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Compliance with EU vs. extra-EU labelled geographical provenance in virgin olive oils: A rapid untargeted chromatographic approach based on volatile compounds

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Highlights

- Flash-GC was applied to discriminate VOOs with different geographical origin
- Chemometric data elaborations with untargeted approach were realized
- PLS-DA and ANN classification models were built and validated
- Satisfactory percentages of samples correctly classified were obtained

210 SAMPLES (EVOOs and VOOs)

Oils from countries members of EU

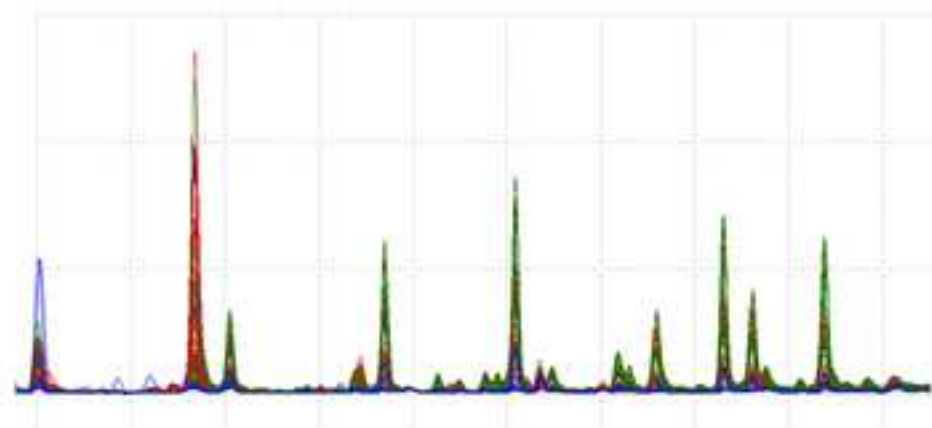


Oils from countries extra-EU



Blends

- ❖ 12 EU
- ❖ 12 EU/Extra-EU



Extraction of raw data

Data pre-treatment:

1. Alignment of raw chromatograms (COW algorithm)
2. Mean centering
3. Unit vector normalisation

PLS-DA

(Partial Least Square – Discriminant Analysis)

ANN

(Artificial Neural Network)

1 **Discrimination of virgin olive oils with different geographical origin:**
2 **a rapid untargeted chromatographic approach based on volatile compounds**

3

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12 **Abstract**

13 Many studies have shown that the geographic origin is one of the most influencing factors in
14 consumers' choice of olive oil. To avoid misleading, European regulation has established specific
15 rules to report the geographical origin of extra virgin (EVOOs) and virgin olive oils (VOOs) on the
16 product label, but an official analytical procedure to verify this information has not been yet
17 defined. In this work, a flash gas chromatography untargeted approach for determination of volatile
18 compounds, followed by a chemometric data elaboration, is proposed for discrimination of EVOOs
19 and VOOs according to their geographical origin (EU and Extra-EU). A set of 210 samples was
20 analyzed and two different classification techniques were used, one linear (Partial Least Square-
21 Discriminant Analysis, PLS-DA) and one non-linear (Artificial Neural Network, ANN). The two
22 models were also validated using an external data set. Satisfactory results were obtained for both
23 chemometric approaches: considering the PLS-DA, 89% and 81% of EU and Extra-EU samples,
24 respectively, were correctly classified; for ANN the percentages were 93% and 89%, respectively.
25 These results confirm the reliability of the method as a rapid approach to discriminate EVOOs and
26 VOOs according to their geographical provenance. The methodology is fast (around 200 sec for
27 each chromatographic run), easy to use (no sample treatment is required), and the data were
28 effectively elaborated by applying both linear and non-linear chemometric techniques.

29

30 **Key words**

31 Virgin olive oil; Geographical origin; PLS-DA; Untargeted approach; Volatile compounds; ANN.

32

33 **Abbreviations**

34 ANN: Artificial Neural Network; EVOO: Extra Virgin Olive Oil; FGC: Flash Gas
35 Chromatography; PLS-DA: Partial Least Square - Discriminant Analysis; VOO: Virgin Olive Oil.

36 **1. Introduction**

37 Over the last 40 years many investigations have been focused on understanding what attributes are
38 important determinants in consumer choice, which have highlighted that the geographic origin is
39 one of the most influencing factors for olive oil (Dekhili, Sirieix, & Cohen, 2011; Del Giudice,
40 Cavallo, Caracciolo, & Cicia, 2015).

41 In order to ensure that consumers are not misled, the fourth article of the EU Reg. 29/2012
42 establishes that “*Extra virgin and virgin olive oil shall bear a designation of origin on the*
43 *labelling*”. This means that for extra virgin (EVOOs) and virgin olive oils (VOOs) commercialized
44 within the EU, it is mandatory to specify the geographical provenance on the label of the product
45 following specific rules. If an oil comes from an EU Member State or third country, a reference to
46 the EU Member State, to the EU, or to the third country must to be reported. In the case of blends of
47 oils originating from more than one EU Member State or third country, one of the following
48 mentions must be used: ‘blend of olive oils of European Union origin’ or a reference to the EU;
49 ‘blend of olive oils not of European Union origin’ or a reference to origin outside the EU; ‘blend of
50 olive oils of European Union origin and not of European Union origin’ or a reference to origin
51 within the EU and outside the EU. An exception is the case where the olives were harvested in an
52 EU Member State or third country other than that in which the mill where the oil was extracted is
53 located. In this case, the designation of origin shall contain the following wording: ‘*(extra) virgin*
54 *olive oil obtained in (the Union or the name of the Member State concerned) from olives harvested*
55 *in (the Union or the name of the Member State or third country concerned)*’.

56 However, the regulation does not specify an official analytical procedure to verify the conformity of
57 the label-declared geographical origin, and this has raised the interest of researchers to develop a
58 reliable and effective method for purposes of authentication (Conte et al., 2019). During the last
59 years, different analytical techniques have been applied in order to find potentially useful markers
60 and efficient instrumental approaches that are able to discriminate olive oils according to their
61 geographical origin.

62 In this regard, traditional chromatographic techniques, analyzing both major and minor compounds
63 either individually or in a combined way, coupled or not with specific statistical chemometric data
64 elaboration, have been investigated. A study in 2009 (García-González et al., 2009) proposed the
65 application of artificial neural network (ANN) models for different levels of geographical
66 classification (country, region, province, PDO) on a set of 687 EVOOs and VOOs from Spain, Italy,
67 and Portugal, which were chemically characterized for the content of fatty acids, hydrocarbons,
68 sterols, and alcohols. Other researchers evaluated the triacylglycerol (TAG) content and
69 composition to discriminate Moroccan oils (Bajoub et al., 2016) and Croatian samples (Peršurić,
70 Saftić, Mašek, & Kraljević Pavelić, 2018). In addition, the stereospecific distribution of fatty acids
71 in TAGs was reported to be useful in discriminating olive oils from different areas of North-Eastern
72 Italy (Vichi, Pizzale, & Conte, 2007). Specific metabolites such as sterols and phenolic compounds
73 have been investigated to identify the optimal markers, and may be a promising approach to
74 discriminate oils according to geographical origin (Giacalone, Giuliano, Gulotta, Monfreda, &
75 Presti, 2015; Ben Mohamed et al., 2018; Ghisoni et al., 2019). Interesting findings have also been
76 recently reported on sesquiterpene hydrocarbons as geographical markers (Quintanilla-Casas et al.,
77 2020). Moreover, volatile compounds have been amply studied by applying different instrumental
78 techniques combined with chemometric data elaborations (Kosma et al., 2017; Bajoub et al., 2018;
79 Lukić, Carlin, Horvat, & Vrhovsek, 2019).

