Food Chemistry **46** (1993) 137–141

Evaluation of a static headspace gas chromatographic method for the determination of lipid peroxides

F. Ulberth & Dorothea Roubicek

Department of Dairy Research and Bacteriology, Agricultural University, Gregor Mendel-Str. 33, A-1180 Vienna, Austria

(Received 20 December 1991; revised version received 28 March 1992; accepted 29 March 1992)

Lipid peroxides were decomposed to volatile components (pentane, hexanal, *trans*-2-heptenal) on heating to 140°C for 30 min. Volatiles were quantified by means of headspace gas chromatography (HSGC). Thermal treatment of the oil samples was achieved by means of an air-forced oven instead of heating the oil in the HS sampler. Therefore, less sophisticated instruments can be used. An inert atmosphere (N_2) in the vials during the thermal pre-treatment was a prerequisite for obtaining reproducible results. Since leakage of crimp-capped HS vials occurred after cooling down the hot vials in some cases, flame-sealed ampoules instead of the HS vials were used. This modification improved the repeatability of the method (overall relative standard deviation <7%). External standards were used for calibration purposes. Thermostating the oil samples for 1 h at 60°C before HSGC was necessary to reach equilibrium. This treatment did not initiate further peroxidation of the samples.

INTRODUCTION

The application of headspace gas chromatography (HSGC) for examining the extent of oxidative and/or light damage to vegetable oils has received much attention. In one of the first reports dealing with this subject, the direct injection of the oil sample into the hot injection port of the GC and the subsequent separation of the heat-induced volatiles was described (Scholz & Ptak, 1966). The original idea was improved and modified in several ways (Dupuy *et al.*, 1973, 1985; Jackson & Giacherio, 1977; Warner & Frankel, 1985). Although not a very potent odorant *per se*, the pentane formed in these direct GC methods is reportedly a good marker for detecting deterioration of oils (Scholz & Ptak, 1966; Evans *et al.*, 1969; Jackson & Giacherio, 1977; Pongracz, 1986). Besides hydrocarbons various volatile saturated and unsaturated aldehydes are produced under the test conditions, and their amounts have been linked to sensorically determined oil rancidity (Warner *et al.*, 1978; Grosch, 1989). More recently, the method of directly injecting the oil has been substituted by static (Marsili, 1984; Snyder *et al.*, 1985) or dynamic HS sampling (Selke & Frankel, 1987; Raghavan *et al.*, 1989) of the volatiles. Unfortunately, the sampling conditions chosen in these reports varied to a great extent with respect to sample volume, sampling temperature, sample conditioning time, etc. Therefore, a comparison of quantitative data is sometimes hindered or even impossible. The aim of the present study was to establish a simple and reliable static HSGC method for assessing vegetable oil quality. The heat induced generation of volatiles from their involatile precursors was physically separated from the subsequent HS thermostating step. This enables the analyst to use less sophisticated instrumentation, which is readily available in many oil laboratories.

Food Chemistry 0308-8146/92/$05.00

MATERIALS AND METHODS

Materials

A freshly refined and deodorized soybean oil sample was a kind gift from Unilever Austria and used for calibration purposes. Other oil samples were bought in supermarkets. *n*-Pentane (reference substance for GC) was from Merck (Darmstadt, Germany), hexanal from PolyScience Corp. (Niles, USA) and *trans*-2-heptenal from Aldrich-Chemie (Steinheim, Germany). Linoleic acid and lipoxidase (type I-S, 48000 units per mg solids) were purchased from Sigma (Munich, Germany). Silica gel GF_{254} (Merck) was used for TLC separation of linoleic acid hydroperoxides from unreacted starting material. Other chemicals were of analytical grade.

Preparation of linoleic acid hydroperoxides

Hydroperoxides were prepared from linoleic acid as described by Grosch (1976). The purified linoleic hydroperoxides were dissolved in a freshly deodorized soybean oil at a concentration of 0·84 μmol g^{-1}.

Determination of the peroxide value (POV)

POV was determined by an iodometric procedure (Timmen, 1975).

