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Detection of Virgin Olive Oil Adulteration with Refined Oils by Second-Derivative Spectrophotometry

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ABSTRACT

Weak absorption maxima of conjugated tetraenoic systems (285–315 nm) can be quantitated by second-derivative spectrophotometry. The distance of two consecutive extremes (maximum–minimum) of the second-derivative reflection at 315 nm, measured in units of absorptivity (ΔK_{315}) shows the most characteristic differences between virgin olive oil and refined olive, olive kernel and seed oils. The ranges of $\Delta K^{1\%}_{315}$ values were found to be 0·008–0·015 and 0·010–0·030 for virgin olive oils of the last and older crops, respectively, whereas $\Delta K^{1\%}_{315}$ values of refined olive and olive kernel oils exceed 0·450 and 0·990, respectively. It is demonstrated that 5% adulteration of virgin olive oil by refined oils may be detected effectively by this technique.

INTRODUCTION

The detection of adulteration of virgin olive oil with low proportions of refined olive or olive kernel oils is not yet possible with the required safety and sensitivity. So far, UV-spectrometry is the most satisfactory technique in common use for this purpose, but its sensitivity is limited because virgin olive oils themselves show a wide range of absorptivity at 270 nm ($K^{1\%}_{1\,cm}$), which, in many cases, does not allow the unequivocal detection of such adulterations to the extent of 10–20%.

Substantial progress on this problem was achieved by studying the features of the UV spectra of virgin and refined oils in the region of

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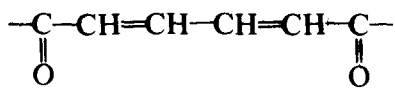
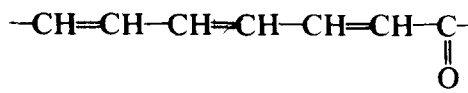


(I)



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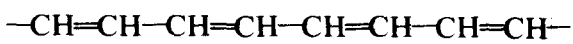

(III)

310–320 nm, where conjugated tetraene systems present their absorption maxima. Such tetraene systems of the types (I), (II) and (III) are secondary oxidation products of some chain reactions during auto-oxidation of oils (Galanos *et al.*, 1968*a*). As a result, the UV spectra of oils containing these oxidation products show either a distinct peak at 315 nm, or a distinguishable disturbance of the smoothness of the curves in the region between 310 and 320 nm, depending on their concentration (Galanos *et al.*, 1968*a*). A quantitative estimate of this property is given by the value $R_s = (K^{4\%}_{315} - K^{4\%}_{320})/(K^{4\%}_{310} - K^{4\%}_{315})$. By using this coefficient, the detection of 2–5% of refined oils in virgin olive oil is possible in most cases (Galanos *et al.*, 1968*a*).

However, experimental errors in measuring K_{310}, K_{315} and K_{320} in low absorbing samples, as well as translocation of the apparent maximum in the region of 315 nm in virgin olive oils towards shorter or longer wavelengths (V. M. Kapoulas and S. Passaloglou-Emmanouilidou, unpublished data) may be sources of false conclusions (e.g. detection of non-existing adulteration) in some—albeit few—cases of virgin olive oils.

Described in this paper is a new approach to a beneficial utilization of the aforementioned properties based on the advantages of second-derivative spectrometry, which provides much more accurate information when conventional spectrophotometric methods are less informative.

MATERIALS AND METHODS

Virgin olive oil samples of guaranteed purity, with a free fatty acid content of less than 1% (expressed as oleic acid) were obtained from the Special Experimental Laboratory of the Greek Ministry of Commerce, or from oil-producing industries.

Samples of refined olive and olive kernel oils, as well as of olive and seed oils at various stages of their refinement, were obtained from oil industries. All these oil samples were of domestic (Greek) origin.

All oil samples were studied as 0·5–4·0% (w/v) solutions in cyclohexane, UV grade (Merk). The UV spectra were taken by using a Perkin-Elmer UV–VIS spectrophotometer, model 551, connected with derivative

accessory and chart recorder, model 550-0110. Operating conditions were: slit, 2 nm; scan speed, 120 nm/min; chart speed, 12 cm/min (10 nm/cm): mode, 3. The full width of the recorder's paper corresponds to 1·0, 2·0 or 4·0 units of optical density at input voltages of 50, 100 and 200 mV, respectively.

