Trace Metal Content of a Herring Oil at Various Stages of Pilot-Plant Refining and Partial Hydrogenation

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ABSTRACT

Samples of a typical Atlantic herring oil at various stages of pilot-plant processing were analyzed for cadmium, selenium, arsenic, mercury, copper, lead, and zinc. The FAO/WHO Codex Alimentarius requirements for low levels of specific metals in edible oils were always difficult to meet completely in either a washed and bleached oil or in two lots of oil processed from one crude oil by the additional steps of partial hydrogenation and deodorization. The mercury content of the crude oil was relatively low and was not greatly affected by processing. The selenium level of 47 ppb in the crude oil was significantly lowered by hydrogenation and deodorization. Arsenic was removed by alkali refining. The lead content was reduced by only 40% upon refining, probably because lead was present as an organometallic material. The concentration of the other heavy elements was generally lowered during processing.

INTRODUCTION

A series of partially processed oil samples derived from Eastern Canadian herring *(Clupea harengus)* were recently examined for residues of chlorinated hydrocarbons (1). The oil samples were collected from a pilot processing plant at various stages of refining of the crude oil and of hydrogenation to a hardness such that it could be used in a margarine (iodine value 79). This report documents the results of analyses of the same oil samples for seven trace elements: mercury, zinc, lead, copper, arsenic, selenium, and cadmium. These elements are known to be toxic to man at high concentrations. Furthermore, trace levels of some of the metals (e.g., lead and cadmium) have been linked to illnesses such as coronary heart disease and hypertension (2). Selenium, on the other hand, is an essential micronutrient, and a dietary deficiency of this element may be linked to the occurrence of many common forms of cancer (3).

The samples most thoroughly examined were crude oil, bleached oil, and hydrogenated plus deodorized oil. This study, therefore, reports the levels of several trace elements in a typical commercial marine oil and also how these levels were altered during normal refining of an oil for edible use.

EXPERIMENTAL PROCEDURES

Reagents

All solutions were prepared from reagent grade materials in glass-distilled water. Glassware was cleaned with either $KMnO_4/H_2SO_4$ or hot 1:1 HNO_3. All acids and bases used for preparation of solutions were ultra high purity (Ultrex grade, J.T. Baker, Co.).

Preparation of Samples

The oils were stored in a refrigerator and melted in a water bath prior to sampling. The samples were digested in one of two ways: (a) for the determination of arsenic and mercury, 0.2-0.3 g was heated in a Teflon bomb with 3 ml of HNO_3 at 70 C for 2 hr, at 120 C for 16 hr, and finally at 140 C for 5 hr; (b) for the analysis of the other elements, 0.5 g was digested with 6 ml HNO_3 plus 2 ml $HClO_4$ under reflux in Pyrex Kjeldahl flasks and subsequently heated to fumes of $HClO_4$.

Instrumentation

Stripping analysis: The polarographic analyzer (Model 174, Princeton Applied Research Corp.) was coupled to a X-Y recorder (Omnigraphic 2000, Houston Instruments Ltd.). Connections to the cell were made through a platinum wire counter electrode, a saturated NaCl calomel reference electrode, and a hanging mercury drop micrometer working electrode.

Atomic absorption spectrophotometry: The spectrophotometer, (Model 403, Perkin Elemer Ltd.), was equipped with a deuterium background corrector, a graphite furnace (Model HGA-2100, Perkin Elmer Ltd.), and the appropriate lamps.

Cold vapor atomic absorption spectrometry: The mercury detector was a 30 cm path length, dual beam, UV photometer (LDC Mercury Monitor supplied by Pharmacia Ltd.). The output of the detector was displayed on a two-pen, integrating recorder (Omniscribe, Houston Instruments Ltd.).

Stock Solutions

The standard solutions (1000 ppm) were prepared from high purity metals, H_2SeO_3 (Alfa Products, 99%), As_2O_5, and $HgCl_2$ dissolved in dilute acids, and serially diluted to provide solutions suitable for "spiking." All metals, except selenium and mercury, were quantified using the method of standard additions.