Headspace gas chromatographic analysis

HSGC analysis was performed by means of a syringe-type headspace autosampler (Carlo Erba HS 250) coupled to a Carlo Erba Mega 5300 GC (Carlo Erba Strumentazione, Milan, Italy). Peak areas were evaluated by means of a SP 4270 integrator (Spectra Physics, San Jose, USA). The volatiles were separated by using a 25 m × 0·32 mm ID fused silica capillary column coated with SE-54 (Macherey & Nagel, Düren, Germany). The thick-film stationary phase (d_f 1·0 μm) was chemically bonded. A 1·25 ml HS gas sample was injected by means of a heated gas-tight syringe (80°C) at an oven temperature of 38°C. The split ratio was *c.* 1:15. After an initial hold of 3 min, the column temperature was programmed at 6°C min^{-1} to 170°C. The injector temperature was 135°C and the FID temperature 235°C. Hydrogen at 0·5 bar was the carrier gas.

Oil portions (1 g) were weighed into HS vials (10 ml nominal capacity) and sealed with a PTFE lined septum. The vials were thermostated in the water bath of the HS 250 for 1 h at 60°C. A standard oil sample containing *c.* 1000 ppm pentane, hexanal and *trans*-2-heptenal was prepared by a capillary weighing technique (Bassette, 1984).

Thermal decomposition of lipid hydroperoxides

Lipid hydroperoxides were decomposed by heating the oil sample contained either in 10 ml HS vials or in flame-sealed 5 ml brown-glass ampoules in a forced-air oven (Salvis TSW 120 ED, Reussbühl-Luzern, Switzerland).

RESULTS

Headspace gas chromatographic conditions

To establish proper thermostating conditions an oil based standard sample (1 g) containing *c.* 20 ppm pentane, 200 ppm hexanal and 200 ppm *trans*-2-heptenal was equilibrated at different temperature/time combinations. Peak areas increased in exponential order with increasing temperature, as expected from the Clausius–Clapeyron equation (Hachenberg & Schmidt, 1979). A temperature of 60°C was selected for further experiments. After thermostating for 1 h at this temperature, subsequent changes in component peak areas were negligible, indicating that the vapour phase was in thermodynamic equilibrium with the liquid sample. The volatiles above an oil containing 0·84 μmol linoleic acid hydroperoxides per g exhibited the same time-dependent behaviour to reach equilibrium and, moreover, the peak areas were essentially constant when incubated at 60°C for up to 2 h.

Table 1. Mean values ± standard deviation (×10^3) of pentane, hexanal and *trans*-2-heptenal peak areas. Five replicates were run on three separate working days (series A, B, C)

Series	Pentane	Hexanal	*trans*-2-Heptenal
A	1479 ± 60·8	235 ± 8·4	100 ± 2·4
B	1455 ± 44·1	238 ± 9·1	100 ± 1·0
C	1399 ± 33·4	235 ± 8·0	99 ± 1·0
Overall RSD (%)	3·88	3·37	1·65

The precision of the HSGC method was tested by replicated injections of a deteriorated oil sample on three different working days (Table 1). Analysis of variance indicated that in each case the within-series variability of the peak areas was not statistically different from the between-series variability ($P > 0·05$). Mean values of peak areas of all series did not differ by more than 5%. The overall relative standard deviation (RSD) was 3·88% for pentane, 3·37% for hexanal and 1·65% for *trans*-2-heptenal, respectively.

