Determination of Selenium, Arsenic, Iodine and Bromine in Fish, Plant and Mammalian Oils by Cyclic Instrumental Neutron Activation Analysis

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ABSTRACT

The concentrations of arsenic, selenium, iodine and bromine in a series of fish, plant and mammalian oils have been determined by cyclic instrumental neutron activation analysis (CINAA). Crude fish oils contain between 0.047 and 0.151 μg Se g^{-1}, 2.36–14.5 μg As g^{-1}, 2.36–9.63 μg Br g^{-1} and 0.97–4.76 μgI g^{-1}. Seal oil contains the same four elements, but at levels below the lower end of the fish oil ranges. Iodine, bromine and arsenic were not detected in rapeseed or soybean oils and the concentration of selenium varied from < 0.010 to 0.042 $\mu g\ g^{-1}$. The levels of selenium, iodine and bromine are reduced markedly by hydrogenation of the menhaden oils. The CINAA method yielded results which were in agreement with published values obtained by other methods. The technique was rapid, requiring minimal sample manipulation, and was essentially free from interferences.

INTRODUCTION

The application of neutron activation analysis (NAA) to the determination of selenium in oil samples is an attractive analytical method since it is nondestructive and requires minimal sample manipulation. However, earlier work employing NAA involved the nuclide ^{75}Se ($t_{½}$ = 120 day) which was produced by lengthy irradiations (up to 24 hr) and the nuclide was measured after decay times of 4–6 weeks (1, 2). Apart from the time required to complete the analyses, a second drawback was the production of ^{32}P in crude oil samples. Phosphorus-32 ($t_{½}$ = 14.3 day) emits β-particles which contribute to the background activity of the γ-spectrum and therefore reduce the sensitivity of the technique. In a recent report, the sensitivity of the long-lived nuclide, ^{75}Se, was found to be only 2.6 times that of the short-lived nuclide, ^{77m}Se ($t_{½}$ = 17.4 sec) (3).

We have, therefore, investigated the applicability of cyclic instrumental neutron activation analysis (CINAA) to the determination of selenium and included bromine, iodine and arsenic, in a series of menhaden oils collected at different stages of pilot-plant refining and partial hydrogenation as well as in several other crude fish, plant and marine mammal oils. The technique of CINAA is ideally suited to the analysis of short-lived ($t_{½} < 60$ sec) nuclides, since samples are irradiated for a short period, rapidly transferred by a pneumatic system to a detector, counted for a short interval, and then returned to the neutron source to be carried through the same cycle for a specified number of times. The total analysis time is reduced to a few minutes and interferences form long-lived nuclides such as ^{32}P are virtually eliminated. The theory of CINAA has been described in detail by Spyrou and Kerr (4).

EXPERIMENTAL

Standards

An individual standard aqueous solution containing 0.50 ppm Se, 30.0 ppm As, 10.0 ppm Br and 10.0 ppm I was prepared by dilution of 1000 ppm atomic absorption standard solutions (Ventron Corp.). Aliquots (0.10 mL) of this standard solution were used to "spike" oil samples in the method of standard additions. Weighed portions (0.12–0.19 g) of lyophilized bovine liver (NBS Standard Reference Material 1577) were sealed in 2/5 dram polyethylene vials. This weight of bovine liver half-filled the vial and gave the same counting geometry as the oil samples.

Preparation of Samples

All crude marine oil samples were commercial scale production lots. The 8 menhaden oil samples represented one lot of oil from whole *Brevoorita tyrannis* commercially produced in the USA and refined stepwise as described elsewhere (5). The production of Newfoundland cod liver oil involves simple steam rendering (6) of livers from *Gadus morhua.* The seal oil was similarly produced (6) by rendering the thick blubber of seals, mostly juvenile (pup) Atlantic harp seals *Phoca (Pagophilus) groenlandicus* (7). The crude Canadian fish body oils were produced from the whole bodies or body scrap (after removing edible fillets or roe) by operations of cooking, pressing and centrifuging (6, 8). The species were, respectively, Atlantic herring *Clupea harengus,* Pacific herring *Clupea pallasii,* Atlantic redfish *Sebastes marinus,* and Atlantic mackerel *Scomber scrombrus.*

The rapeseed oils included one lot produced by solvent extraction in Western Canada (WCSP) from a high erucic acid variety of rapeseed, probably cv. Target, in 1971. Some analytical details are given elsewhere (9–11). The LEAR (low erucic acid) or canola oils (cv. Tower) included two commercial lots which were also solvent-extracted, and one lot of oil expelled mechanically on a small scale without solvent at the POS Pilot Plant Corporation, Saskatoon (12, 13). The crude soybean oil came from commercially solvent-extracted seed.

