Determination of Sulfur Contents of Vegetable and Marine Oils by Ion Chromatography and Indirect Ultraviolet Photometry of Their Combustion Products

R.C. Wijesundera[a,1], R.G. Ackman[a,*], V. Abraham[b] and J.M. deMan[b]

[a]Canadian Institute of Fisheries Technology, Technical University of Nova Scotia, P.O. Box 1000, Halifax, Nova Scotia, Canada B3J 2X4, and [b]Department of Food Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1

A simple method for the determination of total sulfur content in vegetable and marine oils is described. The method involves combustion of the oil sample in an oxygen bomb to convert all forms of sulfur to sulfate ions with subsequent determination of the sulfate by ion chromatography and indirect ultraviolet detection. The ultraviolet system described is more sensitive than conductivity detection and enables the method to be applied more widely.

Application of the method to a variety of vegetable and marine oils showed the general occurrence of sulfur in fats and oils, albeit often at a low level. Among the samples examined, crude Canola oil had the highest sulfur content (25.0 mg/kg) followed by the marine oils (5.8–15.2 mg/kg) and the non-Cruciferae vegetable oils (2.0–6.1 mg/kg).

Vegetable oils belonging to the Cruciferae family, such as rapeseed and mustard seed oils, are known to contain sulfur compounds (1–3) which are thought to arise from the breakdown of glucosinolates present in the seed (4). Although these sulfur compounds occur in trace quantities they have attracted considerable attention in the recent past (5) because they are known to inhibit oil hardening processes (6) and to impart characteristic odors to the oils (7, 8).

Though the origins may be different (9), fish oils also have been shown to contain sulfur compounds (10). Consequently, the fish oil hardening industry has paid increasing attention to the presence of sulfur in the oil. According to one report (11), the sulfur content in fish oils is on its way to becoming a measure of quality in the same way as are free fatty acids and color, and it is possible to foresee a situation where the sulfur content implies a reduced commercial value for the oil, and in more pronounced cases could prevent its sale for use for food. There is an urgent need, therefore, to develop simple and reliable methods for the quantitative determination of sulfur compounds in fats and oils.

The method of Raney nickel reduction (12), though widely used, does not measure the total sulfur content in fats and oils (11, 13, 14). Abraham and deMan (14) recently reviewed methods and reported on a method based on the initial conversion of all forms of sulfur in the oil to sulfate by combustion in an oxygen bomb. The sulfate so derived was separated by ion chromatography and measured by means of a conductivity detector. In the present work, the detection and quantification of the sulfate has been executed with the more commonly available ultraviolet (UV) detector. This paper describes the application of the UV photometric method and compares it to conductivity measurement of sulfur in marine oils. The general occurrence of sulfur in vegetable oils, both crude and refined, is also demonstrated.

EXPERIMENTAL PROCEDURES

Materials. Canola oil, industrially expelled but unrefined, was obtained from a Canadian oil mill. Soybean oil was extracted in the laboratory with hexane from a retail sample of beans. The rest of the vegetable oils were of refined, edible grade and were purchased from retail outlets in Halifax. The marine oils examined were taken from a collection of samples submitted by various producers to the Canadian Institute of Fisheries Technology. All working samples were carefully withdrawn by pipette from four-l glass containers left undisturbed for several months.

Double-distilled, deionized water filtered successively through a NORGANIC™ trace organic removal cartridge and a 0.45-micron filter (Millipore Corp., Bedford, Massachusetts), was used for preparing the phthalate buffer.

Heptyl isothiocyanate was purchased from Eastman Kodak Co., Rochester, New York. Standard sulfate solutions were prepared from potassium sulfate (BDH, AnalaR). A stock solution containing 1000 mg/l of sulfate was diluted appropriately with the eluent buffer to obtain the required standard concentrations. Potassium hydrogen phthalate (Fisher certified reagent) was used for preparing the buffer.