Thermal decomposition of lipid peroxides

In order to study the influence of heat on the decomposition of peroxides, purified linoleic acid hydroperoxides dissolved in a freshly refined and deodorized soybean oil contained in crimp-capped HS vials were heated for 30 min at different temperatures in an oven. A non-fortified oil sample served as a blank. The pentane concentration in the HS gas was used as an indicator

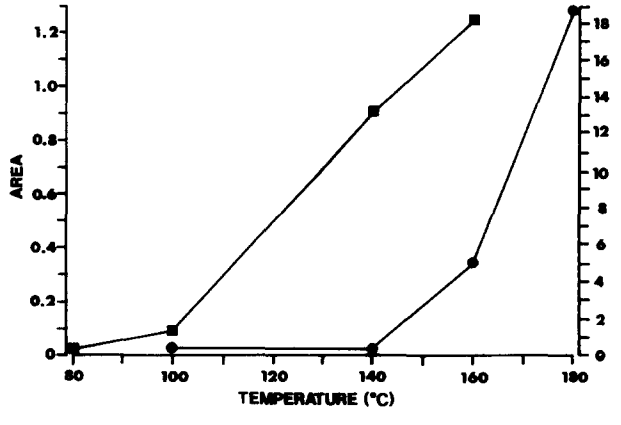

Fig. 1. Pentane peak area after thermal treatment (30 min) of an oil containing purified linoleic acid hydroperoxides (right *y* axis) and an unfortified oil serving as the blank (left *y* axis).

for the extent of peroxide break-down (Fig. 1). The blank showed no increase in pentane when changing the oven temperature from 100 to 140°C. On the contrary, the same temperature rise increased the pentane concentration above the peroxide containing sample by a factor of 10. At 160°C a further increase in pentane values was observed for both sample types. Therefore, 140°C was selected as the pre-treatment temperature for subsequent experiments.

The effect of the vial atmosphere (nitrogen or air) on the production of volatiles during the thermal treatment was tested by heating 1 g samples of a freshly deodorized soybean oil in HS vials at various incubation conditions (Fig. 2). As expected, the presence of oxygen in the HS led to an increased formation of volatiles due to oil oxidation. The more drastic the incubation conditions (time/temperature) the more pronounced was the effect of oxygen. Oil oxidation was retarded by flushing the vials with nitrogen before heating. Although effective at all temperatures tested, the inert vial atmosphere was not able to prevent completely the onset of oil oxidation. Compared with heating the oil in nitrogen at 120°C for 30 min, the pentane area increased by a factor of 3·6 when heated at 150°C. In accordance with the results shown in Fig. 2, no increase in pentane values was observed at 140°C.

Occasionally, outliers were observed in these experiments due to leaking crimp-caps of the HS vials. They were found not to be airtight after heating to 140°C and subsequently cooling down to room temperature or 60°C. To circumvent this source of error, experiments were also carried out with sealed brown-glass ampoules serving as sample containers during the incubation period. The ampoules were loaded with 1·3 g of oil, flushed with nitrogen, flame-sealed and treated as given above. After cooling

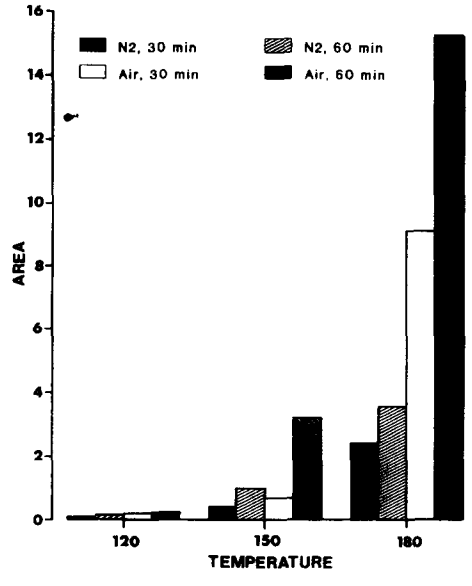

Fig. 2. Influence of the vial atmosphere (air or N_2) on the heat induced formation of pentane measured by HSGC (peak areas are reported).

