Food Chemistry **39** (1991) 249–256

Application of a New, Simple and Economic Colorimetric Method for the Determination of Non-esterified Fatty Acids in Vegetable Oils

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(Received 2 February 1990; accepted 23 April 1990)

ABSTRACT

The amount of non-esterified fatty acids in eight different vegetable oil samples has been determined by two different methods: (a) with a standard titration procedure, and (b) with a new colorimetric method which utilizes phenol red solubilized in reverse micelles. The results obtained by the two methods are in good agreement, although the standard deviation in the case of the phenol red method is significantly higher compared with the conventional titration. The main advantage of the new colorimetric method is an economic one: only small amounts of oil samples (less than 0·1 ml) are required and a comparably small amount of organic solvent (less than 5 ml isooctane) is needed.

INTRODUCTION

The content of free (non-esterified) fatty acids in vegetable oils is usually determined by titration with standard alkali by using, for each measurement, 10 g oil in 50 ml of a solvent consisting of equal volumes of ethanol and diethyl ether (Gunstone *et al.*, 1986).

Recently, a new colorimetric titration method for the determination of the concentration of non-esterified fatty acids in vegetable oils has been described (Walde, 1990). This method uses reverse micellar solutions and takes advantage of their optical transparency and of their solubilization capacity for water-insoluble as well as for water-soluble compounds.

Food Chemistry 0308-8146/90/$03·50

In the following, we will briefly describe the principle of the method and apply it to eight different vegetable oil samples. It will be shown that the new method allows us to estimate the acid value of commercially available oils by using less than 100 µl and not more than 5 ml solvent in total.

Whenever the term 'fatty acid' is used in this paper, we use it as a synonym for 'non-esterified' fatty acid; as suggested in the literature (Glatz & Van der Vusse, 1988), the term 'free fatty acid' is avoided.

MATERIALS AND METHODS

Bis(2-ethylhexyl) sodium sulfosuccinate (AOT) was a product for Sigma and used as obtained. Isooctane (for UV spectroscopy), tris(hydroxymethyl) aminomethane (abbreviated as 'tris'), phenolphthalein, phenolsulfonephthalein (phenol red), octanoic acid (caprylic acid, purum), hexadecanoic acid (palmitic acid, puriss.), and *cis*-9-octadecenoic acid (oleic acid, puriss.) were from Fluka, Switzerland. The cold pressed vegetable oils from olives, soybeans, peanuts, walnuts, corn germs, sesame seeds, safflower, and sunflower seeds were a gift from Morga AG, Switzerland.

The absorption spectra were recorded at 25°C on a Uvikon 810 spectrophotometer from Kontron, Switzerland. The pathlength of the quartz cells was 1 cm.

The density measurements were carried out with a DSA 48 density and sound analyzer from Paar, Austria.

The conventional titration of fatty acids was performed at room temperature in the following way: 50 ml diethylether/ethanol (96%) (1:1, v/v), containing three drops of a 1% (w/v) phenolphthalein solution in ethanol, were first neutralized with 0·1N NaOH. Ten grams of the vegetable oil were then added and the solution was titrated from yellow (colour of the oil) to pink with 0·1N NaOH. Each determination was carried out in triplicate.

DESCRIPTION OF THE PHENOL RED METHOD

(a) Description of the system

AOT (the sodium salt of bis (2-ethylhexyl) sulfosuccinic acid) solubilized in isooctane in the presence of a small amount of water forms relatively monodisperse reverse (or inverted) spherical micelles. These aggregates are small water droplets which are surrounded by a layer of surfactant molecules of which polar heads face into the interior polar pool of the

reverse micelles (e.g. Fendler, 1982). If we now dissolve in isooctane, for example, 22·23 g AOT and 9·99 g water per ml, we obtain an optically transparent micellar solution which is 50 mM in AOT and 555 mM in water. The corresponding w_0-value is 11·1 (w_0 is defined as the ratio of the molar concentrations of water and AOT, $w_0 = [H_2O]/[AOT]$). The concentration of reverse micelles in this solution is 0·41 mM, and each micelle has a mean water pool radius of 2·1 nm (Walde & Luisi, 1989); over 95% (w/v) of the solution is isooctane.