80 Furthermore, rapid and innovative instrumental approaches have been developed and tested in order
81 to deal with the need for simple, rapid, and environmentally friendly techniques (Valli et al., 2016).
82 This critical review (Valli et al., 2016) reports an overview of the principal applications of optical
83 techniques (UV-Vis, NIR, MIR, RAMAN, NMR, and fluorescence spectroscopy), methods based
84 on electrical characteristics, and instruments equipped with electronic chemical sensors (electronic
85 nose and tongue) for discrimination of EVOOs and VOOs according to their geographical
86 provenance. In addition to these approaches, other promising techniques include stable isotopes
87 analysis (Angerosa et al., 1999; Chiocchini, Portarena, Ciolfi, Brugnoli, & Lauteri, 2016; Bontempo

88 et al., 2019), multi-element fingerprint (Sayago, González-Domínguez, Beltrán, & Fernández-
89 Recamales, 2018), differential scanning calorimetry (Mallamace et al., 2017), and GC-IMS
90 (Gerhardt, Birkenmeier, Sanders, Rohn, & Weller, 2017).
91 Melucci and co-workers (Melucci et al., 2016) proposed the application of a Flash Gas
92 Chromatography Electronic Nose (Heracles II) and a multivariate data analysis to control the
93 compliance of information on geographic origin declared in the label (“100% Italian” vs “non-
94 100% Italian”) for the first time. This instrumental approach allows to realize the headspace
95 analysis in short time and the results are processed by chemometric tools following an untargeted
96 approach. For this reason, it can be considered as a fingerprint method, since the data can be
97 elaborated for sample classification that is not aimed towards identification and quantification of
98 specific analytes. Following these preliminary results and the actual need for a rapid and effective
99 method for geographical authentication of VOOs, the aim of this work was the application of flash
100 gas chromatography (Heracles II) for rapid discrimination of 210 EVOOs and VOOs according to
101 geographical provenance. In this case, the categories considered for samples classification were EU
102 member states vs third countries, and the data obtained were elaborated by applying two different
103 classification techniques, one linear (Partial Least Square-Discriminant Analysis, PLS-DA) and one
104 non-linear (Artificial Neural Network, ANN).

105

106 **2. Materials and methods**

107 **2.1 Samples**

108 A total of 210 EVOOs and VOOs with a different geographical origin were collected directly from
109 companies that were also asked to provide, when available, information about location of the mill,
110 type of plant used, olive variety, and commercial category (Table S1, Supplementary material).
111 Considering that the indication of the geographical origin on the product label is mandatory for
112 EVOOs and VOOs, samples belonging to both these two categories were included in this study.

113 According to geographical provenance, samples were distributed in 3 classes (Table 1): “EU” for
114 oils coming from EU member states; “Extra-EU” for oils coming from third countries (outside EU);
115 “Blends” for samples obtained by mixing oils coming from different EU state members or oils
116 coming from EU state members and third countries.

117 Aliquots of each sample (50 mL) were stored at -18 °C in plastic dark bottles. Oil were defrosted
118 for at least 12 h and stored at 12°C before analysis.

119

120 **2.2 Volatile compounds analysis by Flash Gas Chromatography**

121 The analysis of volatile compounds was carried out using the Flash Gas Chromatography Electronic
122 Nose Heracles II (Alpha MOS, Toulouse, France). The instrument was equipped with two metal
123 capillary columns (MXT-5: 5% diphenyl, 95% methylpolysiloxane, and MXT-1701: 14%
124 cyanopropylphenyl, 86% methylpolysiloxane, for both columns: 10 m length, 180 µm internal
125 diameter, 0.4 µm film thickness) working in parallel mode and different in polarity of the stationary
126 phase. This permits slight differences in the separation capability of molecules detected by a FID
127 applied at the end of each column.

128 Each sample was analysed in triplicate, weighing 2 ± 0.1 g of oil in a 20 mL vial sealed with a
129 magnetic plug. For analysis, the vial was placed in a shaker oven for 20 min at 40 °C and 500 rpm.
130 Next, 5 mL of the headspace were collected, introduced in a splitless injector (injector temperature
131 200 °C, injection speed 100 µL/sec, carrier gas flow, to ensure a fast transfer of the sample from the
132 inlet to the trap, 30mL/min), and adsorbed on a Tenax[®] TA trap maintained at 40 °C for 60 sec to
133 concentrate the analytes. The syringe temperature was set at 70 °C. Subsequently, desorption was
134 obtained by increasing the trap temperature to 240 °C in 93 sec and the sample was injected
135 (pressure of the carrier gas at columns' head 40 kPa.) and split (split flow 5 mL/min) into the two
136 columns. The thermal program started at 40 °C (held for 2 sec), increased up to 80 °C at 1 °C/sec,
137 and then to 250 °C at 3 °C/sec. Hydrogen was used as the carrier gas with a pressure from 40 kPa to
138 64 kPa, increasing with a rate of 0.2 kPa/sec. At the end of each column, a FID detector (detector

139 temperature 260 °C) was placed and the acquired signal was digitalized every 0.01 sec. The
140 software used to control the instrument was AlphaSoft version 14.5.

141

142 **2.3 Data processing**

143 For the data analysis, the full chromatograms were processed by applying chemometric elaborations
144 with an untargeted approach. The raw data of each chromatogram (intensity values for each point of
145 the chromatogram considering that the signal was digitalized every 0.01 sec, Palagano et al., 2019
146 [Dataset]) were exported from the software of the instrument and the data set with all the samples
147 was imported into MatlabR2018a[®]. As data pre-treatment, chromatograms were aligned by COW
148 (Correlation Optimized Warping) algorithm (Tomasi, Van Den Berg, & Andersson, 2004) and
149 autoscaled (mean-centering followed by division of each column (variable) by the standard
150 deviation of that column). Preliminary tests showed that chromatograms obtained from the MXT-5
151 column had a discriminant power higher than the other one (MXT-1701) and for this reason the
152 classification models were developed considering only this column. Considering the reduced
153 number of samples for the classes “Blend EU” and “Blend EU-Extra EU”, these oils were grouped
154 together with “EU” and “Extra-EU” samples, respectively. This means that for the data elaboration
155 only two sample categories were considered: “EU” and “Extra-EU”.

156 Two different statistical techniques were used to classify samples according to their geographical
157 origin, the first (PLS-DA) based on a linear approach, and the second (ANN) on a non-linear
158 approach.

159 In particular, the PLS-DA model was built using the PLS Toolbox for Matlab2018a[®]: intensity
160 values of each point of the chromatogram, for a total of 19,900 data points, were used as variables
161 X (matrix X), while the origin (“EU” and “Extra-EU”) was implemented as variable Y (binary
162 variables, 0 - 1). The sample data set was split into a calibration/full-cross validation set (75% of the
163 sample) and an external validation set (25% of the sample) using the Kennard-Stone method
164 (selects samples that best span the same range as the original data, but with an even distribution of

165 samples across the same range) (Daszykowski, Walczak, & Massart, 2002). The threshold value
166 useful to define the category of each sample was defined using a probabilistic approach based on
167 Bayes's rule.

168 The ANN model was performed by using the Neural Net Pattern Recognition tool for
169 Matlab2018a[®]. Specifically, a Multi-Layer Perceptron (MLP) neural network was built to predict
170 the specific class to which samples belong using a non-linear method. For input and hidden layers,
171 linear and logistic activation functions, respectively, were used, while for output layer the SoftMax
172 function was applied. From a statistical point of view, with the SoftMax activation function and
173 cross entropy error, the output is interpretable as posterior probabilities for categorical target
174 variables (Bishop, 1995). One nominal output variable is returned, assuming that the target output is
175 1.0 in the correct class output, and 0.0 in the non-correct class. Looking for the best classification
176 ability, different node numbers in the hidden layer and combinations were tested. The convergence
177 of ANN was ruled by a back propagation algorithm. The original data set was randomly divided
178 into a training set (60%), verification set (20%), and test set (20%). The training set was used to
179 calculate the transfer function parameters of the network, the verification set to indicate possible
180 over-learning, and the test set was treated as an unknown, the correct classification of which
181 indicates that the neural network is performing well. It was checked that samples from both classes
182 were contained in the test set.

