Interference of Polar Lipids with the Alkalimetric Determination of Free Fatty Acid in Fish Lipids

Shengying Zhou and Robert G. Ackman*

Canadian Institute of Fisheries Technology, Technical University of Nova Scotia, Halifax, Nova Scotia B3J 2X4, Canada

ABSTRACT: An examination of the suitability of an alkalimetric method for the determination of free fatty acid (FFA) contents in fats, oils, and lipid extracts was conducted by comparing AOCS method Ca 5a-40 with a method based on a Chromarod–Iatroscan thin-layer chromatography–flame-ionization detector (TLC–FID) system. The FFA contents determined by the alkalimetric method were consistently higher than the genuine FFA contents obtained by the Iatroscan TLC–FID method. Phospholipids were found to be the major components that contributed to the alkali-titratable, nongenuine FFA in the total FFA determined alkalimetrically. Contributions from other polar lipid components were smaller, but they dominated as the proportion of phospholipids fell. The other alkali-titratable polar components may include oxidized lipids and their by-products bound to protein fragments. The accurate determination of FFA contents by alkalimetric methods may only be applicable to those commercially refined fats and oils that contain negligible amounts of phospholipids. Corrections for the alkalimetrically determined FFA contents should be made for those fats and oils with relatively high phospholipid contents by correlating the nongenuine FFA contents and the phospholipid contents.

JAOCS 73, 1019–1023 (1996).

KEY WORDS: Alkalimetric titration, free fatty acid, free fatty acid content, Iatroscan TLC–FID, oils, lipid class, phospholipid, polar lipid.

The free fatty acid (FFA) content is one of the major quality indicators for fats and oils; it is also the key parameter that controls alkali and physical refinery processes. Conventional procedures for the determination of FFA content are based on the direct titration of diluted fats and oils with standardized alkaline solution. This is the principle of various official methods of organizations, such as AOCS, IUPAC, and AOAC (1–3). The advantages of these alkalimetric methods are simplicity, speed, and low cost. However, fats and oils, especially those not carried through refining processes, are composed of various lipid components, and free fatty acids may not be the only lipid class that would contribute to the acidity of total lipids. Minor components of crude oils include phospholipids, oxidized lipids, amino acids, peptides, or other impurities that might exhibit acidity when neutralized with an alkaline solution. With the endorsement of various official methods, the alkalimetrically determined FFA content has long been accepted as the true FFA content in fats and oils, and the interference of other alkali-titratable components on the determination of genuine FFA content has largely been ignored. It has been reported (4,5) that isolated phospholipids gave a high "FFA content," but the combined effect of all possible contributors to the alkalimetrically determined "FFA content" are still not clear because the other components that could also make contributions are seldom part of the analysis. Furthermore, the "natural acidity" of phospholipids in fats and oils might have been slightly altered after the complicated isolation processes. In this study, we have used fish tissue extracts to examine the apparent and genuine FFA contents by comparing the traditional alkalimetric method with the method of Chromarod thin-layer chromatography with Iatroscan flame-ionization detection (TLC–FID). The differences between the apparent and genuine FFA contents will be discussed and correlated with the amount of polar lipids.

EXPERIMENTAL PROCEDURES

Materials. Atlantic salmon of aquaculture origin, live-held in the Dalhousie University Aquatron Laboratory, provided salmon materials of known history. They were fed a diet of Fundy Choice feed (Corey Mills Ltd., Fredericton, Manitoba, Canada) and killed by a blow on the head. The muscle tissue of the Atlantic salmon was immediately dissected into regions designated white muscle, dark muscle, belly flaps, and lower flank (6). All salmon muscle tissues were frozen at −35°C for four months before analysis. Fresh cod fillets and livers, and fillets of silver hake and herring were obtained from local fish markets. All samples were stored in a −35°C freezer until analysis. Refined canola oil (Crisco Vegetable Oil, Procter & Gamble Inc., Toronto, Ontario, Canada) was purchased from a local store and kept under nitrogen once opened. Herring silage was prepared by ensiling whole fish mince with 3.5% formic acid at room temperature for 31 days in the presence of ethoxyquin (7). Authentic standards of palmitic acid, tripalmitin, cholesterol, 1,2-distearin, 1-mono-palmitin, dipalmitoyl phosphatidylcholine (PC), dipalmitoyl phosphatidylethanolamine (PE), sphingomyelin (beef brain), and palmitoyl lysophosphatidylcholine (LPC) of >99% purity were purchased from Serdary Research Laboratories, Lon-

*To whom correspondence should be addressed at Canadian Institute of Fisheries Technology, Technical University of Nova Scotia, P.O. Box 1000, Halifax, Nova Scotia B3J 2X4, Canada.

don, Ontario, Canada (now Doosan Serdary Research Laboratories, Englewood Cliffs, NJ).

