	
PCA−LDA test set number	CP th (%)	not classified (%)	correct classification (wrong defect)	misclassified
1	39	10.0	82.9 (21.4)	17.1
2	40	6.9	89.3 (11.6)	10.7
3	42	7.7	85.0 (13.3)	15.0
4	39	6.9	79.3 (9.1)	20.7
5	41	6.2	82.8 (10.7)	17.2
6	42	7.7	74.2 (11.7)	25.8
7	43	8.5	79.0 (13.4)	21.0
8	42	2.3	78.7 (13.4)	21.3
9	42	3.1	88.1 (11.1)	11.9
10	43	5.4	82.1 (8.9)	17.9
mean ± sd	41.3	6.5 ± 2.4	82.1 ± 4.6 (12.5 ± 3.5)	17.9 ± 4.6

[a]Each row shows results of each round of the cross-validation process, with the averaged results in the last row. CP th (%) is the selected threshold value of post-probability used for allocating samples in the different categories. Not classified are samples that the model was not able to classify according to the selected CP th values. Classified samples are split into those correctly classified (with samples correctly classified as VOO but with a misidentified defect in parentheses) and those misclassified.

of variables giving the best linear fit able to separate categories of samples. Both PCA and LDA were run using OriginPro 2018 (OriginLab Corporation, Northampton, MA, U.S.A.; http://www.originlab.com).

For the defective samples, when LDA was applied for discriminating between OX and MI samples, those oils characterized by both oxidative and microbiological defects according to paragraph 2.3 were considered twice, one time as MI and one time as OX. Consequently, the total data set increased from 1218 to 1295 samples and was randomly split in a training set (1000 samples) and a test set of 295 independent samples.

RESULTS AND DISCUSSION

The final goal of this work is to propose reliable tools for supporting the panel test in virgin olive oil classification, easily applicable by quality control laboratories for their routine assessments. Our approach is based on HS−SPME−GC−MS quantitation of 73 VOCs in more than 1200 oil samples to obtain a data set for a successive statistical analysis. The HS−SPME−GC−MS method uses nine internal standards for area normalization and 72 six-point linear least squares calibration lines for VOC quantitation. The method is a slightly modified version of the method recently validated.[7] In particular, methanol, ethanol, and acetic acid were added to the set of the quantified VOCs to obtain a more suitable system for virgin olive oil classification.[19,26−28] Furthermore, to simplify the original method,[7] hexanoic acid-d_{11} and ethyl hexanoate-d_{11} were removed from the pool of internal standards because they were used for area normalization of only two and one analytes, respectively. In light of these slight modifications, the method has been validated again, and parameters for validation are listed in Table S1 of the Supporting Information.

The volatile fraction of all samples was analyzed by such a method, and simultaneously, the same oils were analyzed by the panel test and classified as extra virgin (EV) or defective (DE) and defective for oxidative (OX) or microbiological (MI) defects (paragraph 2.3). Almost all of the analyzed samples were representative of the marketplace; most of them had a median of defect lower than 1.5 and had been recognized as difficult to be classified with accuracy by the panel test. In our opinion, this sample set was the most suitable for building reliable models for virgin olive oil classification, also considering that the huge number of samples allows for the acquisition of very robust predictive models.

The chemical and sensory data were used to develop four different chemometric approaches, validated using a group of independent samples (test set). Results obtained with each approach were compared to the panel test to confirm the capability of the models to correctly predict sample classification; finally, results from the different models were compared.

Approach 1: PCA−LDA. This approach considers all of the variables initially available, and none of the available information is *a priori* excluded when the model is created; thus, it likely gives the best results. PCA was applied on the 72 variables (the quantified VOCs) of both the training set and test set to reduce the dimensionality of the data. The scree plot in Figure S1 of the Supporting Information shows that the elbow point is reached with approximately 15 PCs; we selected a slightly higher number of PCs to be sure to retain all of the useful variance but excluded those PCs that only caused noise (20 PCs with a total explained variance of 72.7%). Then, the predictive capability of the LDA model was internally validated by a full 10-fold cross-validation procedure, working on the input information given by a data matrix (1000 × 20) containing the scores of the 1000 virgin olive oils of the training set on the 20 selected PCs. To this goal, 10 equal sized subset of samples were created, starting from the entire data set, and the cross-validation procedure was repeated 10 times using each of the 10 subsets once as the test set (Scheme S1 of the Supporting Information). The model was then externally validated using the test set (295 independent samples). The proposed model was set for classifying samples in the categories extra virgin (EV), defective for oxidative defects (OX), and defective for microbiological defects (MI); both MI and OX were considered defective samples (DE). Samples were allocated in the different categories based on the suitable threshold value of post-classification probability (CP th %) relative to each category. This threshold value was selected by an iterative process to give the best results in terms of lower percentages of non-classified samples and greater percentages of correctly classified samples (Figure S2 of the Supporting Information shows an example of how these parameters change as a function of the selected threshold value). Non-classified