183

184 **3. Results and discussion**

185 A set of 210 EVOOs and VOOs were analyzed for their volatile profile by flash gas
186 chromatography. Considering the large amount of data and aim of this work, chemometric
187 elaborations following an untargeted approach were carried out.

188 For elaborations, samples were grouped into two categories: "EU", that included oils from single
189 EU state members and blends of oils from different EU countries, "Extra-EU" that consisted of oils

190 from single countries outside the European Union and blends of oils from the EU and third
191 countries.

192 In Figure 1-a the mean chromatogram of “EU” and “Extra-EU” categories, obtained averaging the
193 intensity of each variable for all “EU” or “Extra-EU” samples, is reported: even if almost all peaks
194 are concentrated in the initial part of the chromatogram (between 2000 and 10000 variables), a clear
195 difference, in terms of variable intensities, exists between the two groups, thus confirming the
196 discriminating power of the volatile profile with respect to the geographical origin (Melucci et al.,
197 2016; Lukić, Carlin, Horvat, & Vrhovsek, 2019).

198 Concerning the PLS-DA results, the values of the estimated Y variable (geographical category) by
199 the model in cross and external validations are shown in Figure 2. The dotted line identifies the
200 threshold value used to define the attribution of samples to different classes. Regarding the location
201 of each sample, a greater distance from the threshold line can be interpreted as a better classification
202 capacity of the model.

203 The results, in terms of percentage and number of samples correctly classified, are reported in Table
204 2. The percentage ranged from 80.8% to 91.2%. The values obtained for the “EU” category were
205 higher, likely because of the greater number and variability of samples used to build the model. The
206 external validation percentages were lower compared to those obtained for the cross-validation as
207 expected, but the results can be considered more robust since they were obtained considering the
208 25% of samples that were not used to build the model.

209 The VIP (Variable Importance in Projection) score obtained by the PLS-DA confirmed that the
210 section of the chromatogram ranging from 2000 to 10000 variables has a major contribution to
211 sample discrimination (VIP values greater than 1) according to geographical origin (Figure 1-b).

212 Focusing on those incorrectly classified samples, a specific trend as a function of characteristics that
213 could usually affect the volatile profile of the oil (such as the commercial category, olive cultivar, or
214 country of origin) was not seen.

215 Results related to the probabilistic approach are shown in Figure 3. The graph refers to the category
216 “EU”: this means that higher a sample is located, the higher the probability for which it is classified
217 as member of the “EU” category. As a consequence, oils classified as members of the other
218 category (Extra-EU) are located in the bottom area of the graph. In this case, the threshold value is
219 fixed at 0.5, corresponding to a probability of 50%: a sample classified with a probability lower
220 than this is considered as not correctly grouped. It is also interesting to note that most of samples
221 were correctly classified with a probability between 90% and 100%.

222 Regarding ANN, an early stopping technique was used to select the number of training cycles
223 (epochs) to avoid over-fitting, using the test set to monitor the prediction error. An example of this
224 procedure is reported in Figure 4, where the best ANN training was characterized by 18 epochs.
225 Above this point, the error increased further indicating that the ANN tends to overfit. Consequently,
226 the results of ANN are related to these iterations.

227 Training was repeated 5 times and the network’s predictions were averaged, since with ANNs
228 convergence is influenced by the initial weight value and the randomized split of data in training,
229 validation, and test sets. The best prediction results were obtained with a three layers network,
230 having 5 nodes; a larger number of nodes did not increase the network performance.

231 The classification results, in terms of percentage of samples correctly classified, are summarized in
232 Table 3. Means and standard deviations (in brackets) were taken into account.

233 As reported for the PLS-DA model, even in this case higher percentages (from 93.2% to 98.7%)
234 were achieved for the “EU” category in all the three data sets.

235 Comparing the results of the external validation (PLS-DA) and testing (ANN), it is possible to note
236 that higher percentages were obtained in the second case for both the “EU” and “Extra-EU”
237 categories. In particular, an increment of 4.7% and 8% of samples correctly classified was obtained.
238 This is probably due to the fact that the ANN model is based on a non-linear approach.

239 In general, the percentages obtained were slightly lower than those reported by other studies based
240 on volatile compounds and chemometric untargeted data elaboration (Gerhardt, Birkenmeier,

241 Sanders, Rohn, & Weller, 2017; Bajoub et al., 2018; Lukić, Carlin, Horvat, & Vrhovsek, 2019).
242 This aspect can be explained by the great variability, in terms of geographical origin, olive variety,
243 commercial category, of the samples analyzed, which represents a strong point of this work.
244 The results described herein confirm the suitability of flash gas chromatography for checking
245 geographical traceability of EVOOs and VOOs, even using untargeted chromatographic signals of
246 the volatile fraction as variables for multivariate analysis (Melucci et al., 2016). An in-house
247 validation of this analytical method, carried out to verify that a repeatable and reproducible signal,
248 with sufficient sensitivity to collect the valuable information from the samples, has been carried out
249 which underlined the good performance of the technique; this will be discussed in more detail in a
250 subsequent publication.

251

252 **4. Conclusions**

253 In this work, the application of flash gas chromatography for volatile compounds analysis combined
254 with untargeted chemometric data elaborations (PLS-DA and ANN) to discriminate EVOOs and
255 VOOs with different geographical origin was presented.

256 For both elaborations, satisfactory results, in terms of percentages of samples correctly classified,
257 were obtained: PLS-DA (external validation) allowed classification of around 89% and 81% of
258 “EU” and “Extra-EU” samples, respectively; for ANN (testing set) the percentages were equal
259 93.2% and 88.8%, respectively.

260 It is important to highlight that these promising results were achieved by analyzing a set of samples
261 that are representative of the large variety of parameters (olive cultivar, country of origin,
262 commercial category) that can describe olive oil product and affect its chemical characteristics. The
263 results obtained herein sustained the use of multivariate chemometrics with untargeted detection of
264 volatile compounds as a powerful tool to discriminate EVOOs and VOOs of different origin. Other
265 studies have already reported that the analysis of volatile compounds is suitable for tracing the
266 geographical origin of VOOs. However, the methodology proposed herein presents some

267 advantages in comparison with other techniques generally applied for this analysis, as it is very
268 rapid (only 200 sec are needed for each chromatographic run) and easy to use since no sample
269 treatment is required.

270

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281

282 **Declaration of interest**

283 None.

284

285 **References**

286

287 Angerosa, F., Bréas, O., Contento, S., Guillou, C., Reniero, F., & Sada, E. (1999). Application of
288 stable isotope ratio analysis to the characterization of the geographical origin of olive oils. *Journal*
289 *of Agricultural and Food Chemistry*, *47*, 1013-1017.

290

291 Bajoub, A., Medina-Rodríguez, S., Ajal, E. A., Cuadros-Rodríguez, L., Monasterio, R. P.,
292 Vercammen, J., Fernández-Gutiérrez, A., & Carrasco-Pancorbo, A. (2018). A metabolic

293 fingerprinting approach based on selected ion flow tube mass spectrometry (SIFT-MS) and
294 chemometrics: A reliable tool for Mediterranean origin-labeled olive oils authentication. *Food*
295 *Research International*, 106, 233-242.

296

297 Bajoub, A., Medina-Rodríguez, S., Hurtado-Fernández, E., Ajal E. A., Ouazzani, N., Fernández-
298 Gutiérrez, & Carrasco-Pancorbo A. (2016). A first approach towards the development of
299 geographical origin tracing models for North Moroccan olive oils based on triacylglycerols profiles.
300 *European Journal of Lipid Science and Technology*, 118, 1223-1235.

301

302 Ben Mohamed, M., Rocchetti, G., Montesano, G., Ben Ali, S., Guasmi, F., Grati-Kamoun, G., &
303 Lucini, L. (2018). Discrimination of Tunisian and Italian extra-virgin olive oils according to their
304 phenolic and sterolic fingerprints. *Food Research International*, 106, 920-927.