Extraction of lipids. Lipids were extracted from fish tissues, cod liver, and from prepared silage by following the method of Bligh and Dyer (8). The chloroform–methanol extracts were allowed to settle overnight at room temperature to completely separate the organic and aqueous layers. The interfacial layer of fines, if any, was removed from the chloroform layer. It was dried with anhydrous sodium sulfate, and the lipids were stripped of solvent in a rotary evaporator, first under aspirator, then under mechanical vacuum.

Alkalimetric quantitation of FFA content. FFA contents were determined according to AOCS Method Ca 5a-40 (1). The percentage of FFA in the lipids was calculated as oleic acid. Each sample was analyzed in triplicate.

Separation and quantitation of FFA and lipid classes by Iatroscan TLC–FID. Separation of lipid classes was accomplished by chromatography on silica gel Chromarods-SIII, followed by quantitation with an Iatroscan TH-10 Mark III (Iatron Laboratories Inc., Tokyo, Japan; Canadian Distributor, Scientific Products & Equipment, Concord, Ontario, Canada) equipped with an FID and an SP-4270 recording integrator, as described by Parrish (9). The Iatroscan was operated with a hydrogen flow rate of 110 mL/min and an air flow of 2000 mL/min.

The actual separation of lipid classes was conducted with a development sequence of three different solvent systems. The extracted lipids were dissolved in chloroform at an appropriate concentration, and this lipid solution was then spotted onto Chromarods-SIII in 1 μL volumes from glass Microcap disposable pipettes (Drummond Scientific Co., Broomall, PA). The Chromarods were then conditioned in a constant humidity chamber for 5 min. The first development was carried out for 55 min in hexane/chloroform/isopropanol/formic acid (80:14:1:0.2, by vol). The Chromarods were then dried at 100°C for 1.5 min and partially scanned from the top to a point just below the diacylglycerol (DG) peak (Fig. 1, I). The Chromarods were then redeveloped in acetone for 15 min, dried at 100°C for 1.5 min and partially scanned to below the acetone-mobile polar lipid position (Fig. 1, II). Finally, the Chromarods were again developed in chloroform/methanol/water (70:30:3, vol/vol/vol) for 60 min, dried at 100°C for 3 min and completely scanned to reveal different phospholipids (Fig. 1, III). The quantitation calibration of the system was conducted under the same conditions with authentic standards. Each sample was spotted on 10 rods treated as one lot, and each was analyzed in triplicate. The precision and accuracy of the determination of FFA contents were determined by spiking refined canola oil with authentic palmitic acid. The TLC–FID analyses of FFA-spiked oil samples were conducted under the conditions already described.

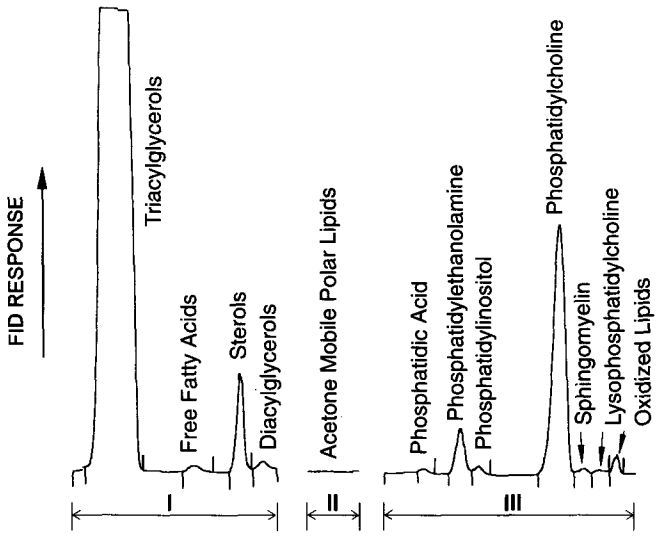

FIG. 1. Sequential Iatroscan thin-layer chromatography–flame-ionization detector (TLC-FID) (Iatron Laboratories, Inc., Tokyo, Japan) profiles of the lipid classes extracted from silver hake muscle tissue. I, II, and III represent partial chromatograms from the three-stage development sequence of total lipids on Chromarod-SIII (Iatron).

