✤ Determination of Nitrogen Content of Fats and Oils by the Method of Combustion/Ion Chromatography

R.C. Wijesundera[1] and **R.G. Ackman***
Canadian Institute of Fisheries Technology, Technical University of Nova Scotia, P.O. Box 1000, Halifax, Nova Scotia B3J 2X4

A method of combustion/ion chromatography (C/IC), initially developed for the determination of sulfur, has been extended to determination of total nitrogen in fats and oils. It is shown that nitrogen gas dissolved in the sample interferes with the determination, and that it is necessary to degas the sample before analysis. Moreover, there is a procedural blank for the nitrogen in the cylinder oxygen employed. Despite these drawbacks, the method has advantages over conventional methods, in that (i) measurements can be carried out with standard laboratory equipment, and (ii) nitrogen and sulfur, and possibly halogens and selenium, can be determined simultaneously.

Compounds of nitrogen and sulfur, when present in oils and fats, even at levels as low as several parts per million, may exert undesirable effects on oil processing. Sulfur compounds, in particular, are known to poison hydrogenation catalysts very readily (1).

Recently we reported on a method for the determination of low concentrations of sulfur in oils and fats (2). The method was based on combustion of the sample in an oxygen-bomb in the presence of water to convert the organically bound sulfur to sulfate ions, and subsequent determination of the sulfate quantitatively by ion chromatography and indirect ultraviolet detection. During this study it became apparent that this combustion process produced nitrate ions from nitrogen compounds in the sample in the same way as sulfate ions were formed from sulfur compounds. The present paper describes an extension of the method to the simultaneous determination of nitrogen and sulfur when present in oils and fats.

EXPERIMENTAL PRCEDURES

Materials. The crude canola oil was obtained from a Canadian oil mill. The refined vegetable and marine oils were purchased from retail outlets. Heptyl isothiocyanate and the phospholipids were purchased from the Eastman Kodak Co., Rochester, New York, and Serdary Research Laboratories, London, Ontario, Canada, respectively. Triacetin (Sigma Chemical Co., St. Louis, Missouri) was distilled under vacuum before use. Potassium hydrogen phthalate (Fisher certified reagent) was used to prepare the ion chromatography eluent, which was made up in deionized, double-distilled water filtered successively through a NORGANIC trace removal cartridge and a 0.45-micron filter (Millipore Corp., Bedford, Massachusetts). The standard nitrate and sulfate solutions were prepared using their respective sodium salts (BDH, Analar) after drying overnight at 110°C. 'Zero' grade oxygen (Canadian Liquid Air, unspecified nitrogen content) was used for all the operations.

Preparation of liquid samples for combustion. Prior to combustion, about three g of the sample was sonicated under vacuum (2–3 millibar) in order to remove dissolved air. After one hr, the vacuum was broken with oxygen and the sample was subjected to combustion immediately.

Combustion. 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. Approximately five ml of the eluent used for ion chromatography (1.0 mM potassium hydrogen phthalate, pH 6.5) was sonicated under vacuum for 10 min and placed in the bottom of the bomb. The stainless steel capsule crucible was placed on the loop electrode, and the two electrodes were connected with a 10-cm length of nickel alloy fuse wire, care being taken not to touch any of the internal parts with bare hands. The bomb was closed and flushed five times with oxygen and left with a slight positive pressure. The bomb and the flask containing the prepared sample were then placed inside a glove-bag (Model X-27-17, I²R, Instruments for Research and Industry, Cheltenham, Pennsylvania) filled with oxygen, and about two g of the sample was transferred into the crucible, the exact weight taken for combustion being obtained by difference. The bomb was closed, removed from the glove-bag and pressurized with oxygen to 3040 KPa after flushing five times as before. It was then immersed in the calorimeter water bath and the sample ignited by means of the ignition button. The bomb was allowed to cool in the stirred water bath for about 15 min, 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 of the phthalate solution.

The pH of the washings was increased to 6.5 by addition of a few drops of aqueous sodium tetraborate, and the volume was adjusted to 25.00 ml with more phthalate. A portion of the solution was filtered, first through a 0.45-micron filter (SWINNEX-HA, Millipore Corp., Bedford, Massachusetts) to remove particulate matter and then through a SEP-PAK C_{18} cartridge (Waters Associates, Framingham, Massachusetts) to remove any residual organics. Usually 0.05 ml of this solution was injected onto the ion chromatographic column.