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Table 2. List of the 23 VOCs with Averaged p Values of <0.01 after Three Rounds of the t Test[a]

codes of VOCs	VOCs	p value EV−OX	p value EV−MI	p value OX−MI	averaged p value
24D	*E,E*-deca-2,4-dienal	9.24×10^{-13}	3.90×10^{-6}	7.67×10^{-9}	0.0000013
P	propanol	2.22×10^{-7}	1.89×10^{-19}	4.93×10^{-6}	0.0000172
O3OL	oct-1-en-3-ol	1.45×10^{-26}	1.07×10^{-12}	1.21×10^{-4}	0.0000403
HEP	heptanal	7.16×10^{-22}	2.09×10^{-20}	2.04×10^{-4}	0.0000679
2P	pentan-2-ol	4.52×10^{-4}	9.48×10^{-27}	2.06×10^{-9}	0.000151
24N	*E,E*-nona-2,4-dienal	5.65×10^{-12}	4.43×10^{-3}	3.76×10^{-4}	0.000273
IV	isovaleraldehyde	1.06×10^{-5}	3.55×10^{-10}	1.69×10^{-3}	0.000568
4EP	4-ethylphenol	9.73×10^{-9}	7.98×10^{-20}	2.56×10^{-3}	0.000853
Z3HL	*Z*-3-hexenal	7.89×10^{-23}	1.23×10^{-29}	2.57×10^{-3}	0.000858
1P3O	pent-1-en-3-ol	2.13×10^{-17}	1.38×10^{-35}	2.96×10^{-3}	0.000987
E2H	*E*-2-hexenal	2.18×10^{-11}	2.73×10^{-23}	4.11×10^{-3}	0.001371
N	nonanal	1.37×10^{-11}	1.11×10^{-15}	4.13×10^{-3}	0.001377
HEX	hexenal	7.23×10^{-5}	5.60×10^{-3}	7.87×10^{-9}	0.001889
G	guaiacol	6.34×10^{-5}	4.57×10^{-8}	8.57×10^{-3}	0.002879
OE	octane	6.30×10^{-15}	4.58×10^{-32}	9.69×10^{-3}	0.003232
BAC	butanoic acid	4.21×10^{-10}	3.28×10^{-4}	0.0105	0.003605
E2O	*E*-2-octenal	6.19×10^{-15}	4.06×10^{-8}	0.0123	0.004096
E2PA	*E*-2-pentenal	3.08×10^{-4}	2.22×10^{-10}	0.0131	0.004473
EA	ethyl acetate	1.62×10^{-4}	4.43×10^{-9}	0.0139	0.004689
ET	ethanol	1.29×10^{-8}	4.00×10^{-22}	0.0152	0.00506
M	methanol	2.05×10^{-3}	0.0179	1.44×10^{-6}	0.006635
IB	isobutanol	0.0200	1.30×10^{-25}	4.95×10^{-6}	0.006673
EPR	ethyl propanoate	3.05×10^{-5}	2.08×10^{-8}	0.0220	0.007352

[a]For each VOC, p value EV−OX, p value EV−MI, and p value OX−MI indicate its capability in discriminating between EV and OX samples, between EV and MI samples, and between OX and MI samples, respectively.

Table 3. Accuracy of the Classification of Samples during the 10-Fold Cross-Validation by the t Test−LDA Model Based on 23 Selected VOCs[a]

			among the classified samples (%)	
t test−LDA test set number	CP th (%)	not classified (%)	correct classification (wrong defect)	misclassified
1	41	5.4	79.7 (18.7)	20.3
2	42	10.0	82.1 (12.0)	17.9
3	41	4.6	77.4 (8.9)	22.6
4	40	10.8	82.8 (9.5)	17.2
5	40	8.5	80.7 (8.4)	19.3
6	40	5.4	70.7 (12.2)	29.3
7	42	7.7	75.0 (10.8)	25.0
8	40	3.1	76.2 (8.7)	23.8
9	42	6.2	80.3 (9.0)	19.7
10	42	2.3	81.1 (9.4)	18.9
mean ± sd	41	6.4 ± 2.8	78.6 ± 3.7 (10.8 ± 3.1)	21.4 ± 3.7

[a]Each row shows the result of each round of the cross-validation process, with the averaged results in the last row. CP th (%) is the selected threshold value of post-probability used for allocating samples in the different categories. Not classified are samples that the model was not able to classify according to the selected CP th values. Classified samples are split into those correctly classified (with samples correctly classified as VOO but with a misidentified defect in parentheses) and those misclassified.

samples were the samples that, according to the selected CP th %, were classified in two different categories or in none of them. The capability of the model in discriminating between EV and DE was evaluated with the main goal of classifying samples as EVOO or VOO/LVOO. Within the DE samples, the capability of the model in discrimination between OX and MI was also evaluated, with the further goal of understanding the origin of the defects for the lower category samples.

