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Application of supercritical fluid chromatography and supercritical fluid extraction to the measurement of hydroperoxides in foods

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ABSTRACT

The applicability of supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC) for determining lipid peroxide levels in foods containing fats and oils was investigated. Lipid peroxide levels determined by SFC and SFE–SFC were compared with peroxide values (POVs) determined by conventional methods. The retention behaviour with respect to oil components in both the mobile phase of supercritical carbon dioxide modified with ethanol and the stationary phase of silica gel is similar to the retention behaviour in normal-phase high-performance liquid chromatography.

SFC was found to be useful for measuring hydroperoxide compounds in small amounts of sample extracted from peanut oil with diethyl ether. The peak areas of peroxide compounds are in good agreement ($r = 0.9923$) with POV determinations made by potentiometry.

Coupled SFE–SFC provides useful qualitative and quantitative information, and can therefore serve as a simple high-speed method for extracting and separating hydroperoxide compounds. Compared with conventional methods, coupled SFE–SFC offers an advantage with regard to sample preparation by eliminating the need for preextraction when using solid samples.

INTRODUCTION

Detecting the presence of peroxide compounds and determining the lipid peroxide value (POV) in foods containing fats and oils are of great interest because peroxide compounds accelerate the deterioration of the taste and appearance of food. Lipid peroxide compounds are also known to have adverse physiological activity *in vivo*. Lipid peroxide levels are currently determined using the official method of the Japan Oil Chemists' Society (JOCS)[1]. This is an iodimetric titration method based on the stoichiometric reaction between lipid hydroperoxide and potassium iodide. However, the method is relatively insensitive and sometimes yields inconsistent results because

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it is difficult to determine the exact titration end-point when the lipid level is low or when the sample is coloured.

Reliable POV determinations can be made with potentiometry[2,3]. In addition high-performance liquid chromatography (HPLC) has been used to measure levels of hydroperoxide compounds[4–13]. However, the results were not completely satisfactory because these HPLC systems employed either a UV or refractive index detector. The former, although highly sensitive, is effective in detecting hydroperoxide compounds only at a wavelength of 235 nm, where the conjugated double bond in these compounds gives rise to maximum absorption. The latter, although capable of measuring a wide range of compounds, even those without UV absorption bands, is relatively insensitive.

Supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC) using carbon dioxide are known to be very suitable methods for the extraction and separation of labile compounds, because sample compounds can be extracted and chromatographed in an oxygen-free environment at relatively low temperatures. In addition, coupled SFE–SFC may offer a great advantage over conventional methods with regard to sample preparation. The reason is that a coupled SFE–SFC system is capable of accepting solid samples, thus eliminating the need for pre-extraction using a Soxhlet extraction system which is currently required in all conventional methods. We investigated the applicability of the above techniques to the determination of lipid peroxide levels in goods, and the results were compared with POVs determined by conventional methods.

EXPERIMENTAL

Materials

Peanut oil samples were prepared from peanuts commonly used as ingredients in confectionary products. The peanuts were ground and sieved to a 30-mesh fineness and then extracted with diethyl ether (Tabata, Chiba, Japan). Oils containing various POV levels were obtained by storing the ground peanut samples at 63°C for 45–450 h prior to extraction.

Peanut samples for coupled SFE–SFC measurements were ground and sieved to a 60-mesh fineness in order to reduce the extraction time.

Reagents and column

Food-additive grade carbon dioxide (Showa Tansan, Tokyo, Japan) was used as the mobile phase and as the extraction medium for the SFC and SFE–SFC analyses. HPLC-grade ethanol was used as a modifier for the SFC mobile phase. A Super-Pak SIL (5-μm silica gel) (JASCO, Hachioji, Japan) column (250 mm × 4.6 mm I.D.) was used for SFC separation. Stigmasterol was used as an internal standard for determining the lipid peroxide compounds in SFC.