305

306 Bishop, C. M. (1995). Neural networks for pattern recognition. Oxford, UK: Oxford University
307 Press.

308

309 Bontempo, L., Paolini, M., Franceschi, P., Ziller, L., García-González, D. L., & Camin, F. (2019).
310 Characterisation and attempted differentiation of European and extra-European olive oils using
311 stable isotope ratio analysis. *Food Chemistry*, 276, 782-789.

312

313 Brereton, R. G., & Lloyd, G. R. (2014). Partial least squares discriminant analysis: taking the magic
314 away. *Journal of Chemometrics*, 28, 213-225.

315

316 Chiocchini, F., Portarena, S., Ciolfi, M., Brugnoli, E., & Lauteri, M. (2016). Isoscapes of carbon
317 and oxygen stable isotope compositions in tracing authenticity and geographical origin of Italian
318 extra-virgin olive oils. *Food Chemistry*, 202, 291-301.

319

320 Commission implementing regulation (EU) No 29/2012 of 13 January 2012 on marketing standards
321 for olive oil. *Official Journal of European Journal, L 12*, 14-21.

322

323 Conte, L., Bendini, A., Valli, E., Lucci, P., Moret, S., Maquet, A., Lacoste, F., Brereton, P., García-
324 González, D. L., Moreda, W., & Gallina Toschi, T. (2019). Olive oil quality and authenticity: A
325 review of current EU legislation, standards, relevant methods of analyses, their drawbacks and
326 recommendations for the future. *Trends in Food Science & Technology*, in press.
327 doi.org/10.1016/j.tifs.2019.02.025

328

329 Daszykowski, M., Walczak, B., Massart, D. L. (2002). Representative subset selection. *Analytica*
330 *Chimica Acta*, 468, 91-103.

331

332 Dekhili, S., Sirieix, L., & Cohen, E. (2011). How consumers choose olive oil: the importance of
333 origin cues. *Food Quality and Preference*, 22, 757-762.

334

335 Del Giudice, T., Cavallo, C., Caracciolo, F., & Cicia, G. (2015). What attributes of extra virgin
336 olive oil are really important for consumers: a meta-analysis of consumers' stated preferences.
337 *Agricultural and Food Economics*, 3, article number 20.

338

339 García-González D. L., Luna, G., Morales, M. T., & Aparicio, R. (2009). Stepwise geographical
340 traceability of virgin olive oils by chemical profiles using artificial neural network models.
341 *European Journal of Lipid Science and Technology*, 111, 1003-1013.

342

343 Gerhardt, N., Birkenmeier, M., Sanders, D., Rohn, S., & Weller P. (2017). Resolution-optimized
344 headspace gas chromatography-ion mobility spectrometry (HS-GC-IMS) for non-targeted olive oil
345 profiling. *Analytical and Bioanalytical Chemistry*, 409, 3933-3942.

346

347 Ghisoni, S., Lucini, L., Angilletta, F., Rocchetti, G., Farinelli, D., Tombesi, S., & Trevisan, M.
348 (2019). Discrimination of extra-virgin-olive oils from different cultivars and geographical origins
349 by untargeted metabolomics. *Food Research International*, 121, 746-753.

350

351 Giacalone, R., Giuliano, S., Gulotta, E., Monfreda, M., & Presti, G. (2015). Origin assessment of
352 EV olive oils by esterified sterols analysis. *Food Chemistry*, 188, 279-285.

353

354 Kosma, I., Vatavali, K., Kontakos, S., Kontominas, M., Kiritsakis, A., & Badeka, A. (2017).
355 Geographical differentiation of Greek extra virgin olive oil from late- harvested Koroneiki cultivar
356 fruits. *Journal of the American Oil Chemists' Society*, 94, 1373-1384.

357

358 Lukić, I., Carlin, S., Horvat, I., & Vrhovsek, U. (2019). Combined targeted and untargeted profiling
359 of volatile aroma compounds with comprehensive two-dimensional gas chromatography for
360 differentiation of virgin olive oils according to variety and geographical origin. *Food Chemistry*,
361 270, 403-414.

362

363 Mallamace, D., Vasi, S., Corsaro, C., Naccari, C., Clodoveo, M. L., Dugo, G., & Cicero, N. (2017).
364 Calorimetric analysis points out the physical-chemistry of organic olive oils and reveals the
365 geographical origin. *Physica A*, 486, 925-932.

366

367 Melucci, D., Bendini, A., Tesini, F., Barbieri, S., Zappi, A., Vichi, S., Conte, L., & Gallina Toschi,
368 T. (2016). Rapid direct analysis to discriminate geographic origin of extra virgin olive oils by flash
369 gas chromatography electronic nose and chemometrics. *Food Chemistry*, 204, 263-273.

370

371 Palagano, R., Bendini, A., Valli, E., Cevoli, C., Gallina Toschi, T. (2019). OLEUM Project.
372 Analysis of volatile compounds of extra virgin and virgin olive oil with a different geographical
373 origin by flash gas chromatography. <http://doi.org/10.6092/unibo/amsacta/6243> [Dataset]

374

375 Peršurić, Ž., Saftić, L., Mašek, T., & Kraljević Pavelić, S. (2018). Comparison of triacylglycerol
376 analysis by MALDI-TOF/MS, fatty acid analysis by GC-MS and non-selective analysis by NIRS in
377 combination with chemometrics for determination of extra virgin olive oil geographical origin. A
378 case study. *LWT - Food Science and Technology*, 95, 326-332.

379

380 Quintanilla-Casas, B., Bertin, S., Leik, K., Bustamante, J., Guardiola, F., Valli, E., Bendini, A.,
381 Gallina Toschi, T., Tres, A., & Vichi, S. (2020). Profiling versus fingerprinting analysis of
382 sesquiterpene hydrocarbons for the geographical authentication of extra virgin olive oils. *Food*
383 *Chemistry*, 307, Article number 125556.

384

385 Sayago, A., González-Domínguez, R., Beltrán, R., & Fernández-Recamales, A. (2018).
386 Combination of complementary data mining methods for geographical characterization of extra
387 virgin olive oils based on mineral composition. *Food Chemistry*, 261, 42-50.

388

389 Tomasi, G., Van Den Berg, F., & Andersson, C. (2011). Correlation optimized warping and
390 dynamic time warping as preprocessing methods for chromatographic data. *Journal of*
391 *Chemometrics*, 18, 231-241.

392

393 Valli, E., Bendini, A., Berardinelli, A., Ragni, L., Riccò, B., Grossi, M., & Gallina Toschi, T.
394 (2016). Rapid and innovative instrumental approaches for quality and authenticity of olive oils.
395 *European Journal of Lipid Science and Technology*, 118, 1601-1619.

396

397 Vichi, S., Pizzale, L., & Conte, L. (2007). Stereospecific distribution of fatty acids in
398 triacylglycerols of olive oils. *European Journal of Lipid Science and Technology*, 109, 72-78.

Origin class	N	Country of origin
EU	116	29 Spain, 25 Italy, 22 Croatia, 16 Greece, 12 Portugal, 12 Slovenia
Extra-EU	70	42 Morocco, 21 Turkey, 6 Tunisia, 1 Chile
Blends	24	12 EU blends, 12 EU/Extra-EU blends

399 **Table 1.** Number of samples for each origin class considered and geographical origin. EU: oils
400 from EU state members; Extra-EU: oils from countries outside the European Union; Blends: oils
401 obtained mixing EU oils or EU and Extra-EU oils.

Category	Cross validation	External validation
EU	91.2% (93/102)	88.5% (23/26)
Extra-EU	91.1% (51/56)	80.8% (21/26)

402 **Table 2.** Percentages and number (in parentheses) of correctly classified samples for each category
403 using the PLS-DA model. EU: oils from a single state member of European Union and oils obtained
404 by mixing EU oils; Extra-EU: oils from a single country outside the European Union and oils
405 obtained by mixing EU and Extra-EU oils.