Quantitative data of second derivatives are expressed throughout this paper in absorbance units measured in the same scale with the respective UV spectra. They are recorded as $\Delta K_{\lambda}^{1\%}$ values, e.g. $\Delta K_{315}^{1\%}$ is the distance (in units of absorptivity) between the two extremes (minimum–maximum in the side towards longer wavelengths) of the reflection of the second derivative of the peak at 315 nm, corresponding to 1% solution of the oil. When $\Delta K_{315}^{1\%}$ is less than 0·030, accurate results are obtained either by increasing the sensitivity (lower input voltage) or by using oil solutions of concentration higher than 1% (e.g. $c\% = 2$–4%). Then $\Delta K_{315}^{1\%} = \Delta K_{315}^{c\%}/c$. (There is a linear relationship between ΔK values and concentration.) We found that in most cases the 1% oil concentration and 2·0 absorbance units in full scale (AUFS) are the most suitable conditions.

For practical reasons, the absorption maxima between 320 and 255 nm are labelled A, B, C, D, E and F, and the respective second-derivatives are labelled A″, B″, etc. (see accompanying spectra). According to literature data (Galanos *et al.*, 1968*a*; Mitchell & Kraybill, 1942) the respective maxima wavelengths are 315, 300, 285, 278, 268 and 258 nm. However, by inspection of the figures presented herein it is obvious that the apparent wavelengths of these maxima are strongly affected by their relative intensities and the slope of the background absorption at 232 nm.

RESULTS AND DISCUSSION

General features

The UV spectra and their second derivatives depicted in Figs 1–5 are representative examples of a large number of examined samples of olive, olive kernel and seed oils of different crops. These data show that second-derivative spectrophotometry reveals some previously unrecognized features, common for the UV spectra of all the vegetable oils, including virgin olive oils, namely:

(1) Apart from the commonly known triplet of absorption maxima at 278, 268 and 258 nm (peaks D, E, F, respectively) due to conjugated trienoic systems, there is another triplet at 315, 300 and 285 nm (peaks A, B, C, respectively) in all UV spectra of vegetable oils, including virgin olive oil (Fig. 2). The latter triplet, due to conjugated tetraenoic systems, has been

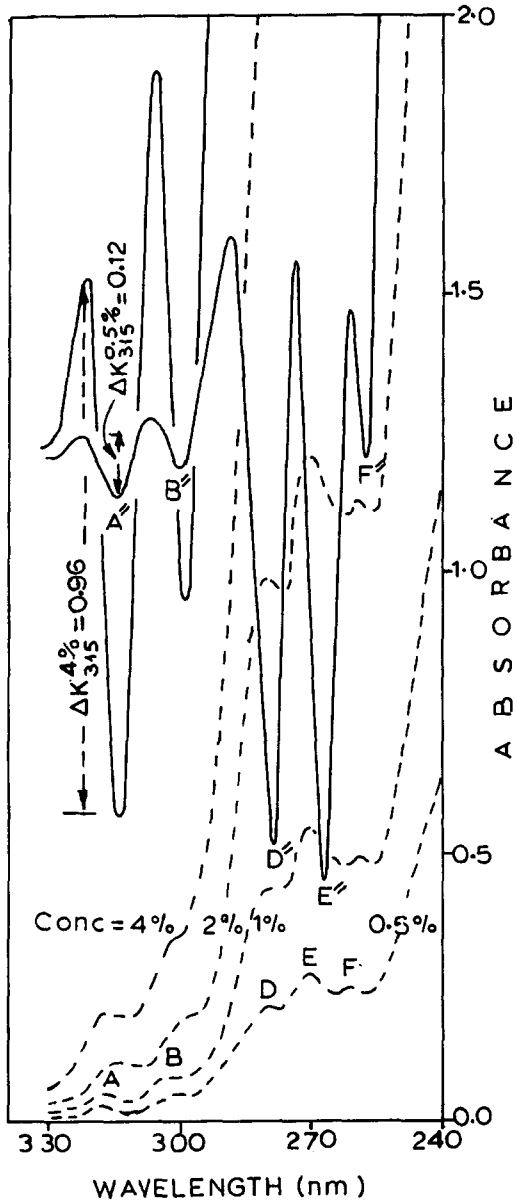

Fig. 1. UV spectra (dashed lines) and second derivatives (full lines) of different concentrations of a representative sample of olive oil 'coupé' (commercial mixture of virgin and refined olive oil).