Ion chromatography. UV photometric ion chromatography was carried out using a system comprising a solvent pump (Waters, Model 6000A), a variable UV detector (Waters Model 450) and a 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 eluent was adjusted with aqueous sodium tetraborate. The UV detector was operated at 290 nm, usually at an attenuation of 0.01 AUFS. Response and calibration data set up standard curves for each lot of buffer used in analyses as this material affected response.

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[1] Permanent address: Ceylon Institute of Scientific and Industrial Research, P.O. Box 787, Colombo, Sri Lanka.

*To whom correspondence should be addressed.

RESULTS AND DISCUSSION

Ion chromatography coupled with indirect UV detection is a convenient method for quantitative analysis of anions (2). Under the conditions used in this study chloride, nitrite, nitrate, sulfate and selenate ions were well separated from each other (Fig. 1) and eluted in less than 15 min. There existed a good linear relationship between peak height and concentration for both nitrate and nitrite ions in the concentration range 0–300 mg/l. Accordingly, concentrations of these ions in the combustion products were determined by referring the heights of the nitrate and nitrite peaks to the respective standard curves.

In a previous study (2), when we analyzed vegetable and marine oils for sulfur, it was observed that their combustion products often contained unexpectedly large concentrations of nitrate ions. Although consistent values were obtained for the sulfate concentration, the apparent nitrate concentration varied appreciably from combustion to combustion. Oils and fats are known to contain significant quantities of dissolved nitrogen gas (3), and we suspected that the extra nitrate in the combustion products originated in the conversion of this dissolved nitrogen to nitrate ions by combustion. To verify this, a series of combustions was carried out on triacetin, an oil with no inherent nitrogen content.

Initially, triacetin was tested according to the previous procedure (2), in which sample degassing was not carried out and no oxygen-filled glove-bag was used. Surprisingly, the combustion products contained nitrite as well as nitrate ions and the total concentration of the two fluctuated somewhat from one combustion to the other (range 34.7–40.4, mean 37.1, standard deviation ±2.3; Figs. 2a and 3a). When samples of triacetin were degassed before

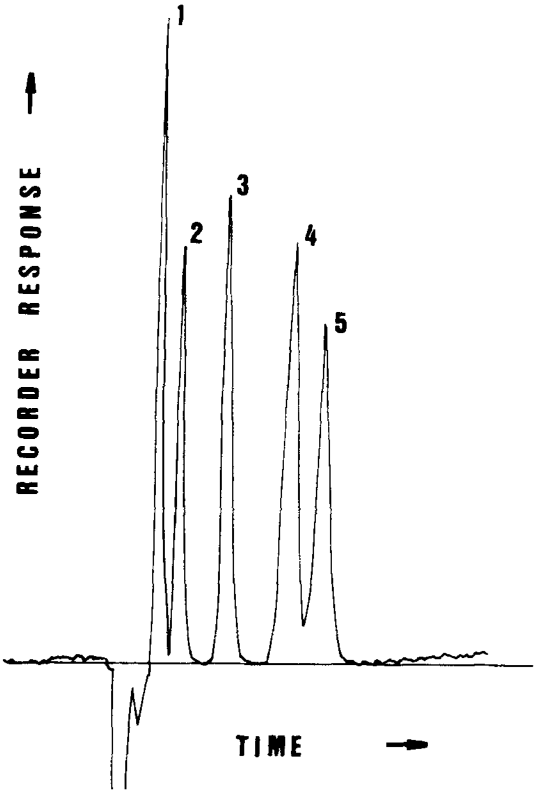

FIG. 1. Separation of anions on a strong anion exchange column using aqueous potassium hydrogen phthalate (1.0 mM, pH 6.5) as eluent. Detection was by indirect UV photometry. 1, chloride; 2, nitrite; 3, nitrate; 4, sulfate, and 5, selenate.

