JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

CORRESPONDENCE/REBUTTAL

Comment on Cluster Analysis Applied to the Exploratory Analysis of Commercial Spanish Olive Oils by Means of Excitation−Emission Fluorescence Spectroscopy

Sir: In a recent work (*1*) the authors use spectrofluorometric data to differentiate extra virgin olive oils (EVOO) from olive and olive-pomace oils. According to the authors' analysis, fluorescence intensities of 56 *undiluted* Spanish oils, collected at 300−400 nm excitation and 400−700 nm emission wavelengths, are able to distinguish the class of the oil samples.

We found that some features of the published spectra in ref *1* reveal inner filter effects (*2*), which strongly alter the true fluorescence bands of EVOO. In particular, the absence of the fluorescence due to chlorophylls, when the excitation wavelength is <320 nm, contrasts with the strong emission of chlorophylls observed when oil samples are excited at λ_{exc} near 350 nm (Figure 1a in ref *1*). It is difficult to explain this lack of fluorescence, because the absorbance of chlorophylls or pheophytins, the probable derivatives of chlorophylls in olive oils, near 300 nm is about the same as that at 350 nm (*3*). As far as extra virgin oils are concerned, we found a minimum of light absorption at, or near, 350 nm and a very high absorption at 300 nm in all of the native samples of Italian EVOO that we have recently analyzed (*4*).

The experimental data in ref *1* were obtained through the commonly used right-angle (RA) fluorescence technique. With such a technique, absorbance effects (*primary and secondary inner filter effects*) (*2*) that greatly affect both the intensity and the spectral shape of the fluorescence bands of EVOO must be taken into account (*4*). To clearly show the effects of these inner filters, and due to the impossibility of using the same oils as in ref *1*, we measured the absorbance and the "vertical beam" RA fluorescence of a Spanish EVOO and progressively diluted solutions of the same oil in a transparent, nonreactive solvent (**Figure 1**).

Curve a of **Figure 1** shows the RA emission (λ_{exc} = 345 nm) of a 1-year-old sample of a Valencian EVOO, together with the emissions of its solutions at 1:3, 1:6, 1:30, and 1:150 with *n*-hexane (curves b−e, respectively, of **Figure 1**). The native oil emission spectrum in the 400−600 nm spectral region (curve a) is very similar to the average spectrum of Spanish EVOO reported in Figure 6a of ref *1*. An immediate observation is that dilution of the oil at 1:3 and 1:6 with *n*-hexane *increases* the fluorescence intensity at λ < 500 nm with respect to the pure oil sample, whereas it *decreases* at λ > 530 nm. This

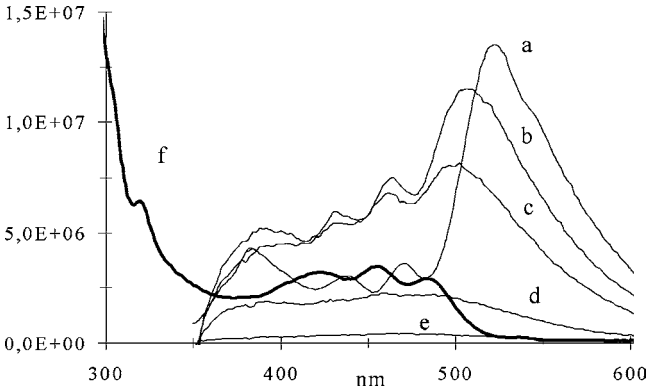

Figure 1. Fluorescence spectra of a Spanish EVOO on 345 nm excitation (a) and of the same oil diluted with *n*-hexane at 1:3 (b), 1:6 (c), 1:30 (d), and 1:150 (e). The absorbance (1 cm path length) of the native oil, multiplied for 10^6, is reported in curve f.

