Internationally accepted methods recommend 500 g as being an adequate analysis sample for Shea-nuts after reduction of the contract sample, whereas in this laboratory a subsample of at least 1 kg is milled and the meal used for routine analysis as described in Methods. We find that our procedure gives results with low standard deviations as shown in Table V. Individual nuts may be grated using a hand grater, but this method is not practicable for kilogram quantities of nuts. Equally, the cutting of pieces from each of a sample of nuts is most unreliable, particularly in view of the localization of FFA.

In our experience, we emphasize that the size of contract samples provided is very often too small. It is only possible to obtain reproducible results from such a heterogeneous material if a large subsample is taken from an adequately large sample, and that the subsample be milled to a relatively small particle size meal in order that thorough mixing is possible before subsequent analysis. Analyses done by different laboratories on contract samples of Shea-nuts representing the same parcel must be expected to differ slightly with such a heterogeneous material, but should not show a consistent bias.

Those who trade in Shea-nuts will be familiar with the respective allowances for oil and FFA, and must be aware of the need for satisfactory methods for the analysis of Shea-nuts.

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TLC-FID Assessment of Lipid Oxidation As Applied to Fish Lipids Rich in Triglycerides[1]

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ABSTRACT

Changes in the polar lipid content of fatty fish oils were studied by quantitative thin layer chromatography-flame ionization detection (TLC-FID) during an accelerated oxidation test. There was a direct linear correlation between increase in polar lipids and increase in weight of the oil samples. Comparison of increases in polar lipids with thiobarbituric acid values gave a hyperbolic correlation curve. However, a linear correlation was obtained in a semilogarithmic system. The simple and time-saving TLC-FID method for the analysis of the polar lipid content of oils as compared to a column chromatographic method suggests that this new method could be used in quality control of edible oils.

INTRODUCTION

Oxidative rancidity of fats and oils in foods is a major problem resulting in a decreased quality and nutritive value of the product. This is especially true for food fish which usually contain highly unsaturated lipids. Autoxidation of unsaturated fatty acids leads to the formation of relatively stable hydroperoxides (1) which later may break down to the lower molecular weight carbonyl compounds responsible for the unpleasant off-flavor in rancid fish oils (2).

Oxidation of lipids consisting mainly of triglycerides results in compounds with a higher polarity than their parent triglycerides (3). The significance of the polar lipid content in edible oils has been recognized by the German Society for Fat Research (DGF, Deutsche Gesellschaft fur Fettwissenschaft) which has recommended that the content of polar artifacts should be taken as a basis for the quality assessment of used frying oils (4). This led us to study the changes in the polar lipid content as a possible indicator of lipid oxidation in some fatty fish lipids during an accelerated oxidation test. Direct analysis of the polar lipid content was achieved by quantitative thin layer chromatography-flame ionization detection (TLC-FID). The results are compared with thiobarbituric acid (TBA) values and weight gain.

EXPERIMENTAL PROCEDURES

Preparation of Fish Oils

Lipids of the skin, including the subcutaneous fat layer, and the meat from common food fishes, Atlantic mackerel (*Scomber scombrus*), Atlantic herring (*Clupea harengus*) and tuna (*Thunus obesus*) were extracted by the method of Bligh and Dyer (5).

Accelerated Oxidation Test

Duplicate samples of ca. 2.0 g of each oil were accurately weighed in glass Petri dishes (id 50 mm). These samples

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were used for the weight gain analyses. At the same time, 24 samples of ca. 30 mg of each oil were accurately weighed in separate petticups (Fisher Scientific Co., U.S.). This oil had approximately the same surface area in ratio to the weight as the samples in Petri dishes. Samples in petticups were used one at a time for determinations of the lipid components and the TBA value.

For the measurement of the rate of lipid oxidation the samples in Petri dishes and petticups were placed in a forced hot air oven at 60 ± 1 C. The increased weights of lipid samples in Petri dishes were regularly recorded and the appropriate petticups removed for further analyses. Cooling and weighing of the samples was completed within 30 min.