Determination of sulfur content. Combustion was carried out in a 350-ml, stainless steel oxygen bomb (Parr Instrument Co., Moline, Illinois, Model 1108) equipped with a Model 2901 ignition system. The two electrodes were connected with a 10-cm length of nickel alloy fuse wire. Approximately five ml of the eluent used for ion chromatography (1.0 mM potassium hydrogen phthalate, pH 6.5) was placed in the bottom of the bomb. The sample (1–2 g) was weighed into the stainless steel capsule crucible, which was then placed on the loop electrode. Tongs were used to handle the capsule. The fuse wire was bent slightly so that it extended to just below the surface of the oil. Gloves were worn during this operation. The bomb was closed and flushed with pure oxygen (Medical Oxygen USP, Canadian Liquid Air) at least five or six times and finally pressurized to 3040 KPa. The bomb was immersed in a calorimeter water bath, and the sample was ignited by means of the ignition button. The bomb was allowed to cool in the stirred water bath for 15 min, and the pressure was released gradually and the bomb carefully opened. The sample capsule, electrodes and the interior surfaces of the bomb were rinsed thoroughly with more chromatography eluent. The washings were transferred to a 25-ml volumetric flask and made up to the 25.00 ml mark. The solution was then filtered through

[1]Permanent address: Ceylon Institute of Scientific and Industrial Research, P.O. Box 787, Colombo, Sri Lanka.
*To whom correspondence should be addressed.

a 0.45-micron filter (Millipore) to remove particulate matter. The pH of the solution was increased to 6.5 by addition of aqueous sodium borate, and a portion (5 ml) was further purified by passing through a SEP-PAK C_{18} cartridge (Waters Associates Ltd., Milford, Massachusetts) to remove any organics. Usually 0.1 ml of this purified solution was injected to the ion chromatography column.

UV photometric ion chromatography was carried out using a system comprising a solvent pump (Waters, Model 6000A), a variable wavelength UV detector (Waters, Lambda-Max, Model 480), and a one-mV pen recorder (Fisher Recordall, Series 5000). A strong anion exchange column (4.6 mm i.d. × 5 cm stainless steel Waters IC-PAK A) was used in conjunction with a guard column (Waters IC-PAK anion Guard-PAK). Aqueous potassium hydrogen phthalate, 1.0 mM, at pH 6.5 was used as the eluent at a flow rate of 1.0 ml/min. The pH of the buffer was adjusted with sodium tetraborate. The UV detector was operated at 290 nm, usually at an attenuation of 0.01 AUFS.

Ion chromatography of combustion products using conductivity detection has been described elsewhere (14).

RESULTS AND DISCUSSION

During the past few years, single-column ion chromatography has emerged as an extremely useful technique for the analysis of anions and cations (15). Interest in the subject has intensified recently, mainly because of the relative ease with which ion chromatography can be performed using standard HPLC equipment. The analysis of strongly ultraviolet absorbing ions is relatively simple, but the analysis of weakly absorbing ions has presented many problems, particularly with regard to detection at trace levels. Inorganic ions such as sulfate which do not absorb sufficiently at accessible UV wavelengths generally are analyzed with conductivity detectors. An alternative method can be found in indirect UV photometry (16). This technique uses a strong UV-absorbing eluent for chromatography, and the UV detector is operated at a wavelength which gives maximum absorbance for the eluent. When a UV-transparent ion such as sulfate enters the flow cell, a decrease in absorbance results, giving rise to a negative peak. Reversing the polarity of the recorder gives a more conventional chromatogram.

We have applied UV photometric ion chromatography to the analysis of anions obtained from combustion of oil samples in an oxygen bomb. Under the chromatographic conditions used, chloride, nitrate and sulfate were well separated from each other (Fig. 1) and eluted in 2.4, 5.8 and 8.4 min, respectively. At pH 6.5, the phthalate is already completely ionized to diphthalate; a further increase in pH does not result in faster elution. However, pH values below 6.5 are best avoided because they tend to give rise to troublesome system peaks and also retard elution. Although the use of higher phthalate concentrations speeds up elution, we preferred to work with 1.0 mM phthalate because it gives good base-line separation of sulfate and nitrate. The latter is usually a major constituent in extracts obtained from combustion of oil samples. Furthermore, at this eluent concentration, the sensitivity of ion detection is greater than at higher concentrations.