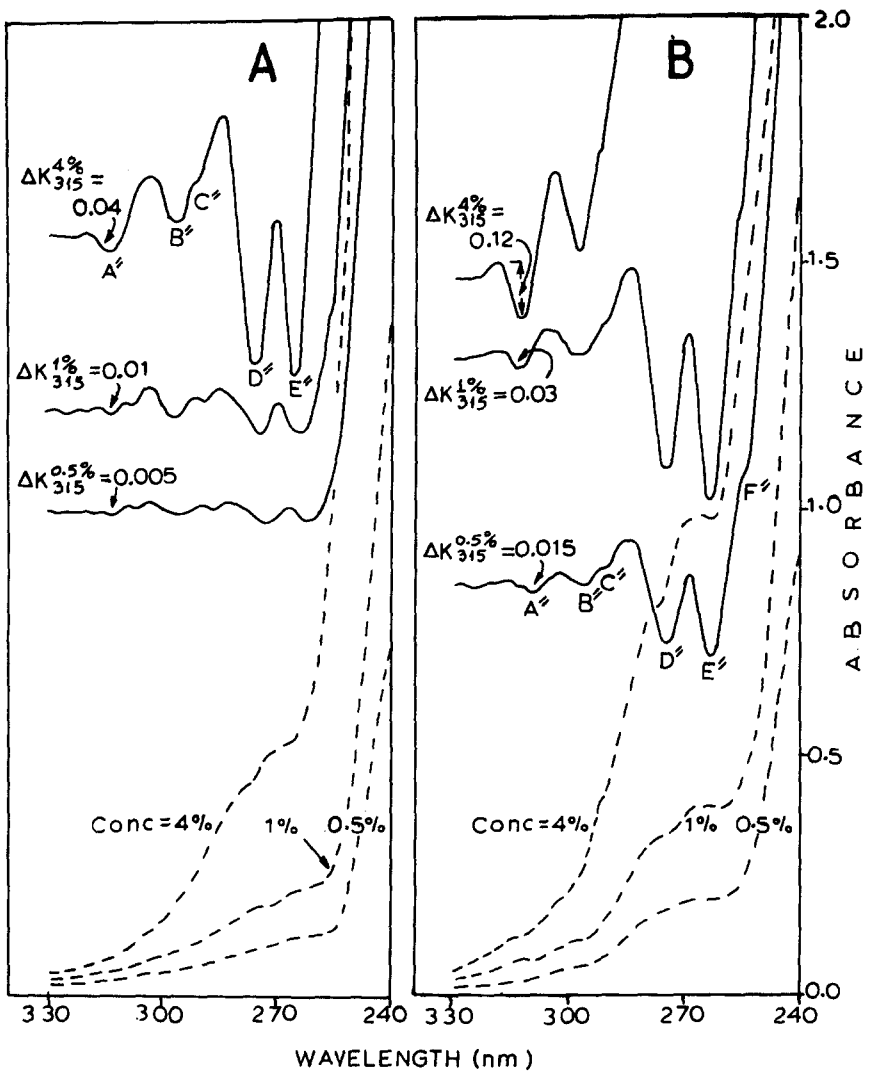

Fig. 2. UV spectra and second derivatives (dashed and full lines, respectively) of virgin olive oils at different concentrations. (A) Representative sample of last crop. (B) Representative sample of old crop.

observed by Mitchell & Kraybill (1942)—almost forty years ago—in extensively autoxidized oils after treatment with decolorizing earths.

(2) Although peaks C and F are not identified in most cases (even by second-derivative spectrophotometry), the above statement is manifested by the appearance of peaks A, B and D, E in the second-derivative spectra of all the examined samples of olive (refined or not), olive kernel and seed oils.