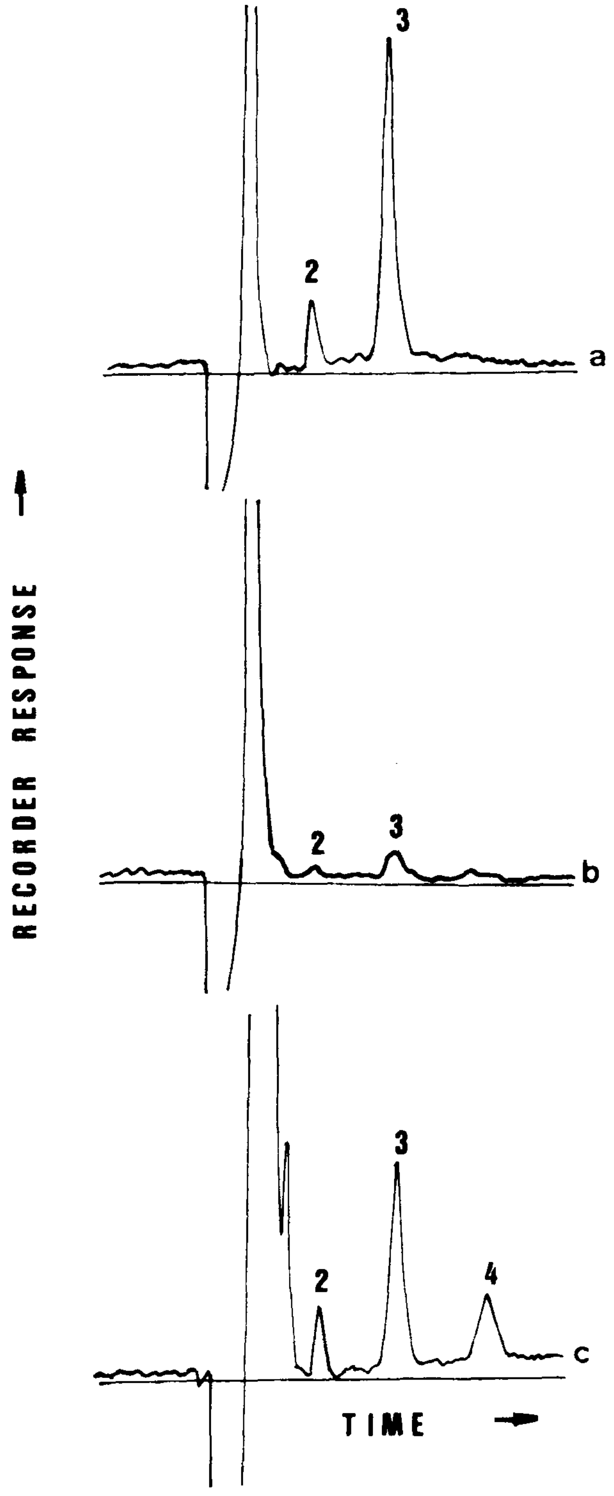

FIG. 2. Indirect UV photometric ion chromatogram of the combustion product of (a) triacetin before degassing, (b) triacetin after degassing, and (c) ω-3 fish oil concentrate (MaxEPA). 2, nitrite; 3, nitrate, and 4, sulfate. Separation conditions were as in Fig. 1.

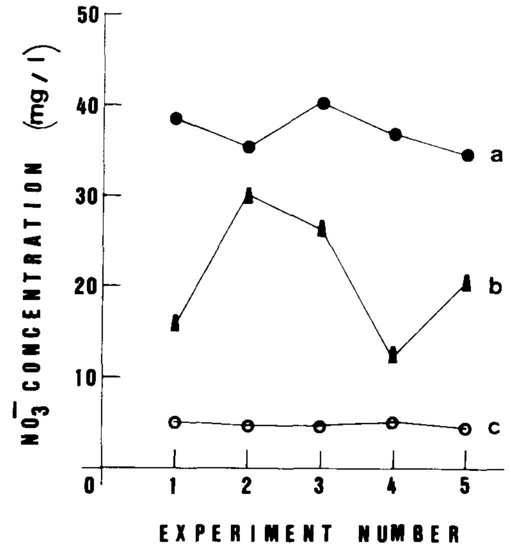

FIG. 3. Effect of sample degassing on the total nitrite and nitrate ion concentration in the combustion product of triacetin. (a) without degassing, (b) degassed, but without oxygen glove-bag, and (c) degassed and oxygen glove-bag.