Category	Training (%)	Validation (%)	Testing (%)
EU	98.7 (1.1)	95.4 (3.9)	93.2 (3.2)
Extra-EU	94.4 (7.0)	88.7 (7.8)	88.8 (5.4)

406 **Table 3.** Percentages (mean of 5 training of the model and standard deviation in parentheses) of
407 samples correctly classified for each category using the ANN model. EU: oils from a single state
408 member of European Union and oils obtained by mixing EU oils; Extra-EU: oils from a single
409 country outside the European Union and oils obtained by mixing EU and Extra-EU oils.

Figure 1. a) Mean chromatogram obtained averaging the intensity of each variable for all “EU” (blue line) or “Extra-EU” (violet line) samples; b) VIP score for each variable (signal digitalized every 0.01 sec).

Figure 2. Graph of the values of the Y variable estimated by the PLS-DA model, in cross and external validations (grey area). Blue squares: EU; violet triangle: Extra-EU.

Figure 3. Probability values of belonging to the “EU” category. 1 = probability of 100%; 0 = probability of 0%. Gray area: test set used for external validation of the PLS-DA model. Blue squares: EU; violet triangles: Extra-EU.

Figure 4. Error graph of validation (green line) and test (red line) set used for the ANN model.

Table S1. Information about country of origin, olive variety and commercial category for all the samples collected and analyzed.

Code	Origin class	Country of origin	Olive variety	Commercial category
1	Blend EU	GRC - ITA	Manaki - Coratina	EVOO ^b
2	Blend EU	GRC - ITA	Manaki - Moraiolo	EVOO ^b
3	Blend EU	GRC - ITA	Manaki - NA	EVOO ^b
4	Blend EU	GRC - ITA - ESP	Manaki - Arbequina - NA	EVOO ^b
5	Blend EU	GRC - ITA - ESP	Manaki - Arbequina - Coratina	EVOO ^b
6	Blend EU	GRC - ITA - ESP	Manaki - Arbequina - Moraiolo	EVOO ^b
7	Blend EU	GRC - ESP	Manaki - Arbequina	EVOO ^b
8	Blend EU	GRC - ESP	Koroneiki - Arbequina - Picual - Cornicabra	EVOO ^a
9	Blend EU	ESP - ITA	Arbequina - NA	EVOO ^b
10	Blend EU	ESP - ITA	Arbequina - Coratina	EVOO ^b
11	Blend EU	ESP - ITA	Arbequina - Moraiolo	EVOO ^b
12	Blend EU	ESP - PRT	Arbequina - Arbosana	EVOO ^b
13	Blend Extra-EU	GRC - TUN	Manaki - Sahli	VOO ^b
14	Blend Extra-EU	ITA - TUN	NA - Sahli	VOO ^b
15	Blend Extra-EU	ITA - TUN	Sahli - Coratina	VOO ^b
16	Blend Extra-EU	ITA - TUN	Sahli - Moraiolo	VOO ^b
17	Blend Extra-EU	ESP - MAR	Picual - Moroccan Picholine	EVOO ^b
18	Blend Extra-EU	ESP - MAR	Picual - Languedoc Picholine	EVOO ^b
19	Blend Extra-EU	ESP - MAR	Picual - Moroccan Picholine - Koroneiki	EVOO ^b
20	Blend Extra-EU	ESP - TUN	Arbequina - Sahli	VOO ^b
21	Blend Extra-EU	ESP - TUN	Picual - Chemlali - Chetoui	EVOO ^b
22	Blend Extra-EU	ESP - TUN	Picual - Chemlali	EVOO ^b
23	Blend Extra-EU	ESP - TUN	Picual - Chemlali	EVOO ^b
24	Blend Extra-EU	ESP - TUN	Picual - Chetoui	EVOO ^b
25	EU	HRV	Picholine	EVOO ^a
26	EU	HRV	Leccio del Corno	EVOO ^a
27	EU	HRV	Istarska Bjelica	EVOO ^a
28	EU	HRV	Rosinjola	EVOO ^a
29	EU	HRV	Leccino - Pendolino	EVOO ^a
30	EU	HRV	Leccino - Pendolino	EVOO ^a
31	EU	HRV	Picholine - Leccio del Corno	EVOO ^a
32	EU	HRV	Istarska Bjelica	EVOO ^a
33	EU	HRV	Oblica	EVOO ^a
34	EU	HRV	Istarska Bjelica - Leccino - Buža	EVOO ^a
35	EU	HRV	Istarska Bjelica - Leccino - Buža	EVOO ^a
36	EU	HRV	Leccino - Pendolino	EVOO ^a

37	EU	HRV	Picholine - Leccio del Corno	EVOO ^a
38	EU	HRV	Ascolana Tenera - Itrana - Frantoio	EVOO ^a
39	EU	HRV	Buža Puntoža - Rosinjola - Bova	EVOO ^a
40	EU	HRV	Istarska Bjelica	EVOO ^a
41	EU	HRV	Ascolana Tenera - Itrana - Frantoio	EVOO ^a
42	EU	HRV	Buža Puntoža	EVOO ^a
43	EU	HRV	Buža Puntoža	EVOO ^a
44	EU	HRV	Picholine	EVOO ^a
45	EU	HRV	Plominka - Simjaca	EVOO ^a
46	EU	HRV	Oblica	EVOO ^a
47	EU	GRC	Koroneiki	VOO ^a
48	EU	GRC	Koroneiki	EVOO ^a
49	EU	GRC	Koroneiki	EVOO ^b
50	EU	GRC	Manaki	EVOO ^b
51	EU	GRC	Koroneiki	EVOO ^b
52	EU	GRC	Koroneiki	EVOO ^b
53	EU	GRC	Manaki	EVOO ^a
54	EU	GRC	Koroneiki	EVOO ^a
55	EU	GRC	Koroneiki	EVOO ^a
56	EU	GRC	NA	VOO ^a
57	EU	GRC	Koroneiki	EVOO ^a
58	EU	GRC	Koroneiki	EVOO ^a
59	EU	GRC	Koroneiki	EVOO ^b
60	EU	GRC	Koroneiki	EVOO ^a
61	EU	GRC	Koroneiki	EVOO ^a
62	EU	GRC	NA	EVOO ^a
63	EU	ITA	Coratina	EVOO ^a
64	EU	ITA	Coratina	VOO ^a
65	EU	ITA	Frantoio	EVOO ^a
66	EU	ITA	Castiglione	VOO ^a
67	EU	ITA	Leccino - Frantoio - Pendolino	EVOO ^a
68	EU	ITA	Leccino - Frantoio - Pendolino	VOO ^a
69	EU	ITA	Arbequina	EVOO ^a
70	EU	ITA	Coratina - Ogliarola	EVOO ^a
71	EU	ITA	Nocellara del Belice	EVOO ^a
72	EU	ITA	Biancolilla	EVOO ^a
73	EU	ITA	Nocellara del Belice	EVOO ^a
74	EU	ITA	Leccino - Frantoio - Moraiolo	EVOO ^a
75	EU	ITA	Coratina	EVOO ^a
76	EU	ITA	Coratina	VOO ^a
77	EU	ITA	Nostrana di Brisighella	EVOO ^a
78	EU	ITA	Leccino - Frantoio - Moraiolo	EVOO ^a