RESULTS AND DISCUSSION

Analysis of the canola oil spiked with three levels of palmitic acid indicated that the current Iatroscan TLC–FID method was suitable for the accurate and precise quantitation of the genuine FFA content in lipids (Table 1). Figure 1 shows the classes of silver hake lipid separated by silica gel Chromarod-SIII with the sequential development of three solvent systems. The extracted total lipids were effectively separated into triacylglycerols (plus traces of hydrocarbons and wax esters), FFA, sterols, diacylglycerols, acetone-mobile polar lipids, different types of phospholipids, and a nonmobile polar group considered to be oxidized lipids. The acetone-mobile polar lipids were not seen in the extracted silver hake lipids but have been observed in marine lipid extracts (9), herring silage (7), oxdized fish meal (10), and most of the lipid extracts in this study.

The free fatty acids derived from the phospholipids of fish muscle fall mostly into two groups, 14:0 + 16:0 and 20:5 ω3 + 22:6 ω3. These will give two separate peaks on Chromarods-SIII under certain TLC conditions (11,12). The problem can be overcome by hydrogenation of total lipids if necessary (12) or simply by the use of the solvent system described for the first step of the three developments (Fig. 1, I). With the current solvent system, both saturated and unsaturated FFA appeared in one peak that was well separated from other lipid classes.

Table 2 displays the FFA contents in lipids from various sources, determined by both the alkalimetric and Iatroscan

TABLE 1
Precision and Accuracy of Analyzing Canola Oil Spiked with Palmitic Acid by Iatroscan TLC–FID[a]

Added FFA (%, w/w)	FFA determined by Iatroscan (%, w/w)
0.0 (nonspiked canola oil)	<0.1
1.9	2.1 ± 0.2
3.3	3.4 ± 0.3
5.8	6.0 ± 0.3

[a]The amount of FFA is expressed as mean ± standard deviation of three determinations (10 rods were treated as one lot).

TABLE 2

FFA Contents Determined by AOCS Alkalimetric Method and Iatroscan TLC–FID, and Amounts of Phospholipids and Total Polar Lipids in Various Types of Extracted Lipids by Iatroscan TLC–FID (%, w/w)[a]

	Lipid content (%, wet weight)	FFA content (% of lipids)			Phospholipids (% of lipids)	Total polar lipids[c] (% of lipids)
		AOCS	Iatroscan	ΔFFA[b]		
Canola oil	—	0.03 ± 0.00	nd[d]	0.0	nd	nd
Salmon mesentery	82.6	0.19 ± 0.00	nd	0.2	nd	0.5 ± 0.3
Salmon belly flaps	82.6	0.41 ± 0.02	nd	0.4	1.0 ± 0.3	1.6 ± 0.4
Crude seal oil	—	3.05 ± 0.00	2.7 ± 0.3	0.4	nd	0.4 ± 0.2
Salmon lower flank	14.0	0.89 ± 0.02	0.3 ± 0.1	0.6	3.3 ± 0.4	4.2 ± 0.5
Herring silage	13.0	6.63 ± 0.04	6.0 ± 0.5	0.6	nd	2.5 ± 0.4
Herring mince	11.9	1.10 ± 0.00	0.3 ± 0.1	0.8	4.0 ± 0.5	5.1 ± 0.6
Cod liver	39.6	12.63 ± 0.00	11.8 ± 1.0	0.8	1.6 ± 0.4	4.9 ± 0.5
Salmon dark muscle	18.4	1.82 ± 0.01	0.6 ± 0.1	1.2	7.2 ± 1.0	7.8 ± 1.4
Salmon white muscle	4.3	2.48 ± 0.00	0.9 ± 0.2	1.6	10.3 ± 1.1	12.0 ± 1.2
Silver hake muscle	2.4	3.67 ± 0.00	0.8 ± 0.2	2.9	20.2 ± 1.8	21.5 ± 2.0
Cod muscle	0.8	10.56 ± 0.00	3.6 ± 0.4	7.0	69.9 ± 5.7	73.1 ± 5.8

[a]TLC–FID, thin-layer chromatography–flame-ionization detector (Iatron Laboratories, Inc., Tokyo, Japan).
[b]Difference in percentage amounts between AOCS methods and Iatroscan method.
[c]Sum of phospholipids and oxidized lipids measured by Iatroscan.
[d]Not detected.