Analytical Method

Oils were stored in a refrigerator prior to use. If solid, they were melted in water bath and mixed thoroughly before sampling. Three individual portions of 0.5 g of each oil sample were weighed into three 2/5 dram polyethylene vials, one of which contained 0.1 mL of the standard solution previously described. The vials were heat-sealed and a

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TABLE I

Concentration of Trace Elements (μg/g)

Oil sample	Se	I	As	Br
Marine oils				
Menhaden oil processing:				
Crude	0.067 ± 0.004[a]	4.76 ± 0.01	13.5 ± 0.1 (10.4 ± 0.7 (5)[b])	2.36 ± 0.07
Degummed	0.046 ± 0.008	3.92 ± 0.77	bdl[c]	2.31 ± 0.10
Degummed plus gums	0.056 ± 0.004	5.04 ± 0.09	bdl	1.91 ± 0.31
Refined	0.051 ± 0.004	4.40 ± 0.02	bdl	2.28 ± 0.15
Refined and bleached	0.052 ± 0.001	4.06 ± 0.04	bdl	2.06 ± 0.08
Hydrogenated and filtered	0.017 ± 0.007	0.35 ± 0.01	bdl	0.39 ± 0.07
Hydrogenated, filtered and bleached	0.015 ± 0.009	0.32 ± 0.01	bdl	0.34 ± 0.12
Hydrogenated, filtered, bleached and deodorized	0.015 ± 0.001	0.23 ± 0.02	bdl	0.34 ± 0.12
Cod liver	0.051 ± 0.002	12.7 ± 0.17	bdl	5.63 ± 0.25
Seal	0.033 ± 0.002	0.73 ± 0.01	1.82 ± 0.06	2.01 ± 0.28
Herring (Pacific)	0.120 ± 0.003	1.45 ± 0.20	14.5 ± 1.8	9.63 ± 1.18
Herring (Atlantic)	0.151 ± 0.003 (0.047 ± 0.008 (43))	0.97 ± 0.05	5.93 ± 0.65 (4.0 ± 0.2 (43))	3.59 ± 0.05
Redfish	0.047 ± 0.003	1.64 ± 0.08	2.36 ± 0.49	3.82 ± 0.22
Mackerel	0.138 ± 0.001	2.19 ± 0.05	7.77 ± 0.51	6.79 ± 0.03
Vegetable oils				
Crude Tower rapeseed	0.035 ± 0.001 (0.008 ± 0.004 (42))	bdl	bdl	bdl
Crude Tower rapeseed	0.042 ± 0.005	bdl	bdl	bdl
Expelled crude Tower rapeseed	bdl (≅.009)	bdl	bdl	bdl
Crude regular rapeseed oil	bdl (≅.008) (0.009 ± 0.003 (42))	bdl	bdl	bdl
Crude soybean	bdl	bdl	bdl	bdl

[a]All samples were analyzed in triplicate. Error limits represent one standard deviation.

[b]Earlier result, reference in text.

[c]bdl = below detection limit (see text).

pinch clamp was attached to the base of the vial containing the standard solution. This vial was then placed in an ultrasonic bath for 3 min to mix the oil and aqueous phases. The samples were irradiated in the reactor and counted using CINAA. Samples of reference bovine liver were also subjected to the same irradiation and counting procedure.

Irradiation and Counting Conditions

Irradiations were performed using the Dalhousie University SLOWPOKE-2 Reactor at a flux of 5×10^{11} n cm^{-2} s^{-1}. The characteristics of the SLOWPOKE flux have been described previously (14). The irradiation, decay and counting times were 60 sec, 2 sec and 60 sec, respectively. Each sample was cycled 5 times. The sample transfer time between the irradiation and counting positions was 0.6 sec. The details of the cyclic system have been reported elsewhere (15). Neutron activated samples were counted using a 40 cm^3 Princeton Gamma-Tech Ge(Li) detector coupled to a Tracor Northern TN-1700 pulse-height analyzer. The Ge(Li) detector had a full width at half maximum of 2.02 keV, a peak to Compton ratio of 30.1:1 and an efficiency of 7.1% (all measured at the 1332 keV photopeak of ^{60}Co). The samples were counted at a distance of 5 mm from the top of the detector. The four elements were determined by measuring the following radionuclides: ^{77m}Se ($t_{½}$ = 17.4 sec, Eγ = 162 keV), ^{128}I ($t_{½}$ = 25.0 min, Eγ = 443 keV), ^{80}Br ($t_{½}$ = 17.6 min, Eγ = 617 keV) and ^{76}As ($t_{½}$ = 26.3 hr, Eγ = 559 keV). The γ-spectra produced during each of the five counting periods were accumulated to yield the final spectrum. The activity of all four nuclides was recorded simultaneously. The nuclides were identified from their gamma-ray energies and by observing their half-lives.