If we then solubilize in the water pool of these reverse micellar aggregates the alkaline form of an acid-base indicator (phenol red), it is possible to transform this alkaline form of the indicator into the acidic form by adding small amounts of fatty acids. While doing so, the reverse micellar solution remains completely clear and the change of the indicator's colour as a function of the acid concentration can therefore be followed spectrophotometrically. One example for caprylic acid is shown in Fig. 1. With increasing fatty acid concentration, the intensity of the band at 560 nm decreases while the peak intensity at 430 nm increases. Isosbestic points are localized at 480 nm and 366 nm. Due to the fact that, for a certain concentration range, a linear relationship exists between the absorption at 430 or 560 nm and the total fatty acid concentration, it is possible to apply this sytem for a quantitative estimation of the fatty acid content in vegetable

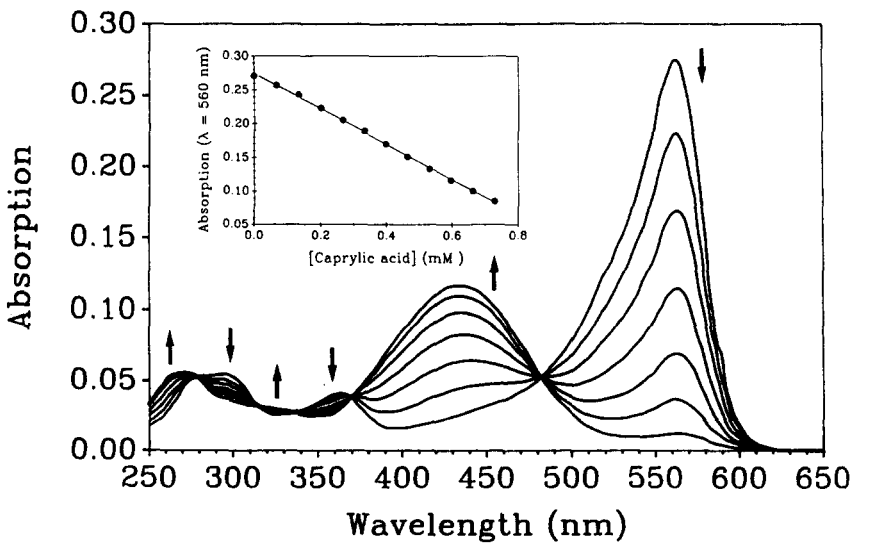

Fig. 1. Effect of caprylic acid on the UV/VIS spectrum of phenol red solubilized in AOT-reverse micelles. Fifty mM AOT/isooctane, $w_0 = 11·1$, $[\text{phenol red}]_{ov} = 5\,\mu M$; 1 = 1 cm. With decreasing absorption around 560 nm (and increasing absorption around 430 nm): $[\text{caprylic acid}]_{ov} = 0$; 0·2 mM; 0·4 mM; 0·6 mM; 0·8 mM; 1·1 mM; 2·0 mM.
Insert: Linear part of the OD_{560} versus caprylic acid concentration diagram.

oil samples, after having carried out appropriate calibration curves with fatty acid solutions of known concentration. As is obvious from Fig. 1, the largest changes in the visible spectrum occur at 560 nm. We therefore limited ourselves to an analysis at this particular wavelength.

(b) The calibration

The calibration was made with three different fatty acids: caprylic acid (C 8:0), palmitic acid (C 16:0) and oleic acid (C 18:1). The curves OD_{560} versus fatty acid concentration were linear up to 0·7 mM for the experimental conditions used. By taking, for each acid, twelve different concentrations, the correlation coefficients for the linear regression were 0·9996, 0·9998, and 0·9996 for caprylic acid, palmitic acid, and oleic acid, respectively. The slope was, in all three cases, the same with a mean value of $-0{\cdot}261 \pm 0{\cdot}001$ (Walde, 1990).

(c) The fatty acid determination in the oil samples

For the fatty acid determinations in the vegetable oils, we then used the following experimental procedure:

First, three stock solutions were prepared: Solution A, which was a 0·1M tris/HCl buffer of pH 9·0; solution B, containing 2 mM phenol red in 0·1M tris/HCl, pH 9·0; and solution C, containing 50 mM AOT in isooctane.

The assay solution D was then prepared by adding 0·375 ml solution A and 0·125 ml solution B to 50 ml solution C. The composition of solution D was the following: [AOT] = 50 mM with $w_0 = 11.1$ (pH 9·0) and an overall phenol red concentration of 5 μM. (Note, that in the case of reverse micellar systems, the concentration of a water-soluble compound can either be given as overall concentration or as local waterpool concentration, depending on whether reference is to the total volume of the solution or only to the water portion.)

The spectroscopic changes caused by non-esterified fatty acids present in vegetable oils were then measured as follows: 1 ml solution D was placed into a 1·5 ml cuvette with a path length of 1 cm and the optical density was measured at 560 nm. Then 5 to 20 μl vegetable oil were added and the optical density was measured again. This procedure was repeated as long as the optical density was not below the linear part of the calibration curves, and as long as the solution was not becoming too diluted (maximum added amount of oil: 40 μl per ml solution).