TLC–FID methods. It is fairly clear that the FFA contents determined for fish tissue extracts by the conventional alkalimetric method are consistently higher than the genuine FFA amounts present in the lipid samples when determined by Iatroscan TLC–FID. The difference in FFA contents between those two methods has its origin in the presence of alkali-titratable non-FFA components, which we term nongenuine FFA, in the lipid samples. Those components could make contributions to the acidity of total lipids when titrated with alkaline solution. The amounts of alkali-titratable nongenuine FFA in lipids went up linearly as the concentration of all polar lipids in total lipids increased, but especially with phospholipids (Fig. 2). The presence of the nongenuine FFA was, to some extent, also related to the lipid sources, their extraction methods, and the handling of raw materials. Commercial canola oil is normally extracted with a nonpolar solvent (hexane) or obtained by mechanical expression. It then undergoes refining processes (13) to remove the nontriacylglycerol components or impurities, such as FFA, phospholipids, pigments, wax esters, and fine particles. Little of the alkali-titratable components would remain in the refined canola oil to contribute to the nongenuine FFA. The difference in the FFA contents between the two methods was therefore essentially zero for this oil (Table 2). The crude seal oil was from grey seal blubber, minced, rendered, and centrifuged for clarity but not otherwise refined. The polar lipids (not phospholipids) accounted for the difference in FFA values and could represent a complex of oxidized fatty acids and/or lipids, with any fine particles of proteins not completely removed by centrifugation.

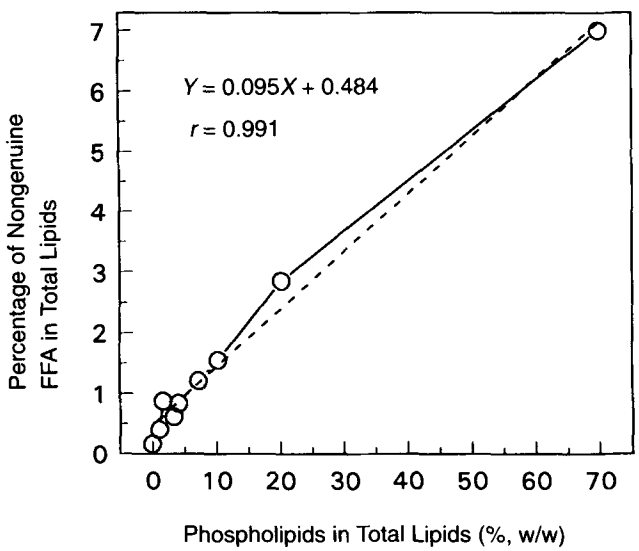

FIG. 2. Correlation of phospholipid concentrations in various marine lipids with the amounts of nongenuine free fatty acids (FFA) in total lipids originated from the alkali-titratable components by employing AOCS method Ca 5a-40 (Ref. 1). The percentage of nongenuine FFA in total lipids is obtained by subtracting the Iatroscan-determined FFA contents from the alkalimetrically determined FFA contents. Company source as in Figure 1.