79	EU	ITA	Nostrana di Brisighella	EVOO ^a
80	EU	ITA	NA	EVOO ^a
81	EU	ITA	Coratina	EVOO ^a
82	EU	ITA	Moraiolo	EVOO ^a
83	EU	ITA	Carolea	EVOO ^a
84	EU	ITA	Dritta - Leccino	EVOO ^a
85	EU	ITA	Frantoio	EVOO ^a
86	EU	ITA	Peranzana	EVOO ^a
87	EU	ITA	Peranzana	EVOO ^a
88	EU	PRT	Arbequina - Koroneiki	EVOO ^b
89	EU	PRT	Arbosana	EVOO ^b
90	EU	PRT	Arbosana	EVOO ^b
91	EU	PRT	Arbosana	EVOO ^b
92	EU	PRT	Koroneiki	EVOO ^b
93	EU	PRT	Arbequina	EVOO ^b
94	EU	PRT	Arbequina	EVOO ^b
95	EU	PRT	Arbequina	EVOO ^b
96	EU	PRT	Sikitita	EVOO ^b
97	EU	PRT	Arbosana	EVOO ^b
98	EU	PRT	NA	EVOO ^b
99	EU	PRT	NA	EVOO ^b
100	EU	SVN	Istarska Bjelica - Leccino - Others	EVOO ^a
101	EU	SVN	Istarska Bjelica - Leccino - Others	EVOO ^a
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123	EU	ESP	Arbequina	EVOO ^a
124	EU	ESP	Picual	EVOO ^a
125	EU	ESP	Arbequina - Hojiblanca	EVOO ^a
126	EU	ESP	Arbequina - Hojiblanca	VOO ^a
127	EU	ESP	Manzanilla	EVOO ^a
128	EU	ESP	Manzanilla	EVOO ^a
129	EU	ESP	Arbequina	EVOO ^a
130	EU	ESP	Hojiblanca	EVOO ^a
131	EU	ESP	Koroneiki	EVOO ^b
132	EU	ESP	Hojiblanca	EVOO ^b
133	EU	ESP	Manzanilla - Hojiblanca - Picual	EVOO ^a
134	EU	ESP	NA	EVOO ^a
135	EU	ESP	NA	VOO ^a
136	EU	ESP	Hojiblanca	EVOO ^a
137	EU	ESP	Hojiblanca	EVOO ^a
138	EU	ESP	Picual	VOO ^a
139	EU	ESP	NA	EVOO ^a
140	EU	ESP	Arbequina	EVOO ^a
141	Extra-EU	CHL	NA	EVOO ^a
142	Extra-EU	MAR	Arbequina	EVOO ^a
143	Extra-EU	MAR	Arbequina	EVOO ^b
144	Extra-EU	MAR	Koroneiki	EVOO ^b
145	Extra-EU	MAR	Arbosana	EVOO ^b
146	Extra-EU	MAR	Arbequina	EVOO ^b
147	Extra-EU	MAR	Arbequina	EVOO ^b
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149	Extra-EU	MAR	Arbosana	EVOO ^b
150	Extra-EU	MAR	Koroneiki	EVOO ^b
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152	Extra-EU	MAR	Arbequina	EVOO ^b
153	Extra-EU	MAR	Arbosana	EVOO ^b
154	Extra-EU	MAR	Koroneiki	EVOO ^b
155	Extra-EU	MAR	Moroccan Picholine - Hojiblanca	EVOO ^b
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164	Extra-EU	MAR	Moroccan Picholine	EVOO ^b
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167	Extra-EU	MAR	Moroccan Picholine	EVOO ^b
168	Extra-EU	MAR	Moroccan Picholine	EVOO ^b
169	Extra-EU	MAR	Picholine - Arbosana	EVOO ^b
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176	Extra-EU	MAR	Arbequina	EVOO ^b
177	Extra-EU	MAR	Moroccan Picholine	EVOO ^b
178	Extra-EU	MAR	Arbequina - Koroneiki	VOO ^b
179	Extra-EU	MAR	Arbequina	EVOO ^b
180	Extra-EU	MAR	Moroccan Picholine - Arbequina	EVOO ^b
181	Extra-EU	MAR	Moroccan Picholine	VOO ^b
182	Extra-EU	MAR	Moroccan Picholine	EVOO ^b
183	Extra-EU	MAR	Arbequina	VOO ^b
184	Extra-EU	TUN	Chetoui - Chemlali	VOO ^a
185	Extra-EU	TUN	Sahli	VOO ^a
186	Extra-EU	TUN	Sahli - Chemlali	EVOO ^b
187	Extra-EU	TUN	Chemlali	EVOO ^b
188	Extra-EU	TUN	Chemlali	EVOO ^b
189	Extra-EU	TUN	Chetoui	EVOO ^b
190	Extra-EU	TUR	Ayvalik - Domat	EVOO ^a
191	Extra-EU	TUR	Memecik - Gemlik	EVOO ^a
192	Extra-EU	TUR	Memecik	EVOO ^a
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200	Extra-EU	TUR	Memecik	EVOO ^a
201	Extra-EU	TUR	Gemlik - Memecik	VOO ^a
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204	Extra-EU	TUR	Memecik	VOO ^a

205	Extra-EU	TUR	Memecik	EVOO ^a
206	Extra-EU	TUR	Saurani - Halhali - Karamani	VOO ^a
207	Extra-EU	TUR	Edremit - Domat - Gemlik	VOO ^a
208	Extra-EU	TUR	Memecik	VOO ^a
209	Extra-EU	TUR	Ayvalik - Edremit	VOO ^a
210	Extra-EU	TUR	NA	EVOO ^a

NA: information not available; EVOO: extra virgin olive oil; VOO: virgin olive oil.

EU: oils from state members of European Union; Extra-EU: oils from countries outside European Union; Blends: oils obtained mixing EU oils or EU and Extra-EU oils.

CHL: Chile; ESP: Spain; GRC: Greece; HRV: Croatia; ITA: Italy; MAR: Morocco; PRT: Portugal; SVN: Slovenia; TUN: Tunisia; TUR: Turkey.

^a Commercial category defined by Panel Test realized in the framework of the OLEUM project.

^b Commercial category declared by the company that provided the sample.