TABLE 1

Variation of Nitrate Ion Concentration[a] in Triacetin Combustion Products with Sample Size

Wt of triacetin (g)	Nitrate ion concentration[b] (mg/l)
0.57	4.6 ± 1.4
1.04	4.9 ± 0.8
1.61	3.8 ± 0.6
2.07	4.3 ± 1.1
2.20	5.1 ± 1.7

[a] Includes contribution from nitrite N.

[b] Mean value and standard deviation for triplicates.

combustion by sonication under vacuum, the values were somewhat reduced but still fluctuated for the nitrogen ion concentration (range 15.5–30.2, mean 20.9, standard deviation ± 7.5, Fig. 3b). Much lower and practically constant values (range 4.3–5.1, mean 4.7, standard deviation ± 0.3; Figs. 2b and 3c) were obtained when the degassed samples were transferred to the bomb inside an oxygen-filled (nitrogen-free) glove-bag. Because the triacetin used for the experiment was distilled before use, it seemed unlikely that the nitrogen detected arose from any nitrogen-containing contaminants. This was confirmed when it was found that the total nitrite and nitrate ion concentration was virtually independent of the weight of triacetin used for combustion (Table 1). Presumably, the small amounts of nitrite and nitrate ions in triacetin combustion products resulted from the oxidation of small amounts of nitrogen gas presnt in the oxygen used for combustion. Indeed, conversion of gaseous nitrogen to oxides of nitrogen in the internal combustion engine is well known (4).

Before application of the combustion/ion chromatography (C/IC) method for the determination of nitrogen content in fats and oils, the blank constant for a nitrogen-free material such as triacetin must be established under the conditions of the experiment. It is likely that the blank constant would vary depending on the quality of the oxygen used and other parameters. In the present study, the actual values for the total nitrite and nitrate ion concentrations in the combustion products of vegetable and marine oils were obtained by subtracting the blank constant value of 4.6 mg/l from the observed value. Although both nitrite and nitrate ions were present in the combustion products, the latter was always the dominant species. For simplicity, the contribution of nitrite N to the total N was expressed in terms of nitrate ions and added to the nitrate ion concentration. The nitrogen content in the sample was calculated from this corrected nitrate ion concentration according to the formula:

$$\text{Nitrogen concent (mg/kg)} = 7V.C/31\ m$$

where V is the volume (ml) of the combustion product, C is the corrected nitrate ion concentration (mg/l) — blank constant, and m is the mass (g) of the sample.

Nitrogen compounds known to occur naturally in oils and fats include several phospholipids, the level of occurrence of which for unrefined oils is significant. They are found in fish oils as well as in other animal lipids and in crude and refined vegetable oils. Seed oils belonging to the Cruciferae family, for example rapeseed oil, contain several alkyl isothiocyanates and other nitrogen-containing substances which are thought to be generated during seed storage and oil expelling (5). To ensure that the C/IC method is applicable to the determination of these types of nitrogen compounds known to occur in fats and oils, the recovery of nitrogen was evaluated for heptyl isothiocyanate and the phospholipids lysophosphatidylcholine $(-\overset{+}{N}Me_3)$, lysophosphatidylethanolamine $(-\overset{+}{N}H_3)$ and sphingomyelin $(-\overset{+}{N}Me_3$ and $-NHCOR)$. Excellent recovery was obtained for the isothiocyanate, while comparatively low but satisfactory recoveries were obtained for the phospholipids (Table 2). The weight proportion of nitrogen in phospholipids depends on the type of fatty acid on the glycerol moiety as well as on general chemical purity.

The nitrogen and sulfur contents of some vegetable and marine oils as determined by the C/IC method are shown in Table 3. The ion chromatographic trace for the combustion product of MaxEPA fish oil concentrate is shown in Figure 2c. Except for the unrefined canola oil, all the other samples were commercially purified retail oils and were considered to be free from proteinaceous and other extraneous impurities. The "capsule" for MaxEPA is, of course, gelatin, but the nitrogen and sulfur values were similar to those of the high quality cod liver oil sold in a glass bottle, suggesting there is no transfer of amino acids.