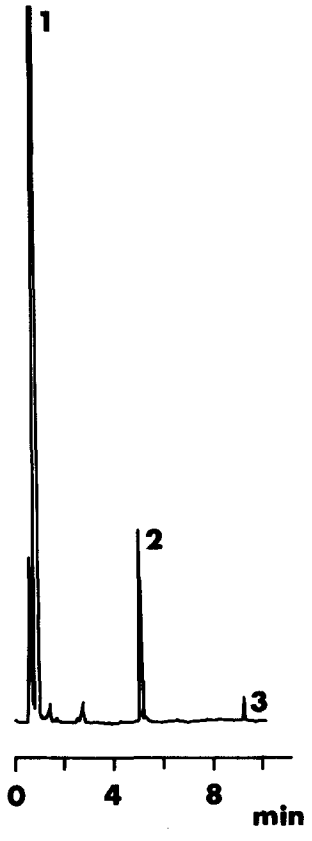

Fig. 3. HSGC separation of oil volatiles. 1, pentane; 2, hexanal; 3, *trans*-2-heptenal.

to room temperature, the ampoules were opened and a 1·0 g portion was tested by HSGC. This modification improved the repeatability of the overall procedure, including sample pre-heating and HSGC estimation of the volatiles. A typical trace of such a separation of the volatiles above an oil sample with a POV of 22·5 is shown in Fig. 3. Precision of the overall method was tested by analysing an oil sample high in POV (12·3), and another sample low in POV (1·8), in duplicate on five consecutive working days. The RSD for the pentane and hexanal area values was 6·79% and 4·43% (high POV), and 5·73% and 3·38% (low POV), respectively.

Calibration and limit of detection

For calibration purposes the standard oil sample containing *c.* 1000 ppm of each volatile was serially diluted with oil and analysed by HSGC as described above. Calibration graphs were constructed by plotting the

Table 2. Calibration functions to relate peak areas to the concentration of oil volatiles (ppm)

	Pentane	Hexanal	*trans*-2-Heptenal
Concentration range (ppm)	0·2–186	1·0–192	4·9–194
Data points	8	7	5
R^2	0·999	0·998	0·999
Slope	88 704	1 279	409

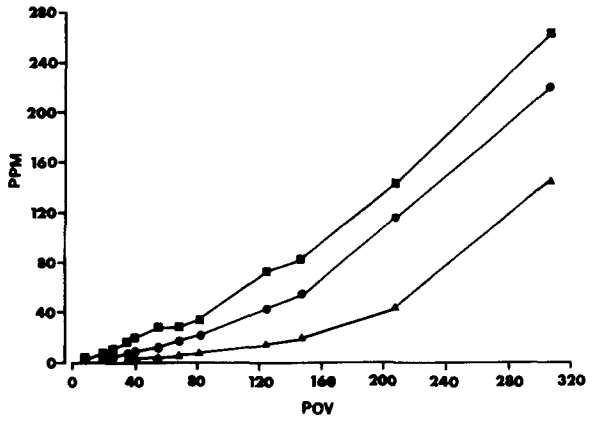

Fig. 4. Relationship between the contents of volatiles and the POV of a salad oil stored at 60°C in air (■, pentane; ●, hexanal; ▲, *trans*-2-heptenal).

peak areas over the corresponding sample amounts. Calibration functions were fitted to the data points by using least squares analysis (Table 2). The slopes of the three regression lines obtained differed considerably from each other. The higher the boiling point of the volatile component the smaller was the regression coefficient. The limit of detection was 0·02 ppm for pentane, 0·2 ppm for hexanal and 1 ppm for *trans*-2-heptenal.

Relationship between POV and volatile contents

A commercially obtained vegetable oil was stored at 60°C in air and sampled periodically. The relationship between POV and the HSGC estimates of pentane, hexanal and *trans*-2-heptenal is given in Fig. 4. Although least squares analysis indicated statistically significant relationships ($P < 0·01$) between the contents of individual volatiles and the POV (correlation coefficients >0·90 in each case), an appreciable curvature of the lines connecting the relevant data points was observed. Logarithmic transformation of the data slightly improved the linearity of the graphs. An excellent fit was obtained by using second-order regression models (Table 3).