Special-reagent-grade sodium thiosulphate, potassium iodide and diethyl ether were used in the potentiometric measurements. Unless indicated otherwise, all chemicals were purchased from Wako (Osaka, Japan).

Apparatus

A JASCO Super-100 SFE–SFC system with a MULTI-320 wavelength UV

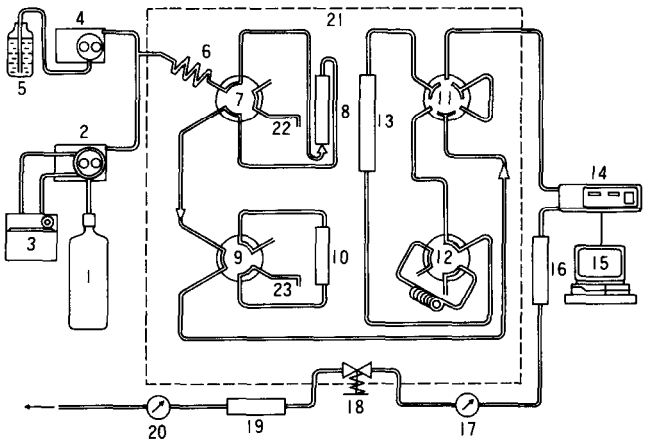

Fig. 1. Schematic diagram of coupled SFE–SFC system. 1 = Carbon dioxide cylinder; 2 = liquefied carbon dioxide delivery pump; 3 = coolant circulating bath; 4 = modifier delivery pump; 5 = modifier solvent reservoir; 6 = heat-exchange coil; 7 = six-way valve for bypassing extraction vessel; 8 = extraction vessel; 9 = six-way valve for bypassing trap vessel; 10 = extract trap vessel; 11 = six-way valve for bypassing injector and separation column; 12 = injector; 13 = separation column; 14 = multi-wavelength detector; 15 = detector data processor; 16 = extract trap column; 17 = back-pressure gauge; 18 = back-pressure regulator; 19 = trap for mass flow meter; 20 = mass flow meter; 21 = oven. (From ref. 14).

detector was used for SFC and SFE–SFC[14]. A schematic diagram of the system is shown in Fig. 1. The volume of the extract trap vessel used for the extraction was 25 ml (50 mm × 8.0 mm I.D.). An AUT-1 automatic titrator (TOA Electronics, Tokyo, Japan) was used for the potentiometric determinations.

Procedures

SFC measurement. Samples for SFC were prepared by weighing 0.1-g samples of fats and oils dissolved in 1 ml of *n*-hexane in 3-ml vials capped with silicone rubber. A 20-μl volume of sample solution was injected into the SFC system. The results of SFC measurements were expressed in terms of peak areas of absorbance at 230 nm.

SFE–SFC measurement. Extraction and chromatography were carried out in a single process using a coupled SFE–SFC system to determine the levels of lipid peroxide compounds in the ground peanut samples. Peanut powder (40 mg) was placed in the extraction vessel (50 mm × 8.0 mm I.D.) and 52.5 μg of stigmasterol in 30 μl of *n*-hexane were added as an internal standard. The extraction was carried out with supercritical carbon dioxide containing 5% ethanol at a pressure of 140 kg/cm^2 at 40°C for 7 min using the constant-pressure delivery mode. During the extraction, the extraction vessel was simply pressurized with carbon dioxide and thus extraction was performed using the stop-flow method.

After 7 min had elapsed, the six-way valve 9 was switched to trap the extracted compounds in the extract trap vessel (10), which contained carbon dioxide gas at atmospheric pressure. When the system pressure had stabilized, indicating that transfer of the extract was complete, the six-way valve 7 was switched to by-pass the extraction vessel 8. Then, six-way valve 11 was switched so that the separation column (13) was included in the flow line in order to equilibrate the system under SFC

conditions at a pressure of 120 kg/cm^2 at 40°C with a carbon dioxide flow-rate of 4.0 ml/min as a liquid at −5°C and an ethanol flow-rate of 0.3 ml/min.