Figure 1
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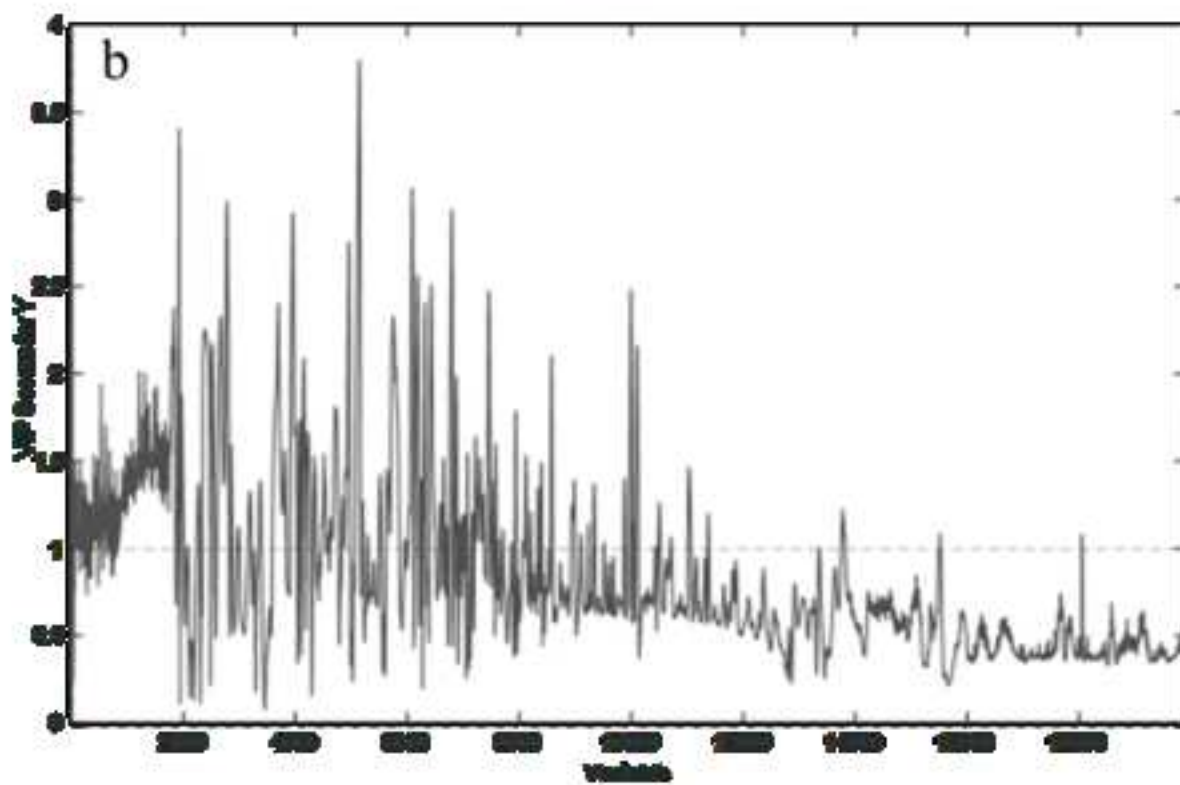
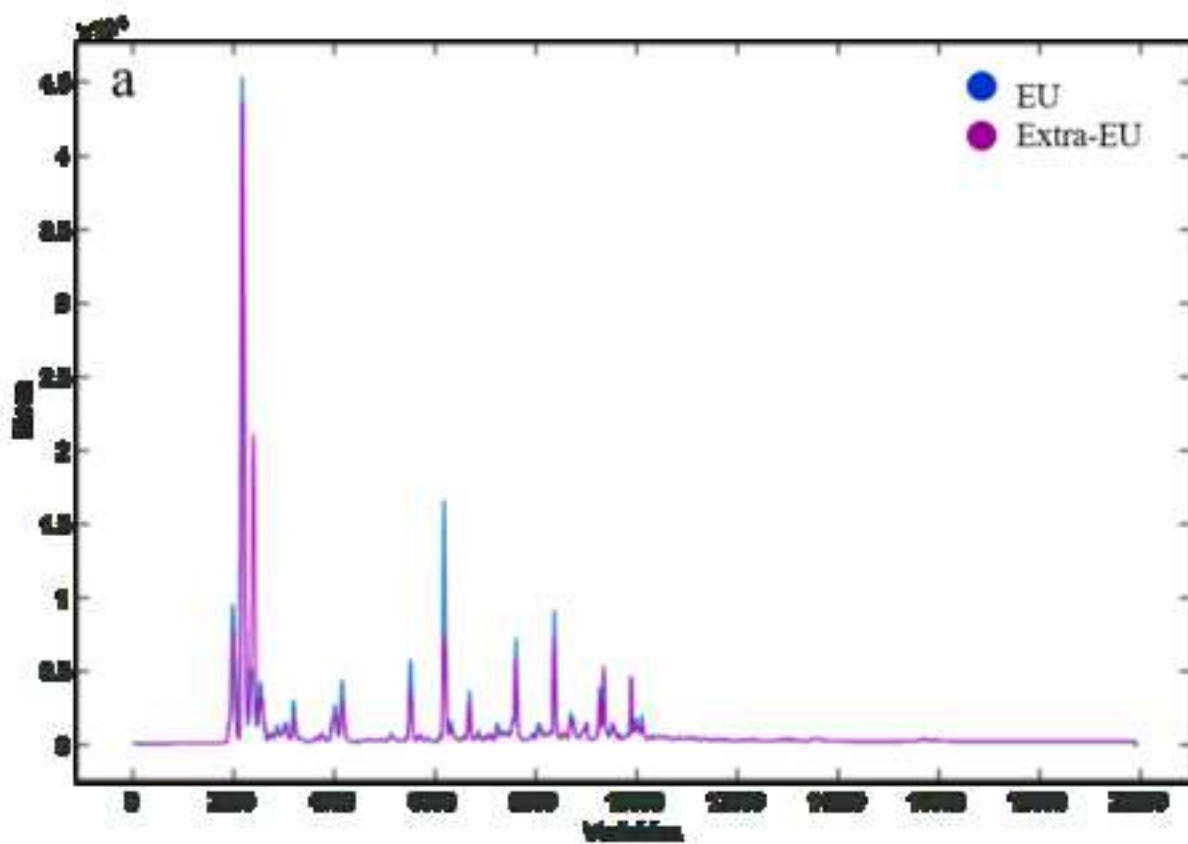


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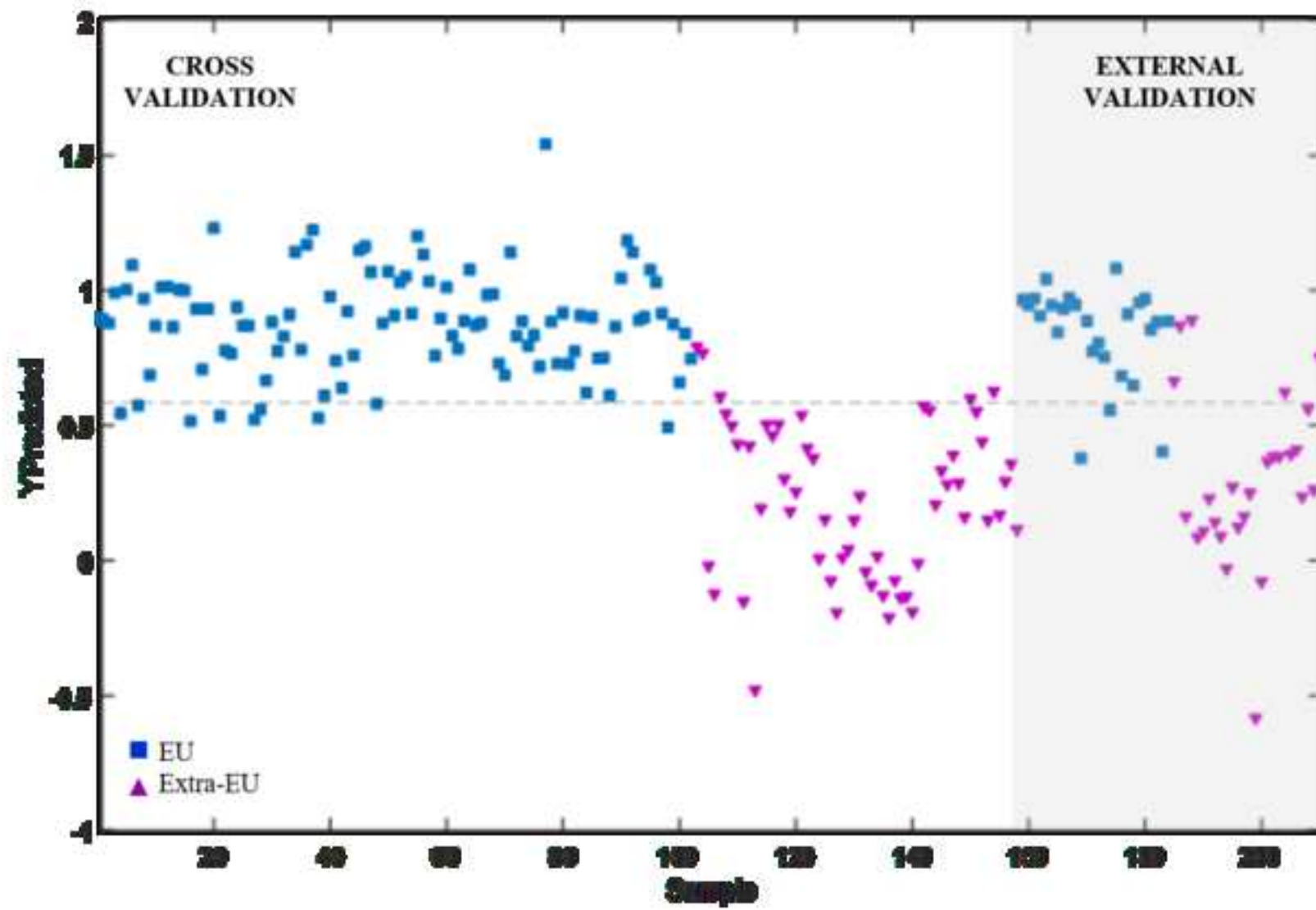