Table 3. First- and second-order regression models to relate the concentration of pentane, hexanal and *trans*-2-heptenal to the iodometrically determined POV value

	Pentane	Hexanal	*trans*-2-Heptenal
First-order			
R^2	0·964	0·932	0·826
Intercept	−14·770	−17·603	−16·349
Regression coefficient, linear	0·811	0·669	0·403
Second-order			
R^2	0·999	0·998	0·989
Intercept	1·562	1·363	5·229
Regression coefficient, linear	0·320	0·098	−0·199
quadratic	0·002	0·002	0·002

DISCUSSION

Lipid peroxides are comparatively unstable substances, which are known to undergo rapid decomposition at elevated temperatures to form a multitude of more or less volatile compounds (Grosch, 1987). In the present study only three substances, i.e. pentane, hexanal and *trans*-2-heptenal, were used for quality grading of vegetable oils. These particular compounds were selected because they exhibit sufficient volatility to be readily detected by means of static HSGC.

Data concerning the precision of lipid peroxide determinations by means of static HSGC are rare. Only Snyder *et al.* (1988) reported repeatability values. Unfortunately, their RSD values (4·5–5·4% depending on POV) were based on the total peak area created by an HS injection, and not on individual areas. RSD of the HSGC estimation of pentane, hexanal and *trans*-2-heptenal *per se* proved to be <4% in the present study. Similar values were reported for the HSGC determination of carbonyls in fermented milk products (Ulberth, 1991). Because of possible errors associated with the heat pre-treatment step, the RSD values of the overall procedure increased to *c.* 7%. When using the technique described, it is of utmost importance to ensure an inert atmosphere in the vials during the high temperature treatment. Otherwise additional peroxides will be formed, thus giving erroneously high values of volatiles and poor reproducibility.

According to Ullrich & Grosch (1988), various C_9 aldehydes and 2,4-decadienal contribute to a large extent to the flavour of autoxidized oils, but they were not detected by the proposed HSGC method. This does not necessarily mean that these components were not formed during the heat induced breakdown of peroxides, rather that their equilibrium concentration in the HS is comparatively small due to their low vapour pressure at 60°C. A small peak with the same retention time as 2,4-decadienal was found sometimes after thermal treatment of badly deteriorated oil samples, but was excluded from quantitation. The estimation of high-boiling compounds by static HSGC has been described (Marsili, 1984; Snyder *et al.*, 1985), but very high thermostating temperatures (180°C) had to be applied in those procedures. Such very high temperatures are not practicable when using less sophisticated instrumentation for sample introduction in HSGC, e.g. a water bath and a gas-tight syringe. Bearing in mind this limitation, the heat-treatment step for the decomposition of lipid peroxides was done separately from the thermostating step necessary for HSGC.

Purge-and-trap techniques or dynamic HS sampling is another means to concentrate oil volatiles before GC separation (Snyder *et al.*, 1988). Even compounds with a high boiling point are stripped off with great efficiency. Compared with static HSGC, dynamic HS sampling reflects to a greater extent the flavour of a particular oil sample. However, static HSGC is a very useful alternative to classical methods for lipid peroxide measurement. In accordance with other reports

(Warner *et al.*, 1974; Snyder *et al.*, 1985), a close relationship was found between POV and the contents of volatiles after extensive heat treatment (Fig. 4). In this context it is noteworthy that both the quality and the quantity of volatiles formed are governed by the type of hydroperoxide precursors present in the sample. Moreover, the quantity of a number of heat-generated oil volatiles correlates well with flavour scores of these oils (Williams & Applewhite, 1977).

REFERENCES

Bassette, R. (1984). Measuring flavor changes with vapor sampling and GLC analysis. *J. Food Prot.*, **47**, 410–13.

Dupuy, H. P., Fore, S. P. & Goldblatt, L. A. (1973). Direct gas chromatographic examination of volatiles in salad oils and shortenings. *J. Am. Oil Chem. Soc.*, **50**, 340–2.

Dupuy, H. P., Flick, G. J. Jr, Bailey, M. E., St Angelo, A. J., Legendre, M. G. & Sumrell, G. (1985). Direct sampling capillary gas chromatography of volatiles in vegetable oils. *J. Am. Oil Chem. Soc.*, **62**, 1690–3.