RESULTS

The levels of selenium, iodine, bromine and arsenic in the various oils are summarized in Table I. The detection limits for each of the four elements, based on a signal equal to twice the square root of the background and a sample weight of 0.5 g, are 0.010 μg Se g^{-1}, 0.082 μg I g^{-1}, 1.7 μg As g^{-1} and 0.185 μg Br g^{-1} and the error limits of Table I represent one standard deviation. The irradiation and counting times employed were not optimized for any of the four elements studied but, instead, represented a compromise between the irradiation time required to achieve a reasonable level of activity of the longer lived nuclides and the total analysis time.

Concentrations of the four elements were determined by comparing the net number of counts in each of the four photopeaks, specified earlier, of the γ-spectra of the samples to the net number of counts of the standard addition to the third sample. The number of counts attributable to the standards in the third vial was determined by multiplying the average number of counts per gram, obtained from the other two samples of the same oil, by the weight of the third sample and subtracting this quantity from the total net counts of the third sample. To test whether the number of counts attributed to the standard addition was valid, the concentrations of selenium and bromine in the NBS bovine liver standard were determined using the results for the standard additions as a reference (iodine and arsenic could not be measured in the liver due to interferences from 439 keV γ-ray of ^{69m}Zn and 556 keV γ-ray of ^{86m}Rb, respectively). Over the six different occasions that analyses were performed, the average, measured contents of the bovine liver were 1.02 ± 0.06 μg Se g^{-1} and 11.6 ± 3.1 μg

Br g^{-1}. These results were in agreement with the NBS certified value of 1.1 ± 0.1 μg Se g^{-1} and the recommended value of 9 μg Br g^{-1} (16), implying that the number of counts allocated to the standards added to the third sample was reliable. The 162 keV γ-ray of ^{77m}Se could have been interfered with by the 164 keV γ-ray of ^{116m2}In ($t_{½}$ = 2.16 sec). However, no such interference was observed in this work; the half-life of ^{77m}Se that we measured through the 162 keV γ-ray photopeak agreed well with the value reported in literature. Moreover, γ-rays emitted by other indium nuclides were not detected in the spectrum.

Values published by this laboratory for the selenium and arsenic contents of the same or similar oils are also included in Table I along with a reference. The agreement in values is reasonable, especially since different analytical methods were used in the earlier studies, where samples were acid digested and analyzed by either atomic absorption spectrophotometry or cathodic stripping voltammetry.

DISCUSSION

The range of concentrations of selenium, arsenic and bromine in the crude fish oils were comparable to the results of Lunde (17) for crude oils produced from fresh herring, mackerel and capelin caught in the North Sea and off the coast of Norway: 0.02–0.09 μg Se g^{-1}, 4.6–9.1 μg As g^{-1} and 3.1–4.4 μg Br g^{-1}. The two sets of results agree reasonably well, especially when it is remembered that the concentration of sulfur, and presumably of related elements in crude fish oils increases considerably as the preprocessing storage time of the fish increases (17).

Apart from the advantages of minimal sample manipulation and speed of analysis, CINAA is ideally suited to the analysis of oils because those elements, e.g., Na, Cl, Mn, Al, etc., that are common interferents in instrumental neutron activation analysis are present at very low concentrations. Indeed, in the present study, the combination of short irradiation times and low levels of interferents produced detector dead times of, at worst, 1%.

Effects of Processing

Commercial hydrogenation conditions are effective in destroying organochlorine compounds of the DDT family but do not affect the more stable halogens of the PCB type (18). In view of the multiplicity of types of lipid-oriented halogen compounds in the marine world (19–21), it is interesting to find that ca. 10% of the bromine and iodine in the crude menhaden oil are removed by the usual sequence of chemical and physical refining steps (Table I). Of the remainder, hydrogenation removes at least 90% of the iodine and over 80% of the bromine. In both cases a persistent residue resists the hydrogenation process, but the chemical form is unknown (17, 22, 23). In a previous report (17) on the hydrogenation of mackerel and herring oils, the residue of unaffected bromine was very similar at 0.2–0.4 μg g^{-1}. The residue of halogens suggests a similarity in chemical form despite variations in origin and processing conditions (24, 25), and confirms the accuracy of irradiation methods for determining bromine.