Equilibration was obtained within a few minutes, then the six-way valve 9 was switched back to include the extract trap loop in the flow line and the extract was then injected into the separation column (13).

RESULTS AND DISCUSSION

Comparison of results obtained by SFC and potentiometry

Peanut oils having different POVs prepared according to the procedure described under Experimental were analysed by SFC. The POVs of these oils were determined by potentiometry prior to SFC analysis. Fig. 2 shows a typical SFC result for peanut oil having a POV of 41.0.

As can be seen in the chromatogram, the retention behaviour of the oil components in both the mobile phase of supercritical carbon dioxide modified with ethanol and the stationary phase of silica gel was similar to the retention behaviour in normal-phase HPLC[6,10].

With regard to controlling the retention in normal-phase chromatography, SFC may prove superior to HPLC. The reason is that the mobile phase strength in SFC can be precisely controlled simply by changing the pressure and temperature of the fluid. Such control cannot be achieved in HPLC by changing the mobile phase composition from a non-polar to a polar solvent.

Glycerides, which are the main constituents of the oil, were eluted at 2.1 min and hydroperoxides were eluted at 3.65 min. The contour plot of this chromatogram (not shown) suggested that the hydroperoxide peak contained at least three components. This corresponds to previous results obtained by HPLC[10].

UV absorption spectra of the peak at 2.10 min containing glycerides and the peak at 3.65 min containing hydroperoxide are shown in Fig. 3. These spectra are in good agreement with those obtained using an ordinary UV spectrometer at wavelengths longer than 215 nm. At shorter wavelengths there is an abrupt fall-off in the spectra owing to UV absorption by ethanol, which is very high in this region, resulting in distortion of the spectra below 215 nm.

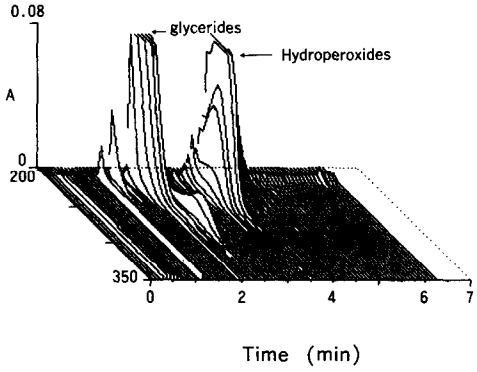

Fig. 2. Chromatogram of peanut oil (POV 41.0) in SFC.

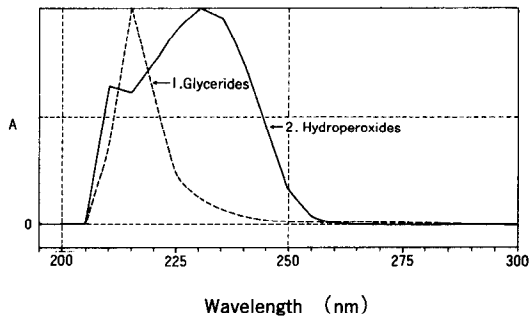

Fig. 3. SFC spectrum for Fig. 2. (1) 2.10 min, 0.2623 a.u.f.s.; (2) 3.65 min, 0.0591 a.u.f.s.

Table I gives quantitative results obtained by SFC and potentiometry. As demonstrated by a linear regression analysis, the correlation between the results obtained by these two methods was excellent. The regression equation was $y = 0.6556x - 0.1031$ and the correlation coefficient was 0.9923.

The reproducibility (relative standard deviation) for SFC and potentiometry for five consecutive measurements of peanut oil having a POV of 53.2 was calculated to be 1.57% ($n = 5$) and 1.63% ($n = 5$), respectively.