Table 1 shows results obtained for each of the 10 rounds of the cross-validation, and the predictive performance of the model is finally given in the last line, where the results of cross-validation are averaged over the rounds. The percentage of non-classified samples never exceeded 10.0%, with a mean of 6.5%. Among the classified samples, an average of 82.1% was correctly classified, with only 12.5% that were VOO samples with a misidentified defect. Noteworthy, the percentage of correctly classified samples reached values of almost 90% in two cases. During external validation, the model was able to classify 94.7% of samples, with 83.5% of correctly classified samples (see Table 8). These results confirm that the PCA−LDA model, easily applicable after VOC quantification, is in agreement with the panel test in prediction of virgin olive oil classification for a very high percentage of samples. In our opinion, this approach, after validation in other laboratories and working with several panels, can be proposed as a useful

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approach to support the panel test in virgin olive oil classification.

Approach 2: *t* Test−LDA. As stated above, the PCA−LDA approach uses all of the available information; thus, the predictive performance is likely better than other models using only parts of the initial information. However, no qualitative information about the volatile molecules able to differentiate between samples are gained by such an approach. This has been the first reason that induced us to develop other models, in which results are related to the chemical profile of samples. The objective pursued by developing other models was to propose simplified approaches, using a reduced number of VOCs and/or shorter statistical procedures. By this way, we were able to compare the predictive performance of several models, all built starting from the same data set.

The *t* test−LDA approach uses the *t* test for reducing the data set to those VOCs that showed the greatest ability in discriminating between EV, OX, and MI samples. The *t* test was run 3 times, for assessing the capability of each of the 72 VOCs in discriminating between EV and OX, between EV and MI, and between OX and MI. For each VOC, the three obtained *p* values (Microsoft Office Professional Plus 2016) were averaged, and those VOCs with an averaged *p* value smaller than 0.01 were considered able to discriminate between EV, OX, and MI and were selected for the following LDA. The 23 VOCs selected by this way are reported in Table 2.

The predictive capability of LDA was then validated using a full 10-fold cross-validation procedure working on the input information given by the data matrix (1000 × 23) containing the quantitative data of the 23 selected VOCs for the 1000 samples of the training set. The model was then externally validated using the test set of independent samples. Again, samples were classified as EV or DE (with the DE samples further classified as OX or MI) based on the selected CP th % values (Scheme S2 of the Supporting Information).

Table 3 shows results obtained in each of the 10 rounds of the cross-validation, and the predictive performance of the model is finally given in the last line, where the results of cross-validation are averaged over the rounds. The percentage of non-classified samples was very similar to that from the PCA−LDA model, with a mean of 6.4%. Among the classified samples, an average of 78.6% was correctly classified, with only 10.8% with a misidentified defect. During external validation, the model was able to classify 95.3% of samples, with 79.7% of correctly classified samples (see Table 8).

The *t* test-LDA model gave a prediction only slightly worst in comparison to the PCA−LDA model, with the advantage that it also gives qualitative information on the VOCs more able in discriminating between the different categories of oils (Table 2). Qualitative aspects of this and the two following approaches will be discussed and compared in a specific paragraph.

Approach 3: *t* Test and Discriminant Value (*t* Test−DSV). The next two approaches were developed for proposing simplified models directly based on the quantitative data of some selected VOCs. Also for developing the approaches discussed in this paragraph, we considered twice the samples characterized by both OX and MI defects. The *t* test was applied twice to the training set (1000 samples) for selecting two small groups of VOCs: one group to discriminate between EV and DE and another group to discriminate between OX and MI. In both of the cases, we selected the 10 VOCs with the lowest *p* values; they are shown in Table 4, together with the mean values of the amount of the selected VOCs in the considered categories of samples.

Table 4. List of 10 VOCs with the Lowest *p* Values after the *t* Test[a]