On the other hand, total lipids extracted by chloroform–methanol from fish tissues rich in lipids, e.g., mesenteric tissue and belly flaps of Atlantic salmon, contained phospholipids and/or small quantities of other materials classed as polar lipids (0.5–1.6% of lipids). The measurement of small quantities of nongenuine FFA (0.2% in the mesentery) was apparently attributable to the presence of these minor components and not to phospholipids. The contribution of polar lipids or nonlipid impurities other than phospholipids to the titratable acidity could be confirmed by comparing the levels of nongenuine FFA in lipids from liquefied herring silage and the parent raw herring mince (Table 2). Although the primary nongenuine FFA contributor, phospholipids, were not detected by Iatroscan TLC–FID in liquefied herring silage due to their total hydrolysis (7), there was still 0.6% nongenuine FFA in the total lipids, whereas in total lipids from raw herring mince, 4% phospholipids and 1.1% other polar lipids produced 0.8% nongenuine FFA. The primary sources of non-

genuine FFA in liquefied silage were probably from lipid oxidation by-products, possibly combined with protein fragments. Attempts have been made in our study to remove the suspected polar components by repeatedly washing the Bligh and Dyer (8) chloroform extracts of herring silage, or salmon white muscle and belly flaps with the Bligh and Dyer solvent upper phase (three times). However, there was no significant difference ($P > 0.05$) in the acidities between the repeatedly washed lipids and lipids extracted after the normal Bligh and Dyer procedures.

Lipid contents in marine organisms show large variations with different species, types of tissues, or even types of subtissue units (6,14,15). Decreases in tissue lipid contents generally have corresponded with the reduction in the neutral storage lipids (6) and resulted in increases in the proportions of polar lipids, particularly phospholipids. Lipids from lean marine organisms, such as cod, silver hake, and white muscle of Atlantic salmon, were composed of high proportions of phospholipids (Table 2). In this case, errors in the alkalimetrically determined FFA contents would be substantial due to the contributions from all polar lipids. For example, silver hake and cod muscle lipids were composed of 20 and 70% phospholipids, respectively, representing the basic cellular membrane lipids; the amount of nongenuine FFA that originated from those polar lipids accounted for 2.9 and 7.0% of the total extracted lipids, respectively (Table 2). Cod muscle lipids have been extensively investigated for phospholipid content and hydrolysis in frozen storage (16–18), but often by titration. However, the amount of phospholipids in cod muscle lipid extract, as measured by Iatroscan, is close to the gravimetric value of 82.3% given by Bligh and Scott (17). Lipid hydrolysis in frozen salmon and rainbow trout muscles was examined by Polvi *et al.* (19) and by Ingemansson *et al.* (20), respectively. Cod liver is of course notorious for enzyme activity (21,22), accounting for the high FFA of Table 2, but intact liver contains phospholipids, protein, and water, as well as triacylglycerol.

Phospholipids were the major source of nongenuine FFA, but other components would also make contributions. Assuming that phospholipids were indeed the only contributor, the calculated values of phospholipid "contributions" to the amounts of nongenuine FFA (as wt% of total phospholipids) in all lipids examined should be approximately the same. However, the actual calculated "contribution" decreased as the proportion of phospholipids in total lipids increased (Fig. 3), suggesting that other components do make contributions and that their relative contributions increase as the proportions of phospholipids in total lipids decrease. Lipids from herring and silver hake contained 4 and 20% phospholipids, but the calculated "contributions" of phospholipids to nongenuine FFA were 20.9 and 14.1% of their weight percentages, respectively. On the other hand, in lipids extracted from cod muscle, the share of phospholipids in the total nongenuine FFA was substantial compared to other polar lipids, and the "contribution" of phospholipids to the nongenuine FFA was only 10% of its weight percentage. This is generally in agreement with the nongenuine FFA contribution determined for phospholipids isolated by de Koning *et al.* (4,5).

Variations exist in the acidity determined by different alkali-

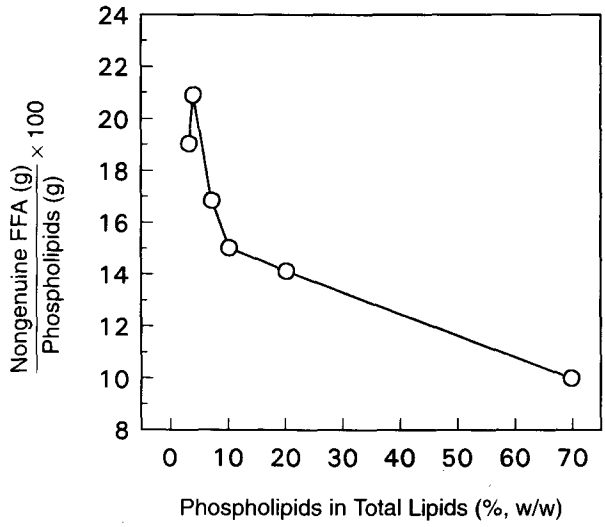

FIG. 3. Correlation of phospholipid concentrations in various marine lipids with their assumed "contribution" to the nongenuine FFA originating from all alkali-titratable components by employing AOCS method Ca 5a-40 (Ref. 1). See Figure 2 for abbreviations.