TLC-FID

The lipid composition of the unoxidized oils, as well as of the samples in petticups removed during the oxidation test, was determined as described earlier (6). TLC of the oil samples was performed on silica-gel-coated quartz rods (Chromarod-S, Iatron Laboratories Inc., Japan) using a two-step development system. The first development (17.5 min) was made with petroleum ether/benzene/formic acid, 92:17:1 (by vol). After development, the solvent was removed by flushing the rods with a stream of nitrogen and drying in air for ca. 5 min. The rods were then placed in the second tank and developed for 17.5 min with a solvent system of petroleum ether/diethyl ether/formic acid, 97:4:1 (by vol). The lipid composition was determined on an Iatroscan Model TH-10 analyzer equipped with a flame ionization detector (Iatron Laboratories Inc., Japan) connected to a Linear Instruments Corp. (Irvine, CA) Model 252 A recorder. The peak areas were converted to weight percent using the appropriate factors established with standard compounds.

TBA Value Determination

The TBA values were determined using the direct spectrophotometric micromethod of Ke and Woyewoda (7). The oil sample in a petticup was dissolved into 10 ml of the TBA reagent in a Teflon-lined, screw-cap tube, incubated for 45 min in a boiling water bath, and cooled in tap water. After addition of the trichloroacetic acid (TCA) solution and centrifugation, the optical density of the aqueous layer was measured at 538 nm. The TBA value was calculated according to the molar absorptivity and expressed as mmol malonaldehyde/kg of oil.

RESULTS

The extracted oils consisted mainly of triglycerides which ranged from 88.9% of the total lipid in mackerel meat oil to 99.1% in tuna skin oil (Table I). Polar lipids, which in fresh fish oils are mostly functional lipids consisting of phospholipids, varied from 0.8 to 10.9% of the total lipid.

TABLE I

Lipid Class Composition of the Unoxidized Fish Oils As Percentages of the Total Lipid

Oil source	PL[a]	TG	Chol	Others
Mackerel skin	3.9	96.0	0.07	0.08
Mackerel meat	10.9	88.9	0.22	0.06
Herring skin	2.4	97.5	0.04	0.06
Herring meat	8.3	91.6	0.06	0.07
Tuna skin	0.8	99.1	0.07	0.08
Tuna meat	2.8	97.1	0.12	0.05

[a]Symbols: PL = polar lipids; TG = triglycerides, Chol = cholesterol.

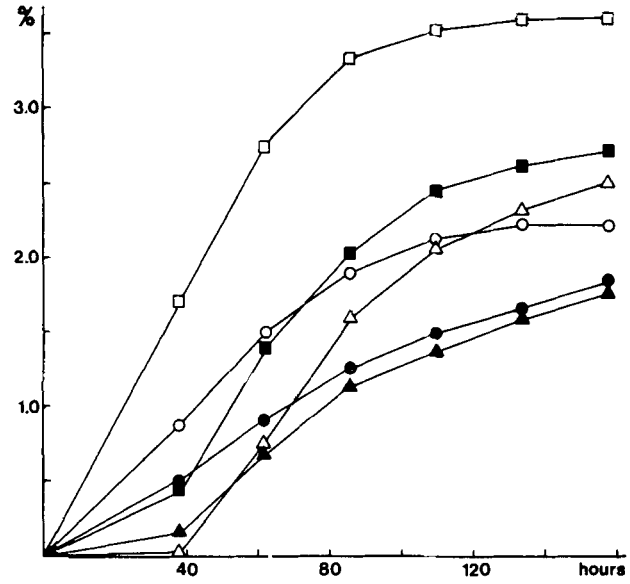

FIG. 1. Development of weight gain of different fish oils during autoxidation at 60 C. Symbols: ○ = mackerel skin oil; ● = mackerel meat oil; △ = herring skin oil; ▲ = herring meat oil; □ = tuna skin oil; ■ = tuna meat oil.