From each absorption reading, the change in optical density at 560 nm caused by 10 μl oil can be determined (ΔOD_{560}/10 μl) and the molar

concentration of (non-esterified) fatty acids in the oil can then be calculated according to the following formulae:

$$\frac{(\Delta OD_{560}/10\,\mu l)}{0{\cdot}261} \times \frac{1010}{10} \times 10^{-3} = [\text{fatty acid}]\ (\text{M}) \qquad (1)$$

On knowing the density of the oil (d_{20}^{20} in g/cm^3) and the molecular weight of the fatty acid (M_r in g/mol), we obtain for the fatty acid content (in wt%):

$$\frac{(\Delta OD_{560}/10\,\mu l)}{0{\cdot}261} \times \frac{1010}{10} \times \frac{M_r}{d_{20}^{20}} \times 10^{-4} = \frac{[\text{fatty acid}]}{(\text{wt}\%)} \qquad (2)$$

The acid value is then calculated in the following way (Gunstone *et al.*, 1986):

$$561 \times \frac{[\text{fatty acid}](\text{wt}\%)}{M_r} = \text{acid value} \qquad (3)$$

For all the oils, we have taken as mean molecular weight for the fatty acid a value of 282 g/mol, the value of oleic acid.

(d) Re-use of the solvent

After carrying out a couple of fatty acid determinations, it is possible to distill the assay mixture and to reuse the solvent (see Fig. 4).

The whole procedure of the phenol red method described above is summarized in Fig. 2.

RESULTS AND DISCUSSION

Eight different cold pressed vegetable oils which are commercially available on the market in Switzerland have been analyzed with respect to the content in non-esterified fatty acids. Two different methods have been compared, a standard titration method and a new method which is based on the colour change of phenol red solubilized in reverse micelles. The results obtained with both methods are summarized in Table 1, together with the determined oil densities.

While the densities of the different oils are very similar—between 0·912 g/cm^3 (for olive oil) and 0·922 g/cm^3 (for safflower and walnut oil)—the fatty acid content varies by a factor of 20: 0·06 wt% for corn oil, 1·26 wt% for peanut oil. Comparing the results obtained by the two methods as

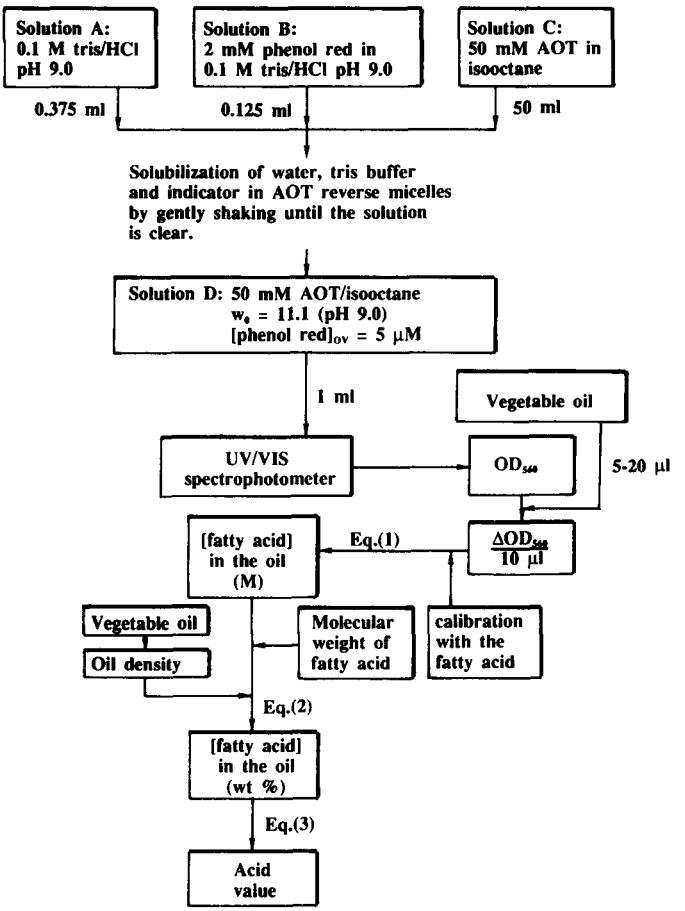

Fig. 2. Schematic representation of the phenol red–reverse micelle method for the determination of the free fatty acid content in vegetable oils.

presented in Table 1, two points are obvious. First, both methods gave approximately the same mean values for the fatty acid content. The largest difference (approximately 11%) was in the case of corn and soybean oil. The correlation between the two methods is illustrated in Fig. 3. Secondly, a clear drawback of our new phenol red method is the relatively large standard deviation which is a direct consequence of the small sample volumes used (see Table 1). The standard deviation (for $n = 12 - 16$) varies between 6·4% of the mean value (for peanut oil) and 18·6% (for safflower oil). The corresponding values in the case of the standard titration method ($n = 3$) are generally below 1%, with the exception of corn oil (8·2%), where the fatty acid content is comparably low.