A VOC EV/DE	codes of VOCs	*p* value EV/DE	mean value EV	mean value DE
octane	OE	6.29×10^{-31}	0.053	**0.194**
E,*E*-hexa-2,4-dienal	24HX	2.3×10^{-29}	**0.220**	0.081
heptanal	HEP	1.06×10^{-28}	0.012	**0.036**
pent-1-en-3-ol	1P3O	5.23×10^{-26}	**0.431**	0.299
heptanol	H	2.50×10^{-24}	0.011	**0.020**
Z-3-hexenal	Z3HL	2.24×10^{-21}	**0.361**	0.132
oct-1-en-3-ol	O3OL	7.94×10^{-20}	0.014	**0.020**
nonanal	N	3.02×10^{-19}	0.280	**0.628**
valeraldehyde	V	9.32×10^{-19}	0.083	**0.129**
4-ethylphenol	4EP	2.39×10^{-18}	0.052	**0.150**
B VOC OX/MI	**codes of VOCs**	***p* value OX/MI**	**mean value MI**	**mean value OX**
butyl acetate	BA	1.15×10^{-7}	**0.005**	0.002
2-pentanol	2P	1.66×10^{-7}	**0.010**	0.006
E,*E*-deca-2,4-dienal	24D	1.66×10^{-7}	0.079	**0.206**
hexanal	HEX	1.71×10^{-7}	0.651	**0.995**
isobutanol	IB	1.3×10^{-6}	0.021	**0.032**
methanol	M	8.9×10^{-6}	**4.595**	3.366
heptanal	HEP	3.72×10^{-5}	0.030	**0.048**
propanol	P	8.4×10^{-5}	**0.022**	0.014
oct-1-en-3-ol	O3OL	1.16×10^{-4}	0.019	**0.022**
E-2-hexenyl acetate	E2HA	1.74×10^{-4}	0.026	**0.046**

[a]For each VOC, (A) *p* value EV/DE and (B) *p* value OX/MI indicate the capability in discriminating between EV and DE samples and between OX and MI samples, respectively. For each VOC, mean value EV is the mean content of that VOC in EV samples, mean value DE is the mean content of that VOC in DE samples, mean value MI is the mean content of that VOC in MI samples, and mean value OX is the mean content of that VOC in OX samples.

Starting from 3, 5, or 10 of these VOCs, we defined two indices ($D_{X\text{-EV/DE}}$ and $D_{X\text{-OX/MI}}$): one for discriminating between EV and DE and another for discriminating between OX and MI. $D_{X\text{-EV/DE}}$ and $D_{X\text{-OX/MI}}$ are the indices for EV−DE and OX−MI samples, respectively, where X is the number of used VOCs (3, 5, or 10). The codes of the used VOCs are explained in Table 4, and the obtained indices are defined according to the following formulas:

$$D_{3\text{-EV/DE}} = 24\text{HX} - (\text{OE} + \text{HEP})$$

$$D_{3\text{-OX/MI}} = 24\text{D} - (\text{BA} + 2\text{P})$$

$$D_{5\text{-EV/DE}} = 24\text{HX} + 1\text{P3O} - (\text{OE} + \text{HEP} + \text{H})$$

$$D_{5\text{-OX/MI}} = 24\text{D} + \text{HEX} + \text{IB} - (\text{BA} + 2\text{P})$$

$$D_{10\text{-EV/DE}} = 24\text{HX} + 1\text{P3O} + \text{Z3HL} - (\text{OE} + \text{HEP} + \text{H} + \text{O3OL} + \text{N} + \text{V} + 4\text{EP})$$

$$D_{10\text{-OX/MI}} = 24\text{D} + \text{HEX} + \text{IB} + \text{HEP} + \text{O3OL} + \text{E2HA} - (\text{BA} + 2\text{P} + \text{M} + \text{P})$$

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We considered the mean amount of the selected VOC in samples belonging to the two considered categories. For each oil, the amount of such VOC was considered with a positive sign when the mean amount was higher in one of the considered categories and with a negative sign when the mean amount was higher in another of the considered categories (used values are in bold in Table 4).

For each of the three defined couples of D_X, we identified a discrimination value ($DSV_{X\text{-EV/DE}}$ and $DSV_{X\text{-OX/MI}}$), selecting the values able to give the best results in terms of lower percentages of non-classified samples and greater percentages of correctly classified samples (Scheme S3 of the Supporting Information). With application of a tolerance factor of 10% with respect to DSV_X, non-classified samples were the samples for which

$$DSV_X \times 0.90 < D_X < DSV_X \times 1.10$$

where D_X is the value of the indices calculated for that sample.

Table 5 shows results obtained applying the model to the test set of 295 independent samples and working with 3, 5, or 10 VOCs. The best results in terms of correctly classifying samples were obtained working with five VOCs: 80.1% of samples, of which 13.8% had a misidentified defect. These results are not so diverse from those from the PCA−LDA approach, so that this could be proposed as a simplified model able to classify virgin olive oil samples based on the quantitative analysis of only 10 selected VOCs: 5 for discriminating between EV and DE samples and 5 for discriminating between OX and MI.

Table 5. Accuracy of the Classification of Samples by the "*t* Test Discriminant Value" Approach, Using 3, 5, or 10 VOCs[a]

	DSV th			among the classified samples (%)	
number of VOCs	EV/DE	OX/MI	non-classified (%)	correct classification (wrong defect)	misclassified
3	0.04	0.20	1.0	74.1 (10.8)	25.9
5	0.40	1.60	8.0	80.1 (13.8)	19.9
10	0.15	0.50	1.7	77.6 (11.5)	22.4

[a]DSV th is the selected threshold discriminant value used for allocating samples in the different categories. Not classified are samples within the range of DSV th × 0.90 − DSV th × 1.10, considering 10% of tolerance. In parentheses, samples were correctly classified as VOO but with a misidentified defect.