metric methods endorsed by various international organizations (23). Investigations on the improvement of method sensitivity and accuracy have been based on changing the indicator (24) or by measuring the reduction prepeak current of FFA (25). The latter group also observed higher acid values with a color (phenolphthalein) indicator method than with voltammetry or potentiometry, confirming our findings. However, considering the obvious interference of known or unknown alkali-titratable components with the FFA determination, alkalimetric methods may only be applicable to commercial refined fats and oils from which phospholipids have been removed. For those fats, oils, or lipid extracts containing more than 1% phospholipids, a correction may be made to the apparent FFA contents determined by titration. This is particularly important for the accurate determination of genuine FFA contents in crude vegetable oils, such as soybean oil, cottonseed oil, and canola oil, that contain relatively high proportions of phospholipids that may be recovered as gums and measured by TLC–FID (26) because the alkali-refining processes are largely based on the determined FFA contents. Corrections of the alkalimetrically determined "FFA content" could be made by subtracting the nongenuine FFA content from the determined apparent FFA content. Because the amounts of nongenuine FFA are well correlated with the concentrations of phospholipids (Fig. 2), the amounts of nongenuine FFA in lipids can be calculated according to Equation 1, that was obtained through regression on phospholipid concentrations between 1–20% in lipids extracted from fish tissues (Table 2):

$$\% \text{ nongenuine FFA} = 0.12 \times \% \text{ phospholipids} + 0.38 \quad [1]$$

Whether this is applicable to vegetable oils requires a separate study. Physical refining of oils may introduce another complication because the severe conditions might destroy

peroxides and similar materials with unknown consequences for determination of FFA by alkaline titration.

ACKNOWLEDGMENT

This work was supported in part by funds from the Natural Sciences and Engineering Research Council of Canada.