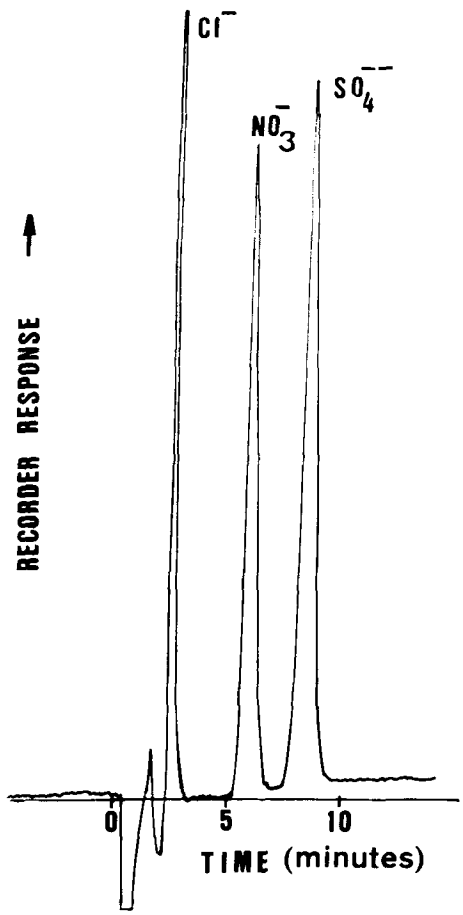

FIG. 1. Ion chromatography with UV photometric detection of chloride, nitrate and sulfate ions. Chromatography on an IC-PAK column with potassium hydrogen phthalate (1.0 mM, pH 6.5) eluent. UV detection at 290 nm.

The retention time for sulfate remained constant throughout the study when the concentration and pH of the eluent were carefully controlled. No appreciable deterioration of peak resolution was observed during a three-month period. For a given batch of buffer solution, there existed a good linear relationship between peak height and concentration of sulfate ions for the concentration range 0-10 mg/l as well as for 10-60 mg/l. The standard curve for the concentration range 10-60 mg/l (Fig. 2) had a correlation coefficient of 0.9985. However, the peak height was sensitive to even the slightest change in the buffer concentration and pH. Therefore, it was necessary to prepare a fresh standard curve for each batch of eluent.

Combustion in the oxygen bomb converts all forms of sulfur in the oil, whether organically bound or otherwise, into sulfate ions. Measurement of the sulfate so formed from a known mass of oil allows the calculation of the total sulfur content in the oil. It is necessary to flush the bomb at least five or six times to eliminate most of the air from the bomb, because an important nitrate peak, presumably resulting in large part from nitrogen in the air or dissolved in the oil, sometimes interfered with the sulfate peak. It was also necessary

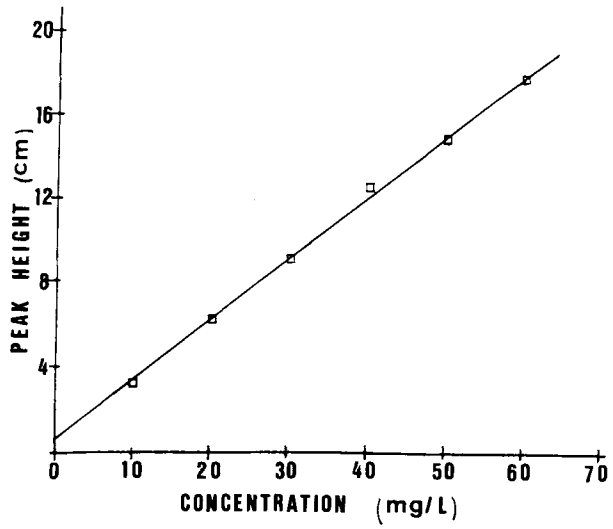

FIG. 2. Plot of the concentration (mg/l) vs peak height (cm) for standard sulfate solutions (10-60 mg/l) obtained by ion chromatography and indirect UV photometric detection. Regression equation is y = 0.290 x + 0.693; correlation coefficient = 0.9985.