Cholesterol ranged from 0.04 to 0.22% and only very minor amounts of unesterified fatty acids, wax and sterol esters, or hydrocarbons were found.

The average weight gain of duplicate analyses as a percentage of the sample weight for each oil during the oxidation test is shown in Figure 1. Tuna skin oil was fastest to adsorb oxygen and also had the highest weight gain. An obvious induction period before an active oxygen adsorption was found with herring skin oil and tuna and herring meat oils. Excluding the induction period, the rates of weight gain of mackerel and herring meat oils were very similar.

All the oils reached their highest TBA values within 62 hr from the beginning of the oxidation test (Fig. 2). Thereafter, TBA values remained at approximately the same level or decreased since decomposition and evaporation rate of small molecular carbonyl compounds responsible for the color reaction exceeded the rate of formation. The development of the TBA values in herring and mackerel meat oils was similar and the same was found between mackerel skin and tuna meat oils. The highest TBA value, 10.0

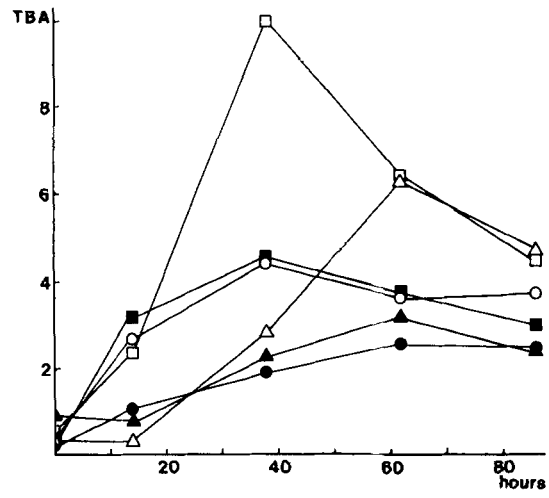

FIG. 2. Development of TBA value (mmol/kg oil) in different fish oils during autoxidation at 60 C. Symbols as in Fig. 1.

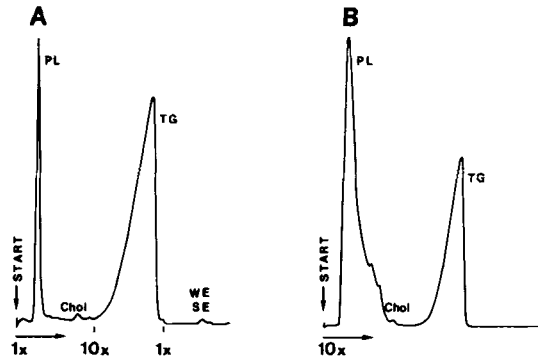

FIG. 3. Separation of lipid classes in fresh herring skin oil (A) and after autoxidation at 60 C with an Iatroscan analyzer. Analytical methods are presented in the text. Note the attenuation changes from 1X to 10X and back to 1X before and after the TG-peak in part A. The PL-peak in part B contains phospholipids and the polar artifacts formed during autoxidation. Symbols: PL = polar lipids; Chol = cholesterol; TG = triglycerides; WE = wax esters; SE = sterol esters.

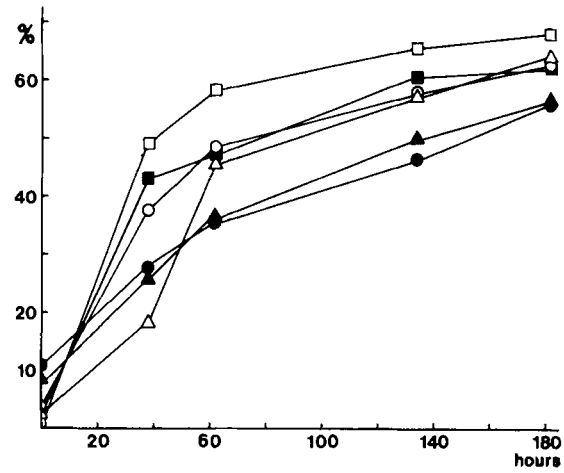

FIG. 4. Changes in the percentages of polar lipids (phospholipids plus polar artifacts) from the total lipid in different fish oils during autoxidation at 60 C. Symbols as in Fig. 1.

mmol/kg oil, was measured as the average of 10.26 and 9.73 mmol/kg oil for duplicate samples of tuna skin oil.