(3) Pertinent to the preamble of the previous statement (2) are the following, previously unrecognized, features: although inspection of most UV spectra indicates that peak D is much weaker than peak E (very often vanishing in the latter's background absorption), by second-derivative spectrophotometry it is demonstrated that the intensity of peak D is very close to that of peak E (and even higher, as in the cases of Figs 2A, 2B and 5).

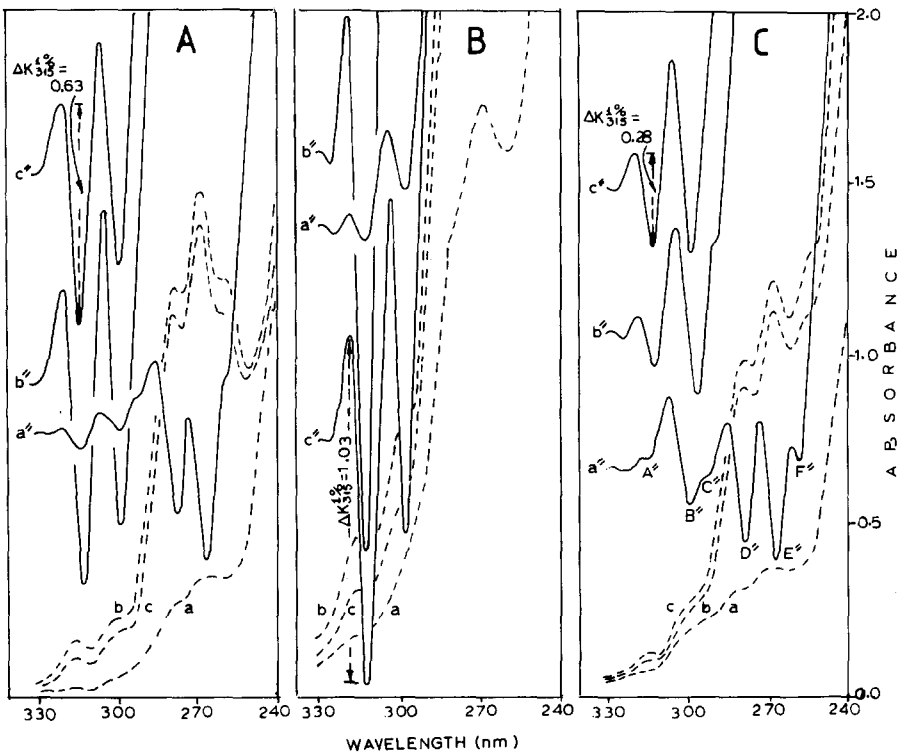

Fig. 3. UV spectra and second derivatives of olive (A), corn (B) and cottonseed (C) oils at consecutive steps of their refinement, i.e. (a) neutralized with alkali, (b) bleached and (c) deodorized. All data refer to 1% concentrations.

Very similar is the relation between the apparent and true intensities of the peaks A and B in most of the examined samples.

Both of these phenomena, as well as the aforementioned usual disappearance of any sign of peaks C and/or F (statement (1)), depend on the intensity of the absorption maximum around 232 nm, due to conjugated dienoic systems: both triplets (D–E–F and A–B–C) fall in the domain of the background absorption of this maximum at 232 nm.