Determination of total nitrogen traditionally has been accomplished by the wet-chemical Kjeldahl method (6), which was developed specifically for proteinaceous

TABLE 2

Recovery of Nitrogen from Some Authentic Nitrogen-Containing Compounds by the C/IC Method, after Correction for Blank

	Actual nitrogen[a] content (mg/kg)	Observed nitrogen[b] content (mg/kg)	%Recovery
Heptylisothiocyanate[c]	200.0	214.2	107.0
Lysophosphatidylcholine[d]	192.3	180.5	93.8
Lysophosphatidylethanolamine[d]	205.8	187.1	90.9
Sphingomyelin[d]	186.7	163.4	87.5

[a] The compounds were either dissolved or suspended in triacetin to obtain concentrations of ca. 200 mg nitrogen/kg triacetin.

[b] Mean value of two determinations.

[c] Compound dissolved in triacetin before degassing.

[d] Compound suspended in degassed triacetin.

TABLE 3

Nitrogen and Sulfur Contents (mg/kg)[a] of Some Vegetable and Marine Oils[b]

	Nitrogen[c]	Sulfur
Canola Oil, retail (Canbra Foods Ltd, Lethbridge, Canada)	1.4 ± 1.0	9.4 ± 0.9
Canola oil (unrefined)	27.3 ± 2.9	24.9 ± 1.8
Corn Oil (Mazola, Best Foods Canada Inc., Etobicoke, Ontario, Canada)	6.3 ± 1.5	8.5 ± 1.1
Vegetable Oil[d] (Crisco, Proctor & Gamble Inc., Toronto, Ont., Canada)	1.6 ± 0.8	6.1 ± 0.7
Lysi Cod Liver Oil (Lysi HF, Reykjavik, Iceland)	28.9 ± 3.8	3.0 ± 0.8
MaxEPA[e,f] (Walgreens MaxEPA 300)	21.5 ± 4.1	3.2 ± 0.6

[a] Mean value and standard deviation of three combustions.

[b] Commercially refined oils except when indicated.

[c] Corrected for blank.

[d] Contains canola and/or hydrogenated soybean oil.

[e] Retail ω-3 concentrate prepared from unspecified marine oils.

[f] Encapsulated oil.

nitrogen. It is, however, time-consuming and is not too reliable at low concentrations. Alternative methods currently available include microcoulometry (7) and combustion/chemiluminescent detection (8), both of which require sophisticated apparatus. The C/IC method has the advantage that it can be carried out with standard laboratory equipment. Moreover, it permits the determination of nitrogen and sulfur simultaneously using the same detector. Halide and selenate ions also can be detected by the ion chromatography system employed in this method (Fig. 1), and it is conceivable that the method could be extended to the measurement of halogen and selenium as well, provided that they are present in levels sufficient for detection (⩾0.1 mg/kg).

Although canola oil has been reported to contain selenium (9), we did not detect any in the samples examined by the C/IC method. Presumably selenium exhibits the same combustion behavior as sulfur, but the 7–8 ng/g concentration measured by atomic absorption spectrometry (9) is not sufficient for detection by the C/IC method.

A major shortcoming of the C/IC method applied to nitrogen content is the need for rather laborious sample preparation to remove dissolved nitrogen from the sample. This could be streamlined for routine use. Also, it is necessary to determine the blank constant for each gas cylinder or set of experiments. Even though unrefined canola oil is known to contain volatile sulfur compounds, our results in the co-analysis for sulfur were 24.9 ± 1.8 mg/kg, whereas in a previous independent study (2) the total sulfur content of the same oil, undegassed, was 25.0 ± 2.6 mg/kg. Thus, no significant fall in the sulfur content was observed as a result of subjecting the oil to degassing. The possibility of losing all or part of any volatile nitrogen or sulfur compounds present in samples during degassing still exists. Nevertheless, the fact that the C/IC method could be performed with standard laboratory equipment and that several elements could be determined simultaneously more than offsets the disadvantages.

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