Typical TLC-FID chromatograms for fresh and oxidized fatty fish oils are presented in Figure 3 using herring skin oil as the reference. In this particular fish oil, the immobile polar lipids composing phospholipids and polar artifacts increased from 2.4 to 64.0% of the total lipid during the oxidation test. The relative amounts of polar lipids in the fish oils vs the oxidation time are plotted in Figure 4, which again shows the similarities between herring and mackerel meat oils as well as between mackerel skin and tuna meat oils. The increase in the polar lipid content was fastest and most extensive for tuna skin oil.

DISCUSSION

Lipid oxidation and its measurement has been recently reviewed (8,9). The thiobarbituric acid value is commonly used to express the level of lipid oxidation. Correlation of the TBA value with flavor score by a taste panel has been reported to be exceptionally good in systems containing fatty acids of greater unsaturation than linoleate (10). However, the relationship between TBA value and change in flavors would have to be established for a given oil before using the TBA value as an index of flavor (8). A simple weight gain method based on the increase in the weight of an oil sample during the oxidation process due to the adsorbed oxygen has been used to establish the oxidative stability, the catalytic effect of some metal ions and the effectiveness of some synthetic and natural antioxidants for inhibiting oxidation in fish oils (11-13). Because of the nonspecificity of the weight gain method, other quality parameters have to be employed to confirm the quality assessment of oil.

The three different methods of assessing oxidation level of fish oils: weight gain, TBA value and polar lipid content, all show similar features for the mackerel, herring and tuna oils during an accelerated oxidation test at 60 C (Figs. 1, 2 and 4). To compare the polar lipid content as an indicator of the oxidation level of fatty fish oils with the other two methods, the increase in the polar lipid content (ΔPL) was plotted against the weight gain (Fig. 5) and against the increase in the TBA value (ΔTBA, Fig. 6) at different stages of oxidation.

Linear regression analysis of ΔPL vs weight gain resulted in the line drawn in Figure 5. The correlation coefficient

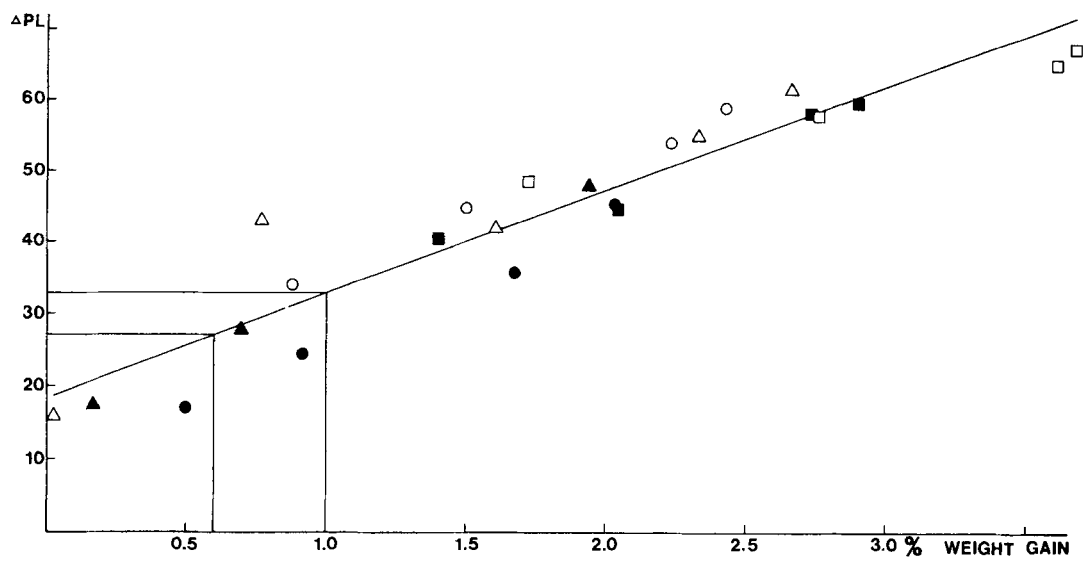

FIG. 5. Correlation between the increase in the polar lipid content (ΔPL) and the weight gain in different fish oils during autoxidation at 60 C. Symbols as in Fig. 1.