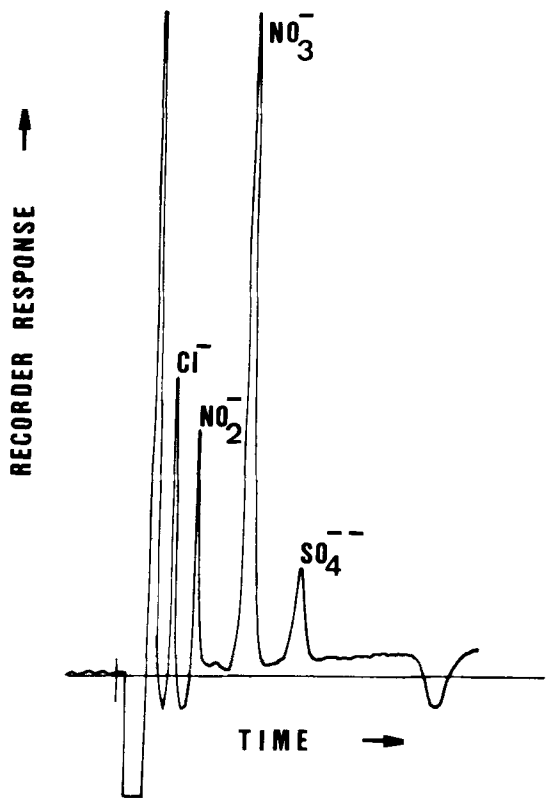

FIG. 3. An ion chromatographic trace typical of fish oil combustion products; the trace shown here is that of channel catfish. Analytical conditions were the same as that of Fig. 1.

to prepare the extracts in phthalate buffer and readjust the pH to 6.5 in order to avoid troublesome system peaks.

Fig. 3 shows a typical chromatographic trace of the ion extract obtained from combustion of a fat, capelin oil. Carbonate elutes first and is sometimes masked by the solvent dip. Chloride, nitrite, nitrate and sulfate follow, in that order. Sulfate and sulfite do not resolve under these conditions, and it is possible that the sulfate peak contains small amounts of sulfite. The dip after the sulfate peak is due to the system peak.

TABLE 1

Recovery of Sulfur from Samples of Canola Oil Spiked with Heptyl Isothiocyanate

Sulfur added (mg/l)	Sulfur recovered[a] (mg/l)	% Recovery
0	22.9[b] ± 2.6	-
10	30.7 ± 1.8	93.3
20	40.6 ± 1.6	94.6
30	50.5 ± 1.3	95.5
40	61.3 ± 2.0	97.5
50	70.0 ± 1.6	96.0
60	79.1 ± 1.3	95.4
	Mean % recovery	95.4

[a]Mean values and standard deviations of three complete determinations.
[b]mg/l; Table 2 units are mg/kg for the same sample.

The level of sulfate in the combustion product was calculated by referring the height of the sulfate peak to the standard curve. The sulfur content in the oil was calculated from the sulfate according to the formula:

$$\text{Sulfur content (mg/kg)} = vc/3m$$

Where m is the mass of oil sample (g), c is the concentration (mg/l) of sulfate in the extract and v is the volume (ml) of the extract.

Recovery of sulfur by combustion was checked with samples of canola oil spiked with heptyl isothiocyanate, which is readily soluble in the oil. Recoveries ranging from 93.3-97.5% were obtained for samples containing 10-60 mg/kg of added sulfur (Table 1).

It is known that the sensitivity of indirect UV photometric detection is greater than with conductivity detector. For example, Cochrane and Hillman (17) found that for sulfate ions, there is a ten-fold increase in sensitivity compared to conductivity detection. With our system, we were able to detect sulfate levels of 0.05 mg/l without pre-concentration (0.2 ml injection, detector sensitivity at 0.005 AUFS). At this level it was possible to obtain a peak at least three times higher than the noise level. This enabled the determination of sulfur contents as low as 0.1 mg/kg in vegetable and marine oil samples. Such low levels cannot be measured with conductivity detectors, which have a detection limit of 0.5 mg/kg (14). Furthermore, the popularity of UV detectors means the equipment is already in place in most laboratories and so indirect UV photometry is also a more accessible method than conductivity detection. We have, however, determined the total sulfur content in crude canola oil and in several marine oils, using the two methods in parallel, and find that the results are in good agreement (Table 2).

It has been reported that sulfur contents as low as 1 mg/kg inhibit catalytic hydrogenation of canola oil (6). The critical sulfur level for fish oil hardening is not known. The five unrefined marine oils examined con-