REFERENCES

1. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn., edited by D. Firestone, American Oil Chemists' Society, Champaign, 1989, Method Ca 5a-40.
2. *Standard Methods for the Analysis of Oils, Fats and Derivatives*, 6th edn., Part 1, International Union of Pure and Applied Chemistry, Applied Chemistry Division, Pergamon Press, Toronto, 1979, Method II.D.1, pp. 52–55.
3. *Official Methods of Analysis of AOAC International*, edited by P. Cunniff, 16th edn., AOAC International, Arlington, 1984, Method 940.28.
4. de Koning, A.J., S. Milkovitch, M. Fick, and J.P.H. Wessels, The Free Fatty Acid Content of Fish Oil—An Analysis of Anchovy Lipids at Different Stages in the Manufacture of Anchovy Meal and Oil, *Fette Seifen Anstrichm. 88*:404–406 (1986).
5. de Koning, A.J., S. Milkovitch, and T.H. Mol, The Origin of Free Fatty Acids Formed in Frozen Cape Hake Mince (*Merluccius capensis*, Castelnau) During Cold Storage at −18°C, *J. Sci. Food Agric. 39*:79–84 (1987).
6. Zhou, S., R.G. Ackman, and C. Morrison, Storage of Lipids in the Myosepta of Atlantic Salmon (*Salmo salar*), *Fish Physiol. Biochem. 14*:171–178 (1995).
7. Zhou, S., A.T. Paulson, and R.G. Ackman, Release of Free Fatty Acids from Lipids During Ensilation of Herring Mince: The Possible Role of Ethoxyquin, *J. Food Lipids 2*:121–134 (1995).
8. Bligh, E.G., and W.J. Dyer, A Rapid Method of Total Lipid Extraction and Purification, *Can. J. Biochem. Physiol. 37*:911–917 (1959).
9. Parrish, C.C., Separation of Aquatic Lipid Classes by Chromarod Thin-Layer Chromatography with Measurement by Iatroscan Flame Ionization Detection, *Can. J. Fish. Aquat. Sci. 44*:722–731 (1987).
10. Gunnlaugsdottir, H., and R.G. Ackman, Three Extraction Methods for Determination of Lipids in Fish Meal: Evaluation of a Hexane/Isopropanol Method as an Alternative to Chloroform-Based Methods, *J. Sci. Food Agric. 61*:235–240 (1993).
11. Ohshima, T., W.M.N. Ratnayake, and R.G. Ackman, Cod Lipids, Solvent Systems and the Effect of Fatty Acid Chain Length and Unsaturation on Lipid Class Analysis by Iatroscan TLC–FID, *J. Am. Oil. Chem. Soc. 64*:219–223 (1987).
12. Shantha, N.C., and R.G. Ackman, Advantages of Total Lipid Hydrogenation Prior to Lipid Class Determination on Chromarods-SIII, *Lipids 25*:570–574 (1990).
13. Mag, T.K., Further Processing of Canola and Rapeseed Oil, in *Canola and Rapeseed: Production, Chemistry, Nutrition, and Processing Technology*, edited by F. Shahidi, Van Nostrand Reinhold, New York, 1990, pp. 251–276.
14. Ackman, R.G., and C. McLeod, Total Lipids and Nutritional Important Fatty Acids of Some Nova Scotia Fish and Shellfish Food Products, *Can. Inst. Food Sci. Technol. J. 21*:390–398 (1988).
15. Heras, H., S. Zhou, and R.G. Ackman, Uptake and Depuration of Petroleum Hydrocarbons by Atlantic Salmon: Effect of Different Lipid Levels, in *Proceedings of the Sixteenth Arctic and Marine Oil Spill Program (AMOP) Technical Seminar*, June 7–9, Calgary, Canada, Vol. 1, 1993, pp. 343–351.
16. Bligh, E.G., Lipid Hydrolysis in Frozen Cod Muscle, *J. Fish. Res. Board Canada 18*:143–145 (1961).
17. Bligh, E.G., and M.A. Scott, Lipids of Cod Muscle and the Effect of Frozen Storage, *J. Fish. Res. Board Canada 23*:1025–1036 (1966).
18. Hardy, R., A.S. McGill, and F.D. Gunstone, Lipid and Autoxidative Changes in Cold Stored Cod (*Gadus morhua*), *J. Sci. Food Agric. 30*:999–1006 (1979).
19. Polvi, S.M., R.G. Ackman, S.P. Lall, and R.L. Saunders, Stability of Lipids and Omega-3 Fatty Acids During Frozen Storage of Atlantic Salmon, *J. Food Process. Preserv. 15*:167–181 (1991).
20. Ingemansson, T., A. Pettersson, and P. Kaufmann, Lipid Hydrolysis and Oxidation Related to Astaxanthin Content in Light and Dark Muscle of Frozen Stored Rainbow Trout (*Oncorhynchus mykiss*), *J. Food Sci. 58*:513–518 (1993).
21. Jangaard, P.M., and H.E. Power, Decomposition of Cod Livers and the Formation of Free Fatty Acids in Cod Oil, *J. Fish Res. Board Canada 23*:21–25 (1966).
22. Ackman, R.G., J.E. Stewart, and H.E. Power, Free Fatty Acid Formation in Cod Livers Sterilized by Irradiation, *Ibid. 23*:155–156 (1966).
23. Horwitz, W., Methods of Analysis Approved by the Codex Alimentarius Commission, I. Acid Value, *J. Assoc. Off. Anal. Chem. 59*:658–661 (1976).
24. Ke, P.J., and A.D. Woyewoda, A Titrimetric Method for Determination of Free Fatty Acids in Tissues and Lipids with Ternary Solvents and *m*-Cresol Purple Indicator, *Analytica Chimica Acta 99*:387–391 (1978).
25. Kusu, F., T. Fuse, and K. Takamura, Voltammetric Determination of Acid Values of Fats and Oils, *J. AOAC Internat. 77*:1686–1689 (1994).
26. Ratnayake, W.M.N., and R.G. Ackman, Rapid Analysis of Canola Gum Lipid Composition by Iatroscan Thin-Layer Chromatography - Flame Ionization Detection, *Can. Inst. Food Technol. J. 18*:284–289 (1985).

[Received August 15, 1995; accepted April 22, 1996]