Comparison of results obtained by SFE–SFC and potentiometry

Peanut samples for SFE–SFC analysis were exposed to heat for different periods of time as described under Experimental section. The POVs of oils from these samples were determined by potentiometry in the same way as for the SFC analysis. In order to obtain reliable quantitative results in SFE–SFC analysis, it is essential that the compound selected for the internal standard should have the same extraction yield as the target compound. SFC analysis provides useful information for selecting a compound for use as an internal standard. The retention behaviour of a compound is highly dependent on its polarity, as is the extraction yield. Therefore, if the retention times of both the internal standard and the target compounds are similar, it is reasonable to assume that the extraction yield in SFE may also be similar under the same extraction conditions. In practice, a longer extraction time tends to minimize errors related to differences in extraction yields.

TABLE I

COMPARISON OF QUANTITATIVE RESULTS OBTAINED BY SFC AND POTENTIOMETRY

Method	*Number of hours of ageing*					
	0[a]	*45*	*65*	*112*	*328*	*450*
SFC: peak area[b]	1.86	2.86	3.16	3.47	4.24	6.31
Potentiometry: POV[c]	19.9	38.4	53.1	60.0	70.2	93.6

[a] Indicates fresh peanuts, tested as supplied without ageing.
[b] Peak areas are given in terms of absorbance (at 230 nm) seconds.
[c] Lipid peroxide values are given in terms of mequiv./kg.

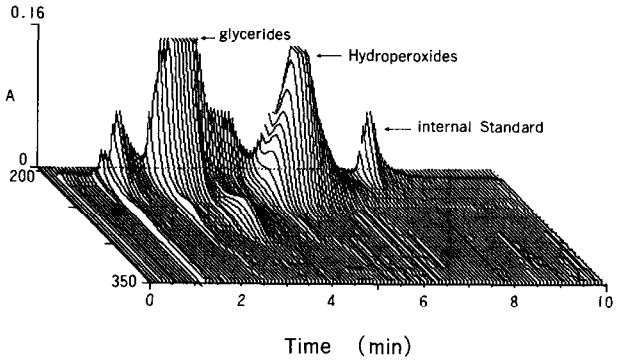

Fig. 4. Chromatrogram obtained by coupled SFE–SFC.

Fig. 4 shows typical SFE–SFC results for oil from a peanut sample containing stigmasterol as the internal standard. The POV of the oil was determined in a separate potentiometric measurement to be 41.0. Glycerides were eluted at 3.12 min, hydroperoxides at 5.54 min and the internal standard at 7.12 min.

The UV adsorption spectrum of each peak component is shown in Fig. 5. Below 215 nm, the absorption curves are distorted for the same reason as given above for Fig. 3.

SFE–SFC analyses were performed on different peanut samples having POVs of 1.95, 40.74 and 89.03. Table II shows the results and reproducibility of the analysis. The peak areas of stigmasterol, the internal standard, were measured at 215 and 220 nm. Hydroperoxide peak areas were measured at 230 nm and were divided by those of the internal standard at 215 and 220 nm to normalize the data for hydroperoxides in the conventional internal standard method. All peak-area data are expressed as absorbance seconds.

The normalized data were then subjected to linear regression analysis to evaluate the correlation between the SFE–SFC results and POV as determined by potentiometry. The correlation coefficients for the 230/215 nm and 230/220 nm ratios were

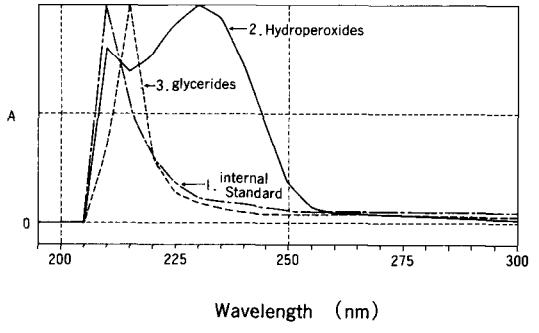

Fig. 5. Spectrum of hydroperoxide compounds and glycerides in peanut oil, together with stigmasterol as the internal standard. (1) 7.12 min, 0.0642 a.u.f.s.; (2) 5.54 min, 0.0875 a.u.f.s.; (3) 3.12 min, 0.2415 a.u.f.s.