Methods of Analysis

Zinc was determined by differential pulse anodic stripping voltammetry (4). The pH of the digest was adjusted to 4.5 by the addition of ca. 1 ml of NH_4OH and 2 ml of 1.25 M $NaCO_2CH_3$/1.75 M HCO_2CH_3 buffer; 1 ml of 100 ppm gallium solution was also added. The solution was diluted to 25 ml and deaerated under N_2 in the polarograhic cell prior to analysis.

Selenium was determined by differential pulse cathodic stipping voltammetry following the method of Blades et al. (5).

Copper, cadmium, lead, and arsenic were determined by atomic absorption spectrophotometry. The digested sample was diluted to 10 ml, and the 10 μl aliquots were injected into the graphite furnace with the aid of an Eppendorf pipet. When arsenic was determined, 25 μl of 0.2% nickel nitrate solution was also placed in the graphite furnace (6).

Mercury was measured by the method developed by the U.S. Environmental Protection Agency (7). Following digestion, the Teflon bomb was rinsed with water, and then the entire sample plus 1 ml HNO_3 was introduced into the stripping cell. The mercury was subsequently reduced by $SnCl_2$ and swept into the analyzer by a stream of N_2.

TABLE I

The Metal Content of a Herring Oil at Various Processing Stages (μg/g) and Comparable Literature Data

Oil stage	Hg	Se	As	Zn	Cd	Pb	Cu
				Original data			
Lot 1 crude	0.010 ± 0.009	0.047 ± 0.008	4.0 ± 0.2	5.1 ± 1.4	0.007 ± 0.001	0.13 ± 0.01	0.21 ± 0.03
Lot 1 bleached	—	0.041 ± 0.005	<0.1	3.4 ± 1.2	0.001 ± 0.001	0.11 ± 0.02	0.10 ± 0.02
Lot 1 partially hydrogenated and deodorized	0.004 ± 0.008	<0.003	<0.1	6.6 ± 1.2	0.004 ± 0.001	0.08 ± 0.01	0.19 ± 0.03
Lot 2 partially hydrogenated and deodorized	—	—	—	—	0.001 ± 0.001	0.08 ± 0.01	<0.05
				Literature data			
Crude marine[a] oil (9)	—	—	8-14	—	—	—	—
Hydrogenated marine oil (9)	—	—	<0.2	—	—	—	0.004 - 0.04
Refined fish oil (10)	<0.01	—	0.02	—	0.008	0.08	—
Crude herring oil (11)	—	0.02 - 0.09	5.3 - 6.5	—	—	—	—
Hydrogenated herring oil (11)	<0.02	—	—	—	—	—	—
Crude fish press oil (12)	0.34	—	0.16	—	0.004	0.05	—

[a]Marine = herring, mackerel, capelin.

RESULTS

The metal content of the processed oils is given in Table I. The errors represent 95% confidence limits based on the results of three determinations for each element. The detection limit (less than symbol) for aresenic is relatively high since small amounts of sample were digested and greatly diluted upon transfer to volumetric ware; also the sensitivity of the atomic absorption method is about 5 ng/ml (6).

A single 10 ng sample of methyl mercury chloride was digested under the same conditions as an oil. Analysis of the digest demonstrated that more than 80% of the mercury had been converted to the inorganic form. The method of digestion was therefore assumed to be at least 80% efficient in releasing mercury from the oils. The mercury values of Table I have not been adjusted for such a factor. The $HNO_3/HClO_4$ wet digestion was not used since breakdown of perchlorate ion to chloride ion would result in the loss of mercury.

The method of Blades et al. for the determination of selenium has been successfully applied to the analysis of other types of oils (5), and a detection limit of 3 ng/g was established.

In addition to the results that appear in Table I, two other oil samples were analyzed for their arsenic content. These oils were collected (a) after degumming (phosphoric acid washing) of the crude oil and (b) after alkali refining of the phosphoric acid treated oil. The acid washed oil contained 1.1 μg/g As, and the content of the alkali washed oil was less than 0.1 μg/g As.

The zinc content of the deodorized oil determined by anodic stripping voltammetry may be exaggerated due to an interference from nickel. The known interference from copper (8) was suppressed by adding gallium to the sample.