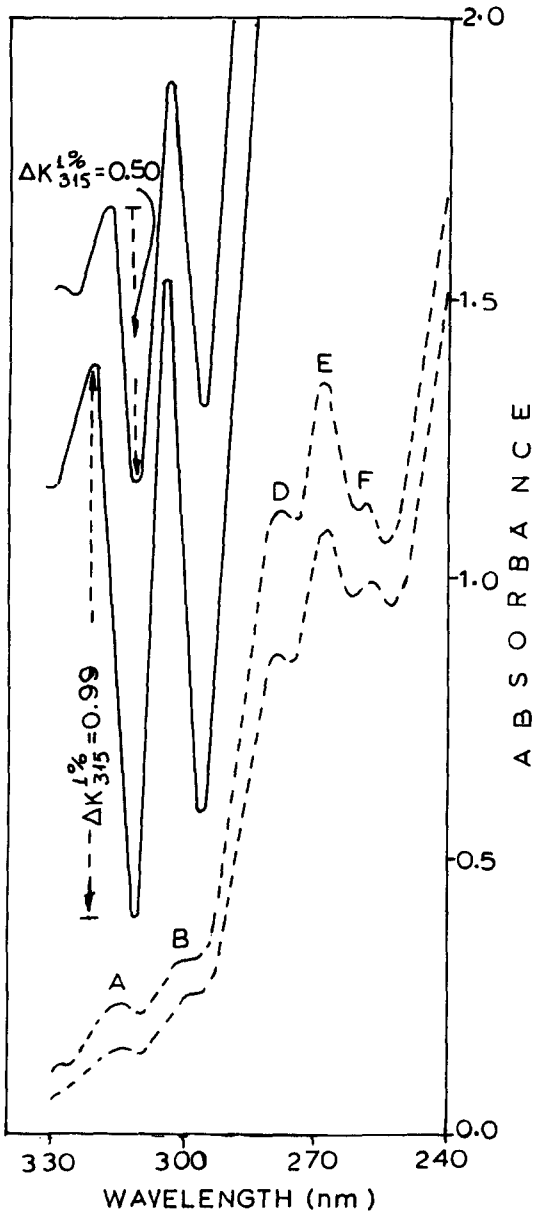

Fig. 4. UV spectra and second derivatives of two representative samples of refined olive kernel oil at 1% concentration.

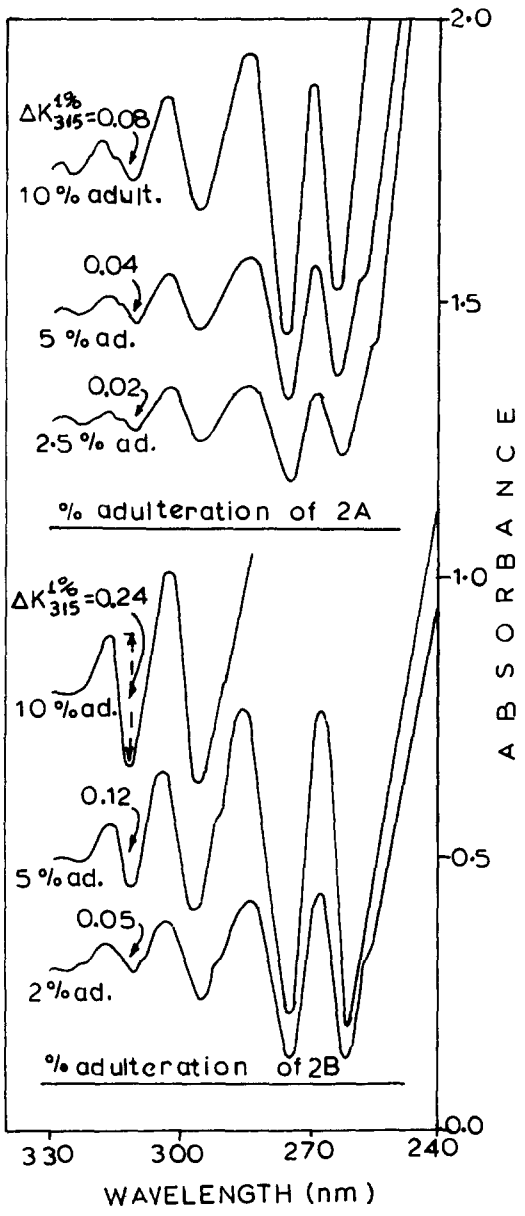

Fig. 5. Representative second derivatives of UV spectra obtained from the virgin olive oils illustrated in Figs 2A and 2B, after their adulteration (to the indicated levels) with an olive kernel oil of $\Delta K^{1\%}_{315} = 0{\cdot}50$ (see Fig. 4). All data refer to 1% concentrations.