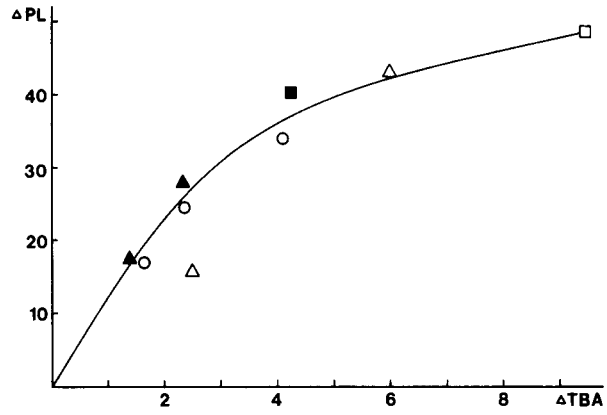

FIG. 6. Correlation between the increase in the polar lipid content (ΔPL) and the TBA values (ΔTBA) in different fish oils during autoxidation at 60 C. Symbols as in Fig. 1.

with 24 values was 0.945, showing a significant linear correlation. Ke and Ackman (12) have demonstrated good linear correlation between weight gain and either peroxide value (POV), volatile carbonyl content, or unsaturation indices. Also, correlation between POV and the oxidative stability (time required for a 0.5% weight gain at 25 C) of capelin and herring oils has been reported (11). In an earlier study, the time required to achieve 1% weight gain at 60 C was used to compare the antioxidative effectiveness of various antioxidants in mackerel skin oil (13). The ΔPL value representing the 1% weight gain as obtained from Figure 5 is 33%. According to this study, the determination of ΔPL value can be used in a way comparable to the weight gain method for the quality assessment of fish oils rich in triglycerides when the phospholipid content of the fresh oil is either known or can be considered negligible. In the latter case, the polar artifact content as such is comparable to the weight gain.

The correlation analysis of ΔPL vs ΔTBA gave a hyperbolic curve shown in Figure 6. However, if plotted on a semilogarithmic system, a straight line was obtained having a good linear correlation ($r = 0.935$; $n = 9$).

The DGF has recommended that 27% of total polar artifacts should be taken as the limit of acceptability for used frying oils (4,14). Their "polar artifacts" correspond to our ΔPL. The 27% level of the ΔPL value, if applied to fatty fish oils, equals 0.6% weight gain at 60 C (Fig. 5). Their recommendation includes the quality assessment of frying oils by a modified column chromatographic method of Billek et al. (3). This method is based on the lipid fractionation on silicic acid, elution of nonpolar lipids with organic solvents and their gravimetric determination from the dried eluate. The difference of the original sample weight and the nonpolar lipids is considered as the amount of polar artifacts formed during oxidation. Thus the method does not take into account the minor amounts of polar compounds present in fresh oils. This defect, however, is usually negligible if the method is used for the quality control of frying oils.

The quantitative TLC determination of the polar lipid increase in the fatty fish oils during their oxidation as described in this study can be used comparably to the earlier weight gain method and can be correlated linearly to the TBA value if plotted on a semilogarithmic system. Although applications are shown only to fish lipid analysis, the method can be used to determine the polar lipid content of any kind of oil samples. As compared to the laborious column chromatographic method to analyze the polar lipid content of an oil, the TLC-FID method is simple and time-saving. Moreover, the TLC-FID analysis reveals the whole lipid pattern of the sample, thus adding to the reliability of the determination.

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