Approach 4: Definition of Chemical Indices. This approach aimed at defining suitable indices for classifying oil samples as EV or DE and for a further discrimination between OX and MI defective samples only based on quantitative data of the 72 quantified VOCs. Data of the different VOCs were initially corrected by suitable multiplicative factors for having equal mean values for all 72 VOCs when they were averaged over all 1218 samples, to guarantee that all of the molecules gave the same contribution independently from their absolute amount. The whole data set of the corrected data was randomly split in a training set (923 samples) and a test set (295 samples), and on the basis of results of the panel test, samples of the training set were labeled as EV, OX, MI-Fu (fusty/muddy defect), MI-Mu (musty/humid/hearth defect), and MI-Wi (winey/vinegary defect); when more than one defect was present, that sample was labeled with all of the defects. Each of the five categories was considered one at a time, and each time samples were split in the three groups: EV, samples in which the considered defect was present, and other samples (when the EV category was considered, the only groups were EV and defective samples). Each time, for each VOC, we calculated mean and standard deviation (sd) in each group, and the means of the different groups were compared dividing, for each defect, the mean of samples with that defect (N_{DE}) by the mean of EV samples (N_{EV}).

$$RV = \frac{N_{DE}}{N_{EV}} \quad (1)$$

By this way, we obtained ratio values (RV) that indicated, on average, how much each VOC was concentrated in samples with the specific defect with respect to EV samples. The molecules with the greater RV were selected as able to discriminate between samples with the considered defect and EV samples. Molecules with too high values of sd within a specific class were excluded. Table 6 shows the VOCs selected for each category. Because the typical defects of VOOs arise from oxidative and microbiological defects, we defined one

Table 6. Selected VOCs for Indices of the Categories Extra Virgin Olive Oil (EV), Oxidative Defect (OX), Fusty/Muddy Defect (MI-Fu), Musty/Humid/Hearth Defect (MI-Mu), and Winey/Vinegary Defect (MI-Wi)[a]

EV	MI-Fu	MI-Wi	MI-Mu	OX
Z-3-hexenal	butanoic acid	acetic acid	6-methylhept-5-en-2-one	octane
E,*E*-hexa-2,4-dienal	octane	ethanol	4-ethylphenol	6-methylhept-5-en-2-one
E-2-hexenal	ethyl butanoate	ethyl acetate	guaiacol	heptanal
E-2-pentenol	phenol		propanol	*E*,*E*-deca-2,4-dienal
isobutanol	4-ethylguaiacol		2-methyl + 3-methylbutan-1-ol	*E*-2-heptenal
pent-1-en-3-ol	isovaleraldehyde		pentan-2-ol	nonanal
	ethyl propanoate			*E*-2-octenal
				E-2-decenal
				octanal
				valeraldehyde
				heptanol
				nonanol
				octanol

[a]For each category, VOCs are ordered starting from those more able in discriminating EV from other samples (for EV category) or samples defective for that defect from EV samples.

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index for oxidative defects and one for microbiological defects (fusty/muddy, musty/humid/hearth, and winey/vinegary were merged together). By this way, we aimed at creating an easy model able to discriminate between EV and DE samples and between defects generated by either oxidative or microbiological activities.

Three indices were thus created on the basis of the selected VOCs (Scheme S4 of the Supporting Information): I_{OX}, sum of the corrected values of the VOCs in the category "OX"; I_{MI}, sum of the corrected values of the VOCs in the categories "MI-Fu", "MI-Wi", and "MI-Mu"; and I_{EV}, sum of the corrected values of the VOCs in the category "EV".

Values of these indices, calculated for all samples of the training set, were used for building a table with decision criteria for virgin olive oil classification (Table 7) based on the threshold values that gave the best results in classification of samples of the training set [i.e., 7.8% of non-classified samples and 79.1% of correctly classified among the classified oils (5.4% with wrong defect)]. The model was externally validated by classifying samples of the test set, and results are reported in Table 8: 8.7% of samples resulted in non-classified according to the decision table, while among the classified samples, 77.0% were correctly classified (5.5% with the wrong defect).

Table 7. Decision Table for Virgin Olive Oil Classification According to the Chemical Indices Defined as Described in Paragraph 3.4[a]

I_{MI}	I_{OX}	I_{EV}	classification (type of defect)
>0.70	>1.00		DE (OX + MI)
>0.70	<1.00		DE (MI)
<0.70	>1.00		DE (OX)
<0.70	<1.00	>0.15	EV
<0.70	<1.00	<0.15	non-classified

[a] I_{OX}, index for oxidative defects; I_{MI}, index for microbiological defects; I_{EV}, index for positive attributes; EV, extra virgin olive oil samples; DE, defected samples; OX, oxidative defects; and MI, microbiological defects.