TABLE II

COMPARISON OF QUANTITATIVE RESULTS OBTAINED BY COUPLED SFE–SFC AND POTENTIOMETRY

Sample	*Stigmasterol*		*Hydroperoxide*		
	Peak area at 215 nm	*Peak area at 220 nm*	*Peak area at 230 nm*	*Ratio of peak areas*	
				230/215 nm	*230/220 nm*
Sample 1: POV = 1.95[a]	145	0.83	2.19	1.48	2.79
R.S.D. (%)[b]	3.40	5.86	3.20	2.92	6.33
Sample 2: POV = 40.74[a]	1.54	0.84	9.97	6.47	11.89
R.S.D. (%)[b]	3.84	2.58	6.65	4.45	4.49
Sample 3: POV = 89.03[a]	1.43	0.81	15.59	10.90	19.28
R.S.D. (%)[b]	2.08	5.02	5.75	4.13	2.74

[a] POVs were determined by potentiometric analysis of peanut samples employing the same extraction method for sample preparation described under Procedures.

[b] Relative standard deviation ($n = 5$).

9.890 and 0.9881, respectively. The relationships in the above data can be expressed linearly as $y = 0.1064x + 1.665$ for 230/215 nm and $y = 0.1894x + 3.188$ for 230/220 nm. Hence the reproducibility of POV determinations by SFE–SFC is as good as that by potentiometry.

The samples for coupled SFE–SFC were solid samples introduced into the system without pre-extraction. Therefore, by eliminating the need for pre-extraction when using solid samples, coupled SFE–SFC can serve as a simple and rapid high-speed method for extracting and separating hydroperoxide compounds from foods.

CONCLUSION

SFC using a multi-wavelength UV detector appears to be a useful method for detecting and measuring hydroperoxide compounds in fats and oils. POVs measured by SFC are in good agreement with those determined by potentiometry.

Coupled SFE–SFC was shown to provide useful qualitative and quantitative information and can therefore serve as a simple and rapid method for extracting and separating hydroperoxide compounds. Compared with conventional methods, coupled SFE–SFC offers an advantage with regard to sample preparation by eliminating the need for pre-extraction when using solid samples.

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REFERENCES

1 *Official and Tentative Methods of the Japan Oil Chemist's Society* (JOCS), Maruzen, Tokyo, 1943, Method 2.4.12-71.
2 S. Hara, O. Washizu and Y. Totani, *Yakugaku*, 31 (1982) 1004.
3 S. Hara and Y. Totani, *J. Am. Oil Chem. Soc.*, 65 (1988) 1948.
4 R. D. Plattner, G. F. Speneer and R. Kleiman, *J. Am. Oil Chem. Soc.*, 54 (1977) 511.
5 R. D. Plattner, *J. Am. Oil Chem. Soc.*, 58 (1981) 638.
6 S. Hara, H. Yamawaki and Y. Totani, *Yukagaku*, 32 (1981) 594.
7 M. W. Dong and J. L. Dicesare, *J. Am. Oil Chem. Soc.*, 60 (1983) 788.
8 D. K. Park, J. Terao and S. Matsushita, *Agric. Biol. Chem.*, 45 (1981) 2443.
9 Y. Totani, *Yukagaku*, 35 (1986) 337.
10 S. Hara, K. Nemoto, H. Yamawaki and Y. Totani, *Yukagaku*, 37 (1988) 541.
11 T. Hoshino, T. Hondo, M. Senda, M. Saito and S. Tohei, *J. Chromatogr.*, 332 (1985) 139.
12 K. Sugiyama, M. Saito, T. Hondo and M. Senda, *J. Chromatogr.*, 332 (1985) 107.
13 K. Sugiyama and M. Saito, *J. Chromatogr.*, 442 (1988) 1213.
14 M. Yoshioka, S. Parvez, T. Miyazaki and H. Parvez, *Progress in HPLC*, Vol. 4, VSP, Utrecht, 1988, p. 101.