Detection of adulteration of virgin olive oils

In accordance with earlier observations (Galanos *et al.*, 1968*a*), a careful inspection of the second-derivative data depicted in Figs 1–5 shows that quantitative measurement of the intensity of peak A (at 315 ± 1 nm) is most useful for the quality control of vegetable oils, with special reference to the detection of adulteration of virgin olive oil by olive kernel oil or by refined olive oil, as well as by seed oils. In the latter case, second-derivative spectrophotometry may serve as an additional tool to predict or verify adulterations that may be effectively detected by argentation chromatography techniques (Galanos *et al.*, 1986*b*; Kapoulas & Passaloglou-Emmanouilidou, 1981) or by reversed phase HPLC (Kapoulas & Andrikopoulos, 1986).

The data of Fig. 2 are representative of all the samples of virgin olive oil examined during this investigation, among which 31 samples were examined within one year from their production (Fig. 2A) and another 17 samples were two years old (Fig. 2B). Their respective ranges of $\Delta K^{1\%}_{315}$ values were found to be 0·008–0·015 and 0·010–0·030 (measured lowest–highest $\Delta K^{1\%}_{315}$ values). These values and the corresponding statistical figures (means and standard deviations) are depicted in Table 1 together with the data obtained from refined olive and olive kernel oils, from commercially available mixtures of virgin and refined olive oil (olive oils 'coupé') and from refined cottonseed and corn oils (see also Figs 3 and 4).

These data show that the $\Delta K^{1\%}_{315}$ values of the refined olive and olive kernel oils are 15–150 times higher than those of virgin olive oils and, in

TABLE 1
$\Delta K^{1\%}_{315}$ Values of Different Vegetable Oils

Oils	*Number of samples*	$\Delta K^{1\%}_{315}$ *values*	
		Mean ± SD	*Range*[a]
Virgin olive oils[b] (last crops)	31	0·011 ± 0·0018	0·008–0·015
Virgin olive oils[b] (old crops)	17	0·019 ± 0·0042	0·010–0·030
Refined olive oils	9	0·725 ± 0·170	0·450–1·020
Olive oils 'coupé'	34	0·319 ± 0·072	0·200–0·450
Refined olive kernel oils	12	0·688 ± 0·146	0·430–0·990
Refined cottonseed oils	14	0·574 ± 0·188	0·250–0·870
Refined corn oils	12	0·929 ± 0·264	0·630–1·420

[a] Observed minimum–maximum values.
[b] For the mixed population of the two crops of virgin olive oils the mean value ± SD = 0·014 ± 0·0054.

addition, that the latter have a quite narrow range of variation. Obviously, such a combination of low absolute values with narrow ranges in virgin olive oils is very advantageous for the detection of their adulteration by refined oils of much higher $\Delta K^{1\%}_{315}$ values.

In statistical terms (Bowker, 1947), the upper tolerance limits of $\Delta K^{1\%}_{315}$ for the 99·5% of the populations of the last and old crops of virgin olive oil with 99% confidence (significance level, $P = 0{\cdot}01$) are 0·018 and 0·037, respectively, while, for the mixed population of last and old crops, the corresponding upper limit is $\Delta K^{1\%}_{315} = 0{\cdot}032$. Therefore, a $\Delta K^{1\%}_{315} = 0{\cdot}040$ (or higher) gives an almost safe indication of adulteration of a virgin olive oil by refined oils.

As shown in Fig. 5, in the extreme case of adulteration of a high quality virgin olive oil with a good quality olive kernel oil (both with low $\Delta K^{1\%}_{315}$ values), it is unequivocally detected at the level of 5%, while the same olive kernel oil in a virgin olive oil with a higher $\Delta K^{1\%}_{315}$ value is detectable at the 2% level. In other words, the detection of adulteration by the present technique is always possible at the level of 5% (or higher), while, in most common cases, it is possible at the level of 2–3% and even at the level of 1% (if the refined oil has a $\Delta K^{1\%}_{315}$ higher than 1·2). On the other hand, it is obvious that for samples declared as virgin olive oils of the last crops, the detection of adulteration is always possible at the level of 1–2% which is indicated by $\Delta K^{1\%}_{315} = 0{\cdot}020$ (or higher) in this case (see above).

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