Table 8. Comparison of the Classification of Samples Obtained during External Validation of the Four Proposed Models, Using the External Set of Independent Samples[a]

		among the classified samples (%)	
model	non-classified (%)	correct classification (wrong defect)	misclassified
1. PCA−LDA	5.3	83.5 (12.0)	16.5
2. *t* test−LDA	4.7	79.7 (10.1)	20.3
3. *t* test−DSV	8.0	80.1 (13.8)	19.9
4. chemical indices	8.7	77.0 (5.5)	23.0

[a] For the "*t* test−DSV" model, the table reports results obtained using five VOCs, while for the other three models, it reports results obtained according to the described procedures.

Qualitative Information on VOCs Responsible for the Classification of Samples. The different approaches developed and proposed in this paper allowed us to gain information on the molecules able to discriminate between the different categories of virgin olive oils and between the different types of defects. The more detailed information was obtained by approach 4 (definition of chemical indices) and is reported in Table 6. For the EV category, in addition to VOCs originating from the LOX pathway (pent-1-en-3-ol, *Z*-3-hexenal, *E*-2-hexenal, and *E*-2-pentenol) and typically associated with fruity notes,[17,28−30] we can see a branched C_4 alcohol (isobutanol) and a C_6 diunsaturated aldehyde (*E*,*E*-hexa-2,4-dienal), molecules never associated with fruity notes to the knowledge of the authors. With regard to the oxidative defects, many of the selected VOCs were already reported (octane, nonan-1-ol, and the aldehydes) or hypothesized (6-methylhept-5-en-2-one) as associated with oxidation processes, while the alcohols heptan-1-ol and octan-1-ol were never associated with oxidative defects. Finally, with regard to microbiological defects, almost all of the selected molecules for fusty/muddy and winey/vinegary defects were already described as associated with these defects, while those selected for the musty/humid/hearth defect (column MI-Mu in Table 6) are different from the C_8 alcohols and ketones usually reported as associated with this defect,[17,19] confirming that further studies are necessary to better clarify the nature of the VOCs responsible for the sensory attributes of virgin olive oils.

The molecules useful for discriminating between the different categories using the *t* test−LDA approach are reported in Table 4. Interestingly, all of the VOCs useful for discriminating between EV and DE samples were also useful for the above "definition of chemical indices", with the only exception of oct-1-en-3-ol (Table 6). Again, 18 of the 23 VOCs reported in Table 2, useful for discriminating between different categories using the PCA−LDA approach, were also useful for the above "definition of chemical indices". In particular, octane, heptanal, pent-1-en-3-ol, *Z*-3-hexenal, nonanal, and 4-ethylphenol were useful for discriminating between EV and DE samples in all of the proposed models and should be considered as a basis pool of VOCs when the aim is classify OO samples as EVOO or VOO/LVOO.

Comparison of the Models. This paper deals with chemicals supporting the panel test in virgin olive oil classification, which is desirable and still strongly required to date. The novelty of the work with respect to models recently proposed in the literature[23,31] is mainly due to (i) the huge number of samples, with most of them considered difficult to be classified with accuracy by the panel test, (ii) the application of a validated HS−SPME−GC−MS method never used before for this purpose, (iii) the comparison of four methodological approaches built using a set of 1000 oils and externally validated with a set of 295 independent samples, (iv) the identification of a reduced set of VOCs suitable for all proposed models, and (v) the possibility to obtain a classification of EV and DE samples in agreement with the panel test with a percentage close to 80% using only 10 selected VOCs within the 72 evaluated VOCs.

When we look at the results in Table 8, we have to take into account that the panel test is a sensorial test that not always gives reliable classification;[7,20,31] thus, the misclassified samples could be misclassified by either our approach or the panel test. Furthermore, our sample set, considered as the most suitable for building a very reliable and robust chemometric approach for virgin olive oil classification, was mainly constituted by oils difficult to be classified with accuracy by the panel test. In light of these considerations, the 83.5% of correct classification obtained with the PCA−LDA model is a very satisfactory result, which allows for the proposal of this model for future official methodology in routine laboratories.

As said, the PCA−LDA model gave the best results in terms of correctly classified samples (Table 8). The differences with

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the other models were lower than expected, pointing out the robustness of the approaches based on VOC evaluation. PCA−LDA and *t* test−LDA models gave similar results in terms of non-classified samples.

According to Table 8, the PCA−LDA model is herein proposed as the more effective model for supporting the panel test in virgin olive oil classification. The *t* test−DSV approach could be a useful alternative for simplifying the analytical work, strongly reducing the number of quantified VOCs. Noteworthy, the model based on chemical indices gave the best results for discriminating samples with different defects.