Evans, C. D., List, G. R., Hoffmann, R. L. & Moser, H. A. (1969). Edible oil quality as measured by thermal release of pentane. *J. Am. Oil Chem. Soc.*, **46**, 501–4.

Grosch, W. (1976). Abbau von Linolsäeurehydroperoxyden zu flüchtigen Monocarbonylverbindungen. *Z. Lebensm. Unters. Forsch.*, **160**, 371–5.

Grosch, W. (1987). Reactions of hydroperoxides—products of low molecular weight. In *Autoxidation of Unsaturated Lipids*, ed. H. W.-S. Chan. Academic Press, London, pp. 95–139.

Grosch, W. (1989). Aromastoffe aus der Lipidperoxidation. *Fat Sci. Technol.*, **91**, 1–6.

Hachenberg, H. & Schmidt, A. P. (1979). *Gas Chromatographic Headspace Analysis.* Heyden & Son, London.

Jackson, H. W. & Giacherio, D. J. (1977). Volatiles and oil quality. *J. Am. Oil Chem. Soc.*, **54**, 458–60.

Marsili, R. T. (1984). Measuring light-induced chemical changes in soybean oil by capillary headspace gas chromatography. *J. Chromatogr. Sci.*, **22**, 61–7.

Pongracz, G. (1986). Determination of rancidity of edible fats by headspace gas chromatographic detection of pentane. *Fette Seifen Anstrichmittel*, **88**, 383–6.

Raghavan, S. K., Reeder, S. K. & Khayat, A. (1989). Rapid analysis of vegetable oil flavor quality by dynamic headspace capillary gas chromatography. *J. Am. Oil Chem. Soc.*, **66**, 942–7.

Scholz, R. G. & Ptak, L. R. (1966). A gas chromatographic method for measuring rancidity in vegetable oils. *J. Am. Oil Chem. Soc.*, **43**, 596–9.

Selke, E. & Frankel, E. N. (1987). Dynamic headspace capillary gas chromatographic analysis of soybean oil volatiles. *J. Am. Oil Chem. Soc.*, **64**, 749–53.

Snyder, J. M., Frankel, E. N. & Selke, E. (1985). Capillary gas chromatographic analyses of headspace volatiles from vegetable oils. *J. Am. Oil Chem. Soc.*, **62**, 1675–9.

Snyder, J. M., Frankel, E. N., Selke, E. & Warner, K. (1988). Comparison of gas chromatographic methods for volatile lipid oxidation compounds in soybean oil. *J. Am. Oil Chem. Soc.*, **65**, 1617–20.

Timmen, H. (1975). Bestimmung der Peroxidzahl und Pruefung der Oxydationsstabilitaet von Butterreinfett. *Milchwissenschaft*, **30**, 329–31.

Ulberth, F. (1991). Headspace gas chromatographic estimation of some yogurt volatiles. *J. Ass. Off. Anal. Chem.*, **74**, 630–4.

Ullrich, F. & Grosch, W. (1988). Flavour deterioration of soya-bean oil: Identification of intense odor compounds formed during flavour reversion. *Fat Sci. Technol.*, **90**, 332–6.

Warner, K. & Frankel, E. N. (1985). Flavour stability of soybean oil based on induction periods for the formation of volatile compounds by gas chromatography. *J. Am. Oil Chem. Soc.*, **62**, 100–3.

Warner, K., Evans, C. D., List, G. R., Boundy, B. K. & Kwolek, W. F. (1974). Pentane formation and rancidity in vegetable oils and in potato chips. *J. Food Sci.*, **39**, 761–5.

Warner, K., Evans, C. D., List, G. R., Dupuy, H. P., Wadsworth, J. I. & Goheen, G. E. (1978). Flavor score correlation with pentanal and hexanal contents of vegetable oil. *J. Am. Oil Chem. Soc.*, **55**, 252–6.

Williams, J. L. & Applewhite, T. H. (1977). Correlation of the flavor scores of vegetable oils with volatile profile data. *J. Am. Oil Chem. Soc.*, **52**, 461–3.