TABLE 2

Sulfur Content (mg/kg)[a] in Some Vegetable Oils[b] and In Marine Oil[c] Samples

	UV	Conductivity
Canola (commercial, unrefined)	25.0 ± 2.6	23.1 ± 1.0
Canola[d] (Canbra Foods Ltd., Lethbridge)	9.4 ± 0.9	n.d.
Soybean (laboratory extracted, unrefined)	4.6 ± 1.7	n.d.
Soybean[d] (HAIN, Hain Pure Food Co., Inc., Los Angeles, California)	2.6 ± 1.3	n.d.
Sunflower[d] (HAIN, Hain Pure Food Co. Inc., Los Angeles, California)	2.0 ± 1.6	n.d.
Coconut[c] (Sunny Crunch Foods Ltd., Markham, Ontario)	6.1 ± 2.1	n.d.
Capelin (*Mallotus villosus*)	12.7 ± 0.2	15.1 ± 0.9
Herring (*Clupea harengus*)	8.2 ± 2.3	8.2 ± 0.4
Menhaden (*Brevoortia tyrannis*)	15.2 ± 0.1	13.1 ± 0.7
Dogfish liver (*Squalus acanthias*)	5.8 ± 1.0	5.5 ± 0.9
Seal blubber (*Pagophilus groenlandicus*)	12.4 ± 0.2	12.8 ± 0.5
Channel catfish (freshwater) (*Ictalurus punctatus*)	7.6 ± 0.1	n.d.

[a]Mean values and standard deviation of three combustions. Values are corrected for 95.4% recovery.
[b]Commercially refined oils except when indicated otherwise.
[c]From commercial production. The menhaden and catfish oils were partially refined.
[d]Retail samples.
n.d., not determined.

tained sulfur at levels ranging from 5.8 mg/kg for dogfish liver oil to 15.2 mg/kg for menhaden (Table 2). The freshwater fish oil also fell in this range. It is of interest that the marine mammal oil also had sulfur levels comparable to the fish oils, but it is usually accepted that their depot fats are essentially fatty acids of fish origin (18) and are not endogenous. The sulfur sites in fish oils may be different from those of canola oil, and the composition of the sulfur compounds present is presumably different. It has been suggested (13) that the catalyst poisoning in low erucic acid rapeseed is caused by the isothiocyanates that arise from the hydrolysis of the seed glucosinolates, whereas the sulfur compounds found in fish oils include sulfides and methyl thioesters considered to be derived from bacterial degradation of methionine (10).

It is noteworthy that all of the four non-Cruciferae vegetable oil samples examined contained sulfur ranging from 2.0 mg/kg for sunflower seed oil to 6.1 mg/kg for coconut oil, compared to 25.0 mg/kg in crude canola oil, although the refined canola oil contained only 9.4 mg/kg (Table 2). Apart from the soybean oil which was solvent extracted in the laboratory, and the canola oil which was commercially expelled, all the other samples were edible grade refined oils. It is known that the oil refining process reduces the sulfur content in such oils (1, 2). The actual sulfur content of the refined oils shown in Table 2 presumably would have been higher in the crude oils.

The low standard deviation for the results from the UV method applied to some samples, as distinct from others, was of interest. A clue to this difference is provided by the fully refined canola oil and reinforced by three of the marine oils. The menhaden oil and the channel catfish oils were partially refined. The seal oil is made from blubber almost free of protein. These considerations suggest that any refining which includes cooking oilseeds or fish, then refining by acid or alkali washing, bleaching and filtration, removes all suspended fine particles of protein and gives a lower standard deviation. We attempted to remove oil samples carefully and with a minimum of disturbance from four-l containers, and the oil samples appeared to be free of turbidity. However, suspended aggregates of such protein particles, or nonhomogeneous distributions of particles from convection, could have influenced each analysis by providing sulfur over and above that native to the fatty acids (19) or unsaponifiable matter.

In a companion paper (20) we record sulfur, as well as nitrogen, contents of six other oils, four vegetable and two marine. For sulfur the results support the above hypothesis: The unrefined canola oil gave 24.9 ± 1.8 mg/kg of sulfur for mean and standard deviations for three further analyses. The actual value is satisfactorily close to that obtained (25.0 ± 2.6 mg/kg) in this initial study. The other fish oils, all refined, had much lower standard deviations.

If this is a valid explanation, then it shows the sensitivity of the method and also provides a cautionary consideration in applying such sensitive methods to oil samples of unknown history as regards refining in future research.

Combustion and ion chromatography coupled with indirect ultraviolet photometry provide a rapid and convenient method for the determination of the sulfur content in fats and oils. The method can be carried out with standard laboratory equipment. Application of the technique to commercial oils and fats shows that sulfur compounds occur in all vegetable oils tested as well as in marine oils.

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