Iodine

The cod liver oil is distinguished by a high iodine content. In all other oils except menhaden there is also more bromine than iodine. The amount of chlorine, bromine and iodine in cod liver oil has exercised the ingenuity of chemists from the first decades of the last century. The early results (26) usually showed that iodine exceeded bromine by factors of 3–10. By 1883, it was suggested that the earlier iodine contents of cod liver oil had been exaggerated, often by factors of 100 or more. The greatly reduced values, 1.38–4.34 μg g^{-1}, proposed for 6 samples analyzed at that time compare with our value of 12.7 μg g^{-1} for one sample of oil (Table I), and with other data (range 5.4–60.4 μg g^{-1}) tabulated for cod liver oils by Lunde (27). The latter study confirmed that iodine levels usually exceeded bromine in the liver oil from mature Arctic cod and also in many smaller cod from the south of Norway.

Selenium

Selenium is chemically similar to sulfur, and the uptake of the latter element by active nickel is, in fact, one way to determine sulfur in edible vegetable or marine oils (28–31). A reduction in selenium content during hydrogenation was therefore expected (17), but it is especially interesting (Table I) to find that, in parallel with the halogen results, about a quarter of the selenium is not removed during refining and thus remains after hydrogenation. The value of 0.015 μg Se g^{-1} is also in accord with the previous value of < 0.02 μg Se g^{-1} for a marine oil hydrogenated in Europe (17).

Arsenic

Arsenic enters the marine food chain via algae because of its chemical similarity to phosphorus, and is detoxified (32–34). Marine animals assimilate the algal phosphorus for phospholipid formation and a proportion of any accompanying arsenic (35–37) would be expected to be included on grounds of the similar chemical characteristics. The toxicity to higher animals is very low (38, 39). The first stage of refining (degumming, i.e., washing with 0.2% phosphoric acid solution) is therefore effective in eliminating arsenic (Table I) in the form of incompletely separated phospholipids and analogous arsenolipids. The low level of arsenic in the Canadian cod liver sample (Table I) is in agreement with the results of a survey which typically gave results of ca. 1.2–1.6 μg g^{-1} for arsenic in cod liver lipids (J. Uthe, private communication). The form of material analyzed is important, as the triglyceride oil content of cod livers is usually ca. 50% (25). The majority of the arsenic compounds in cod livers are in the water-soluble category, and thus would not be found in the oil.

The six fish oils analyzed come from fish caught in a variety of geographical regions. Moreover, the feeding patterns are quite diverse. The menhaden feed directly on phytoplankton, i.e., algae, and together with the herring and mackerel, which feed on the small crustacea which eat the algae directly are probably exposed to the most arsenic. The redfish, which is a slow-growing mesopelagic feeder and the cod, which is an omnivore and more of a bottom feeder, have the least arsenic in their depot fat of the six species examined. The Pacific herring is a more diverse feeder than its Atlantic counterpart (25), perhaps accounting for the higher arsenic, iodine and bromine levels.

Seal Oil

The seal oil elements are not notably different from those of the fish oils except that the levels of all four elements are consistently at the low end of the concentration range of the crude fish oils. The seal blubber layer is almost pure triglyceride and contains minimal phospholipids. Moreover the adult's depot fat is mobilized and transferred to the pup in a very fat-rich milk (7). A degree of biological filtration (40) would be expected to reduce nonfatty acid materials originally present in fish oils, but the exceptional high fat content of seal milk ($\leqslant$ 50%) may be responsible for the partial transfer of such materials to the pup, the likely origin of this sample.

Vegetable Oils

The crude vegetable oils differ from the crude marine oils in having iodine and bromine levels lower by at least an order of magnitude. The concentrations of arsenic and selenium in the crude vegetable oils are also lower. Although arsenic is seldom determined in plant oils, 0.2 $\mu g\ g^{-1}$ has been reported for crude (virgin) rapeseed oils (41). It is of interest that the selenium values obtained by CINAA are very close to those obtained by the more cumbersome, if less capital-intensive, method of cathodic stripping voltammetry (42).

ACKNOWLEDGMENTS

The Natural Sciences and Engineering Research Council of Canada and the Zapata-Haynie Corp., Houston, provided financial support. The Research and Development Division of Canada Packers Ltd., Toronto, processed the menhaden oil.

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[Received August 17, 1982]