This study can help to counteract fraud in the olive oil sector and enriches the literature of qualitative information about VOCs able to discriminate between EVOO and VOOs and between different kinds of defects. The crucial role of several volatile molecules of virgin olive oils clearly emerges in this work. Indeed, chemometric methods only based on VOC quantification can be proposed for future official methodology easily usable in routine laboratories for supporting the panel test. To assess the reproducibility and robustness of the proposed approaches, they have to be validated by the combining results of sensory analysis by several panels and chemical data on volatiles acquired in different analytical laboratories.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.9b03346.

Quality parameters for method validation, followed by a short description (Table S1), scree plot used for selecting the useful PCs (Figure S1), example of accuracy of prediction as a function of the Th CP value (%) during PCA/LDA cross-validation (Figure S2), and summaries of how each approach works (Schemes S1−S4) (PDF)

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Notes

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■ ABBREVIATIONS USED

EVOO, extra virgin olive oil; VOO, virgin olive oil; LVOO, lampante virgin olive oil; LOX, lipoxygenase; IOC, International Olive Council; VOC, volatile organic compound; HS−SPME−GC−MS, headspace solid-phase microextraction−gas chromatography−mass spectrometry; PCA, principal component analysis; LDA, linear discriminant analysis

■ REFERENCES

(1) Beauchamp, G. K.; Keast, R. S. J.; Morel, D.; Lin, J.; Pika, J.; Han, Q.; Lee, C.-H.; Smith, A. B.; Breslin, P. A. S. Ibuprofen-like activity in extra-virgin olive oil. *Nature* **2005**, *437*, 45−46.

(2) De Bock, M.; Hodgkinson, S.; Curtfield, W.; Schlothauer, R. C. Methods and uses of an extract from olive leaf in management of type 2 diabetes. WO Patent 2014038962 A1, March 13, 2014.

(3) Giovannelli, L. Beneficial effects of olive oil phenols on the aging process: Experimental evidence and possible mechanisms of action. *Nutr. Aging* **2013**, *1*, 207−223.

(4) de la Torre-Carbot, K.; Chavez-Servin, J. L.; Jauregui, O.; Castellote, A. I.; Lamuela-Raventos, R. M.; Nurmi, T.; Poulsen, H. E.; Gaddi, A. V.; Kaikkonen, J.; Zunft, H.-F.; Kiesewetter, H.; Fito, M.; Covas, M.-I.; Lopez-Sabater, M. C. Elevated circulating LDL phenol levels in men who consumed virgin rather than refined olive oil are associated with less oxidation of plasma LDL. *J. Nutr.* **2010**, *140*, 501−508.

(5) Covas, M.-I.; Nyyssonen, K.; Poulsen, H. E.; Kaikkonen, J.; Zunft, H.-J. F.; Kiesewetter, H.; Gaddi, A.; de la Torre, R.; Mursu, J.; Baumler, H.; Nascetti, S.; Salonen, J. T.; Fito, M.; Virtanen, J.; Marrugat, J. The effect of polyphenols in olive oil on heart disease risk factor: A randomized trial. *Ann. Intern. Med.* **2006**, *145*, 333−341.

(6) European Commission (EC).. Commission Regulation (EEC) No 2568/91 of 11 July 1991 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. *Off. J. Eur. Communities: Legis.* **1991**, *248*, 1−140.

(7) Fortini, M.; Migliorini, M.; Cherubini, C.; Cecchi, L.; Calamai, L. Multiple internal standard normalization for improving HS-SPME-GC-MS quantitation in virgin olive oil volatile organic compounds (VOO-VOCs) profile. *Talanta* **2017**, *165*, 641−652.

(8) Angerosa, F.; D'Alessandro, N.; Di Girolamo, R.; Vito, R.; Serraiocco, A. Relationship between aroma components and malaxation time of olive paste. *Acta Hortic.* **1999**, *474*, 621−625.

(9) Di Giovacchino, L.; Serraiocco, A. Influenza dei sistemi di lavorazione delle olive sulla composizione dello spazio di testa degli oli. *Riv. It. Sost. Grasse* **1995**, *72*, 443−450.

(10) Ranalli, A.; Angerosa, F. Integral centrifuges for olive oil extraction. The quantitative characterization of products. *J. Am. Oil Chem. Soc.* **1996**, *73*, 417−422.

(11) Morales, M. T.; Angerosa, F.; Aparicio, R. Effect of the extraction conditions of virgin olive oil on the lipoxygenase cascade: Chemical and sensory implications. *Grasas Aceites* **1999**, *50*, 114−121.

(12) Angerosa, F.; Camera, L.; D'Alessandro, N.; Mellerio, G. Characterization of seven new hydrocarbon compounds present in the aroma of virgin olive oils. *J. Agric. Food Chem.* **1998**, *46*, 648−653.