DISCUSSION

For comparison, the concentrations of metals reported for certain other samples of fish oils are also listed in Table I. In general, the results for fully processed oils are in agreement with the possible exception that the level of copper in the Lot 1 partially hydrogenated and deodorized oil is higher than the level in the precursor bleached oil. Since metal levels in crude fish oils are influenced by factors such as species, habitat, oil storage time, processing plant etc., large differences between reported values for unprocessed oils cannot be considered significant. Thus the observation that the mercury content of the crude oil reported here is low is not easily explained. A more important comparison is with the values proposed by the joint FAO/WHO Codex Alimentarius Commission for edible oils (10). The acceptable limits for refined oils are: copper, 0.1 μg/g; lead, 0.1 μg/g; and arsenic, 0.1 μg/g (limits for cadmium, mercury, and selenium have not been established). The products of the full processing of herring oil (bleaching, partial hydrogenation, deodorization), designated Lot 1 and Lot 2, were prepared in the same units by the same operator from the same crude stocks. The values for copper illustrate the difficulty in always meeting the individual Codex requirements. Post-hydrogenation washing of oils, with phosphoric acid, alkali, and bleaching, is required to lower copper levels from 2.6-4.2 μg/g to an acceptable level of 0.3-0.1 μg/g if a certain copper-chromium-manganese catalyst is used with marine oils (13).

Processing of the crude herring oil, in general, reduced the metal content; however, in the cases of mercury and zinc the overlap of the error limits make it difficult to draw definite conclusions. The increased zinc and copper contents of the Lot 1 deodorized oil over those of the bleached oil are difficult to explain since copper and brass are carefully avoided in oil processing plants. Moreover, the zinc concentration of the deodorized oil could be enhanced because the analytical method employed to determine zinc may not discriminate between nickel and zinc.

The bleached oil contains 50% less copper, 15% less lead, 35% less zinc, and significantly less arsenic and cadmium than were found in the corresponding crude oil. The selenium content is virtually unchanged. The arsenic levels of the degummed, alkali refined, and bleached oils indicate that the alkali washing step effectively removes arsenic. Hydrogenating and deodorizing the bleached oil totally removes selenium, reduces the lead content to 60% of that in the crude oil, and leaves the mercury level almost unaltered.

Crude fish oils are expected to contain a certain concentration of metals since during production the oils are in contact with wash water that contains metallic ions, and phospholipids are reported to carry metals into oils (14). However, metals that are complexed by phospholipids would be expected to be removed by degumming and alkali refining since these steps remove phospholipids (11). The results of our study suggest that cadmium may form a phospholipid complex. Copper and zinc may behave similarly, but complications in treating the data arise due to possible contamination from the processing plant. Mercury and lead are still present in the oils following hydrogenation and deodorization, implying that these metals exist as organo-

metallic complexes. Sirota and Uthe (15) have recently reported the discovery of tetraalkylead compounds in the livers and muscle tissue of fish. Such complexes are known to be very resistant to reduction and, therefore, would not be removed during hydrogenation. The mercury content of the oil is considerably lower than that found in tissue samples suggesting that mercurials are preferentially bound to proteins which are destroyed in refining.

The observed removal of arsenic from the oil samples by alkali refining confirms the findings of others (9). The work of Edmonds et al. (16,17) suggests that the arsenic containing species may be arsenobetaine, $As(CH_3)_3CH_2COO^-$, which was isolated from the tail muscle of western rock lobster. Such a compound should be soluble in alkali and, therefore, easily extracted during alkali refining.

Selenium is reported to exist in at least two forms in lipids: as a free or weakly bound organic compound and as part of a lipoprotein (18). The present study demonstrates that very little selenium is present in an inorganic form which can be extracted by acid or base. Selenium is, however, removed by hydrogenating and deodorizing as reported elsewhere for fish oils (11) and rapeseed oils (5).

In summary, the findings indicate that the heavy metal content of a marine oil is generally reduced by processing; however, the degree of reduction is dependent upon the element. Moreover, the removal of the bulk of an element is usually accomplished by one particular refining or processing step.

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