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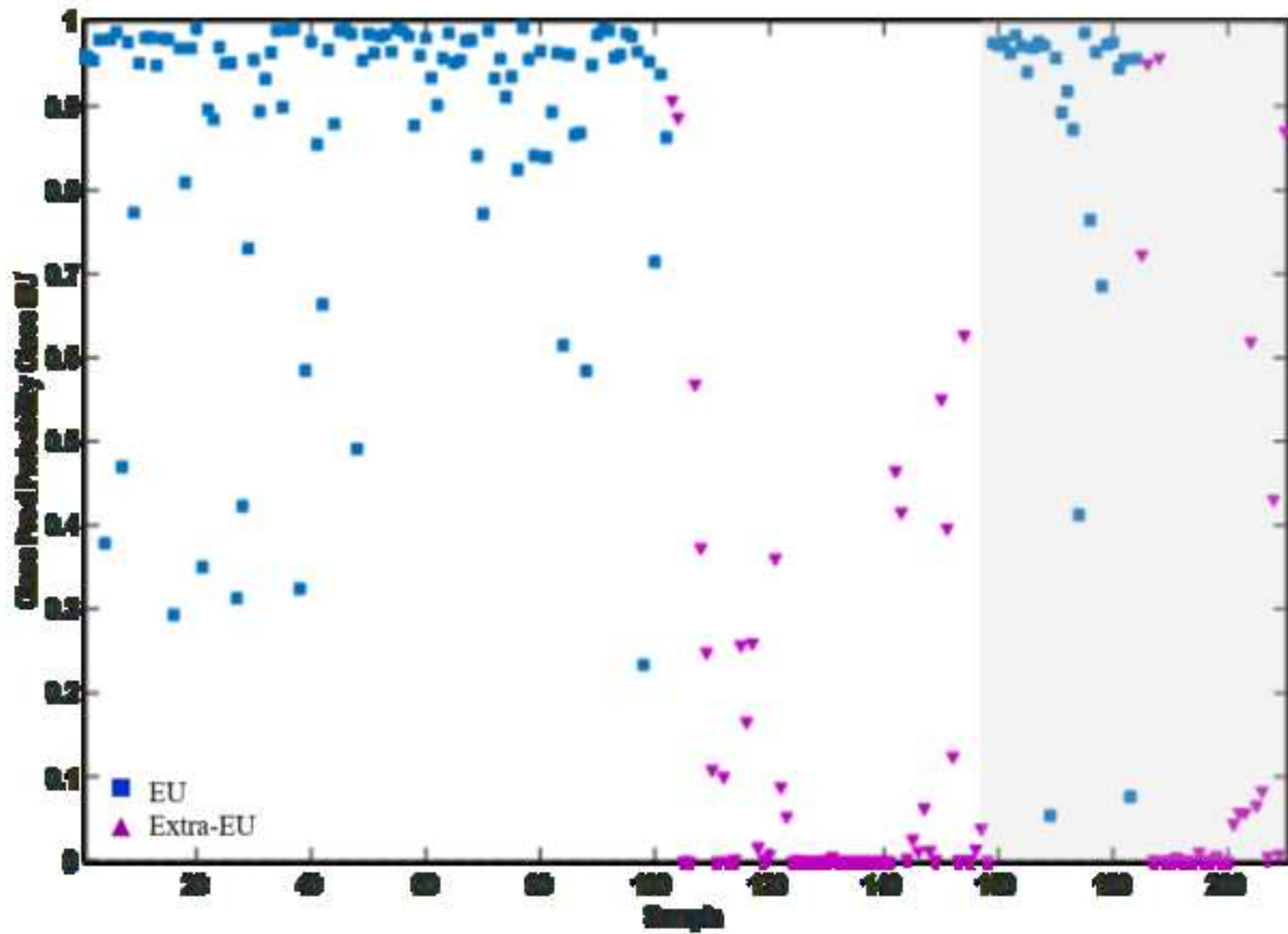
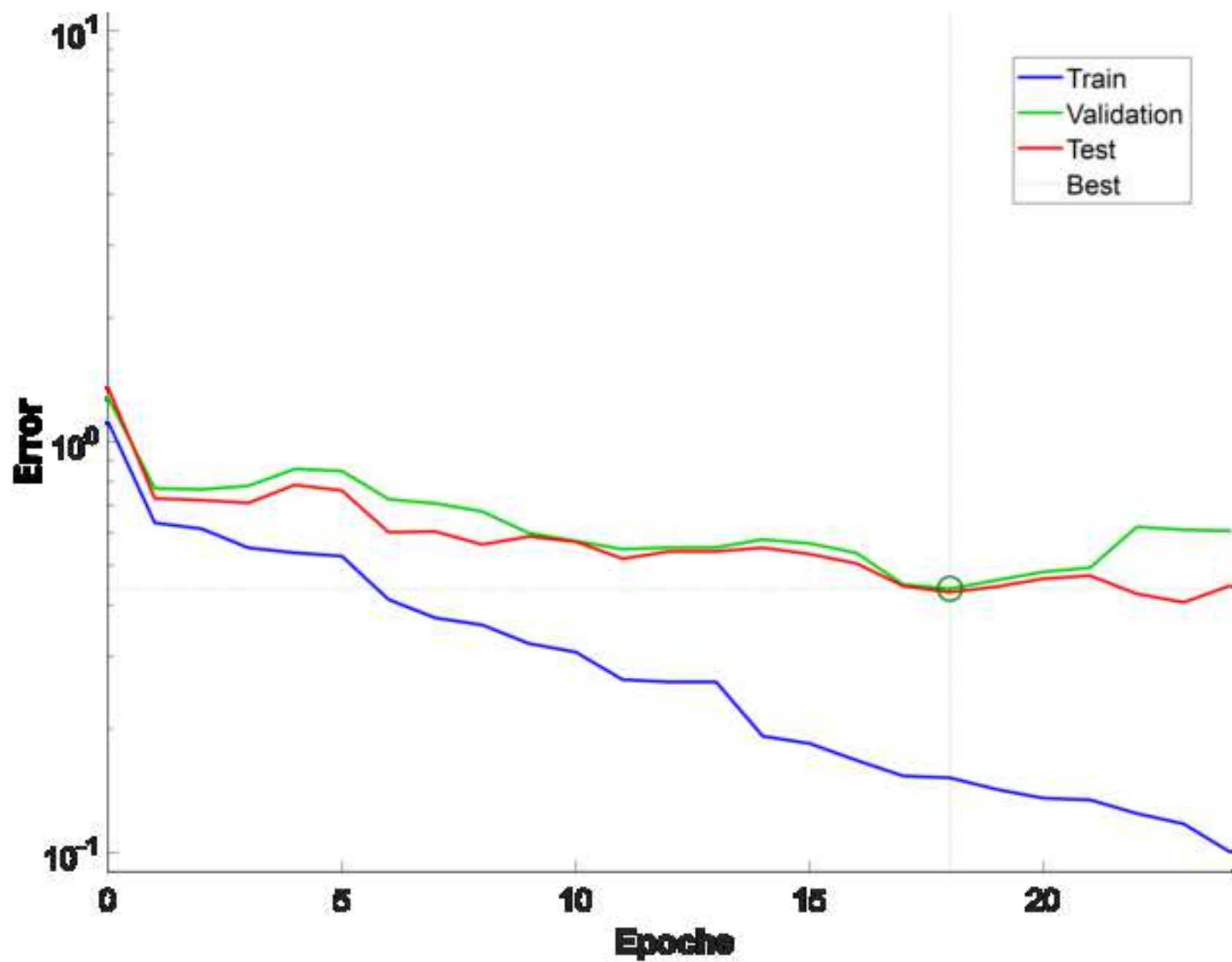


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