(13) Aparicio, R.; Morales, M. T. Characterization of olive ripeness by green aroma compounds of virgin olive oil. *J. Agric. Food Chem.* **1998**, *46*, 1116−1122.

(14) Morales, M. T.; Aparicio, R. Characterizing some European olive oil varieties by volatiles using statistical tools. *Grasas Aceites* **1993**, *44*, 113−115.

(15) Angerosa, F.; Basti, C. Olive oil volatile compounds from the lipoxygenase pathway in relation to fruit ripeness. *Ital. J. Food Sci.* **2001**, *13*, 421−428.

(16) Luna, G.; Morales, M. T.; Aparicio, R. Characterisation of 39 varietal virgin olive oils by their volatile compositions. *Food Chem.* **2006**, *98*, 243−252.

(17) Campestre, C.; Angelini, G.; Gasbarri, C.; Angerosa, F. The compounds responsible for the sensory profile in monovarietal virgin olive oils. *Molecules* **2017**, *22*, 1833−1860.

(18) Kalua, C. M.; Bedgood, D. R.; Bishop, A. G.; Prenzler, P. D. Changes in volatile and phenolic compounds with malaxation time and temperature during virgin olive oil production. *J. Agric. Food Chem.* **2006**, *54*, 7641−7651.

(19) Morales, M. T.; Luna, R.; Aparicio, R. Comparative study of virgin olive oil sensory defects. *Food Chem.* **2005**, *91*, 293−301.

(20) Romero, I.; Garcia-Gonzalez, D. L.; Aparicio-Ruiz, R.; Morales, M. T. Validation of SPME-GCMS method for the analysis of virgin

DOI: 10.1021/acs.jafc.9b03346
J. Agric. Food Chem. 2019, 67, 9112−9120

olive oil volatiles responsible for sensory defects. *Talanta* **2015**, *134*, 394−401.
(21) Sales, C.; Cervera, M. I.; Gil, R.; Portoles, T.; Pitarch, E.; Beltran, J. Quality classification of Spanish olive oils by untargeted gas chromatography coupled to hybrid quadrupole-time of flight mass spectrometry with atmospheric pressure chemical ionization and metabolomics-based statistical approach. *Food Chem.* **2017**, *216*, 365−373.
(22) Lukic, I.; Carlin, S.; Horvat, I.; Vrhovsek. Combined targeted and untargeted profiling of volatile aroma compounds with comprehensive two-dimensional gas chromatography for differentiation of virgin olive oils according to variety and geographical origin. *Food Chem.* **2019**, *270*, 403−414.
(23) Sales, C.; Portoles, T.; Johnsen, L. G.; Danielsen, M.; Beltran, J. Olive oil quality classification and measurement of its organoleptic attributes by untargeted GC−MS and multivariate statistical-based approach. *Food Chem.* **2019**, *271*, 488−496.
(24) International Organization for Standardization (ISO). *ISO3534-1, Statistics, Vocabulary and Symbols*; ISO: Geneva, Switzerland, 1993.
(25) Shrivastava, A.; Gupta, V. P. Methods for the determination of limit of detection and limit of quantitation of the analytical methods. *Chron. Young Sci.* **2011**, *2*, 21−25.
(26) Melucci, D.; Bendini, A.; Tesini, F.; Barbieri, S.; Zappi, A.; Vichi, S.; Conte, L.; Gallina Toschi, T. Rapid direct analysis to discriminate geographic origin of extra virgin olive oils by flash gas chromatography electronic nose and chemometrics. *Food Chem.* **2016**, *204*, 263−273.
(27) Procida, G.; Cichelli, A.; Lagazio, C.; Conte, L. Relationship between volatile compounds and sensory characteristics in virgin olive oil by analytical and chemometric approaches. *J. Sci. Food Agric.* **2016**, *96*, 311−318.
(28) Angerosa, F. Influence of volatile compounds on virgin olive oil quality evaluated by analytical approaches and sensor panels. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 639−660.
(29) Hatanaka, A. The biogeneration of green odour by green leaves. *Phytochemistry* **1993**, *34*, 1201−1218.
(30) Vick, B. A.; Zimmermann, D. C. The lipoxygenase pathway. In *The Biochemistry of Plants*; Stumpf, P. K., Conn, E. E., Eds.; Academic Press: New York, 1987; pp 53−90.
(31) Gerhardt, N.; Schwolow, S.; Rohn, S.; Perez-Cacho, P. R.; Galan-Soldevilla, H.; Arce, L.; Weller, P. Quality assessment of olive oils based on temperature-ramped HS-GC-IMS and sensory evaluation: Comparison of different processing approaches by LDA, kNN, and SVM. *Food Chem.* **2019**, *278*, 720−728.

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J. Agric. Food Chem. 2019, 67, 9112−9120