One advantage of our method lies in the low organic solvent consumption. For all fatty acid determinations reported in this paper, we

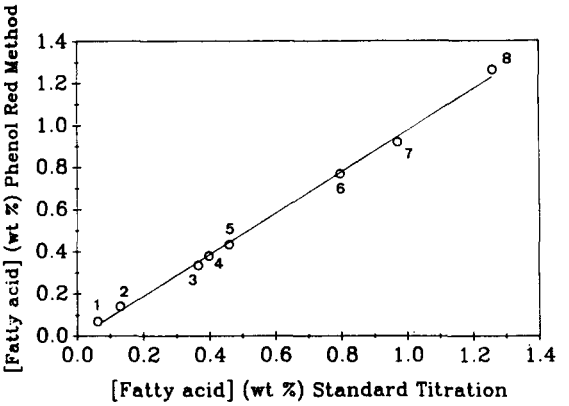

Fig. 3. Comparison of the fatty acid content in vegetable oils determined by standard titration and by the phenol red–reverse micelles method. The vegetable oils used were corn oil (1), safflower oil (2), sunflower seed oil (3), soybean oil (4), olive oil (5), sesame oil (6), walnut oil (7), and peanut oil (8).

used only 40 ml isooctane, while at the same time with the standard method 600 ml diethylether and 600 ml ethanol (96%) were used. The isooctane can easily be reused after distillation of the assay solution as shown in Fig. 4. The consumption of chemicals can therefore be kept at a minimum.

We believe that our new method can be helpful whenever a larger number of different oil samples have to be analyzed for their content in non-esterified fatty acids and where a higher precision than *ca.* $\pm 15\%$ is not required. In

TABLE 1
Fatty Acid Content in Vegetable Oils

Vegetable oil[a]	*Density* (g/cm^3)[b]	*Standard titration method* ($n = 3$)[c]			*Phenol red method*	
		Fatty acid content (wt %)	*Acid value*	n^c	*Fatty acid content (wt %)*	*Acid value*
Corn oil	0·919	0·062 ± 0·005	0·123 ± 0·010	12	0·069 ± 0·010	0·14 ± 0·02
Safflower oil	0·922	0·130 ± 0·001	0·259 ± 0·002	15	0·140 ± 0·026	0·28 ± 0·05
Sunflower seed oil	0·920	0·367 ± 0·003	0·730 ± 0·006	16	0·335 ± 0·050	0·67 ± 0·10
Soybean oil	0·920	0·400 ± 0·001	0·796 ± 0·002	12	0·380 ± 0·049	0·76 ± 0·10
Olive oil	0·912	0·461 ± 0·002	0·917 ± 0·004	12	0·433 ± 0·039	0·86 ± 0·08
Sesame oil	0·919	0·797 ± 0·006	1·586 ± 0·012	14	0·769 ± 0·084	1·53 ± 0·17
Walnut oil	0·922	0·972 ± 0·002	1·934 ± 0·004	12	0·920 ± 0·117	1·83 ± 0·23
Peanut oil	0·915	1·260 ± 0·012	2·507 ± 0·024	13	1·264 ± 0·081	2·51 ± 0·16

[a] All oil samples from Morga AG, Switzerland.
[b] Density measured at 20°C (d_{20}^{20}).
[c] n = number of determinations.

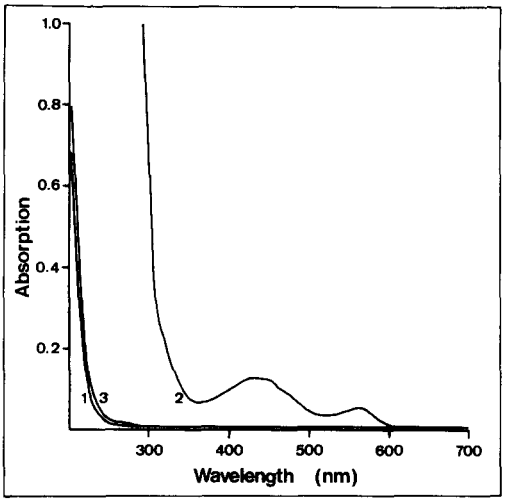

Fig. 4. UV/VIS spectrum of isooctane (1); a mixture of assay solutions containing AOT, water, tris buffer, phenol red and vegetable oil (2); and a distillate of the assay solutions (3). (Note, that the distillate was centrifuged before recording the spectrum, in order to separate water and isooctane, which both have a similar boiling point). Pathlength: 1 cm.

addition, the phenol red reverse micelle method may be advantageous whenever only small amounts of oil samples are available. Fifty microlitres of oil is in principle enough, if we do not include the density determination.

ACKNOWLEDGEMENT

The authors would like to thank Morga AG, Ebnat-Kappel, Switzerland, for the different vegetable oil samples, and Dr Heribert Watzke for helping in the oil density determinations.

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