contrasts with the uniform reduction of the intensity in the whole wavelength range observed in the absorbance spectra of the same solutions and expected also in the fluorescence spectra. Moreover, considerable changes in the shape are observed in the emissions (λ_{exc} = 345 nm) on dilution with *n*-hexane. The maximum shifts from 525 nm, in native oil, to shorter wavelengths upon dilution, finally ending at 460 nm in a structureless band, with the two most diluted oil solutions (curves d and e of **Figure 1**). Only the spectra of these very diluted solutions have an intensity proportional to the oil concentration. Curve f of **Figure 1** presents the absorbance of our Valencian oil, multiplied for 1 million in order to be compared with the fluorescence of the same oil. This curve shows absorbance maxima exactly corresponding to the minima of fluorescence curves a−c and vice versa. With an absorbance of 2 absorbance units/(cm of optical path length) at 345 nm, ∼10% of excitation photons reach the middle of the sample, ∼0.5 cm optical path, but the fluorescence there produced is partially self-absorbed (secondary inner filter effects). This explains why fluorescence minima correspond to absorption maxima. At 300 nm the absorbance is 13, and ∼*1* in *3,000,000* incident excitation photons reaches the middle of the sample. Thus, the emission detected (data not reported) is only noise, a primary inner filter effect.

10.1021/jf047797o CCC: $30.25
Published on Web 06/08/2005

We exclude that the dramatic spectral changes upon dilution in **Figure 1** are due to "solvent effects". In fact, oil solutions, similarly diluted with diethyl ether, present only marginal differences with respect to the spectra in **Figure 1**.

Cluster analysis applied to the RA fluorescence excitation−emission matrices (EEM) of EVOOs, as described by Guimet et al. (*1*), proves to be a potential method to distinguish the class of the oils. Here we point out that the EEM fingerprints in ref *1* are probably due mostly to their absorbance differences rather than to their fluorescence. Thus, it would be of much interest to apply Guimet's analysis methods to the simple absorption spectra of their oil samples or to the fluorescence spectra obtained with the front-face technique in which artifacts due to the absorbance are strongly reduced (*4*, *5*).

LITERATURE CITED

(1) Guimet, F.; Boqué, R.; Ferré, J. Cluster analysis applied to the exploratory analysis of commercial Spanish olive oils by means of excitation−emission fluorescence spectroscopy. *J. Agric. Food Chem*. **2004**, *52*, 6673−6679.

(2) Lakowicz, J. R. Instrumentation for fluorescence spectroscopy. In *Principles of Fluorescence Spectroscopy*, 2nd ed.; Kluwer Academic/Plenum Publishers: New York, 1999; pp 25−60.

(3) Du, H.; Fuh, A. R.; Li, J.; Corkan, A.; Lindsey, J. S. PhotochemCAD: a computer-aided design and research tool in photochemistry. *Photochem. Photobiol.* **1998**, *68*, 141−142.

(4) Zandomeneghi, M.; Carbonaro, L.; Caffarata, C. Fluorescence of vegetable oils: olive oils. *J. Agric. Food Chem*. **2005**, *53*, 759−766.

(5) Sikorska, E.; Romaniuk, A.; Khmelinskii, I. V.; Herance, R.; Bourdelande, J. L.; Sikorski, M.; Koziol, J. Characterization of edible oils using total luminescence spectroscopy. *J. Fluorescence* **2004**, *14*, 25−35.

Received for review December 29, 2004. Revised manuscript received May 18, 2005. Accepted May 19, 2005.

Maurizio Zandomeneghi*,† and Giorgia Zandomeneghi§

Dipartimento di Chimica e Chimica Industriale, Università di Pisa, Via Risorgimento 35, 56126 Pisa, Italy, and Institut für Molekulare Biotecnologie (IMB), Beutenbergstrasse 11, D-07708 Jena, Germany

JF047797O

* Author to whom correspondence should be addressed (telephone +39-050-2219267; fax +39-050-2219260; e-mail zando@dcci.unipi.it).
† Università di Pisa.
§ Institut für Molekulare Biotecnologie.