SYMPOSIUM: METAL-CATALYZED LIPID OXIDATION

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Activation Analysis of Trace Elements in Lipids With Emphasis on Marine Oils[1]

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ABSTRACT

A short introduction to the activation analyses is given where some of the main features of the method, especially when applied on biological material, are described. The following trace elements are analyzed in marine and vegetable oils: arsenic, bromine, sodium, copper, manganese, zinc, nickel and iron. Both arsenic and bromine are present as organic compounds. The arsenic is removed in the alkali refining process. The distribution of trace elements in oils has been studied by use of autoradiography and γ-spectroscopy. The results indicate that this distribution is dependent on the phospholipid content in the oil. A high phospholipid content seems to give a more homogeneous distribution of inorganic trace elements.

INTRODUCTION

The neutron activation analysis combines both high sensitivity and good accuracy for many elements. Today, when high sensitivity is required, the activation is mostly performed by thermal neutrons from a nuclear reactor. The activity is here induced by a (n, γ) reaction. Neutrons are captured by stable isotopes and these are then converted to new isotopes of the same element. At the same time a γ-quant is simultaneously emitted. Some of these isotopes may be unstable, i.e. radioactive, and will disintegrate by emitting characteristic γ and β radiation. The induced activity is measured either by recording the β particles with a Geiger Muller counter or the characteristic γ photons with a multichannel γ-spectrometer. The latter is by far the most used.

The sensitivity of the method will depend on several factors. Among these are the neutron flux (n/cm^2 sec), the irradiation time and the activation cross section, the probability for the isotope to capture a neutron (σ_{act}). In practical analytical work, that is to say, among the elements reported analyzed by this method, the sensitivity has been

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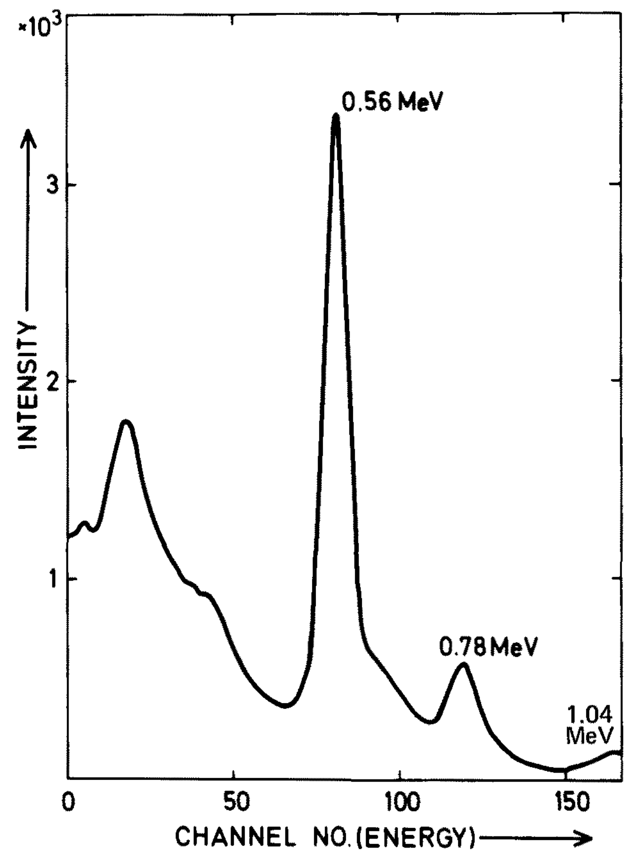

FIG. 1. Gamma-spectrum of neutron activated capelin oil recorded about one day after activation. The main peaks belong to As^{76} with 0.56 MeV and Br^{82} with 0.56 MeV, 0.78 MeV and 1.04 MeV.

from about 10^{-11} to about 10^{-4} g. In addition, the sensitivity will also depend on how the samples are prepared and how they are recorded. Many excellent review articles and books have been published both dealing with the theory for this analytical method and with practical applications (1-3).

In connection with the analysis of trace elements in oils, the following elements are well suited to be analyzed by this method: manganese, iodine, chlorine and nickel, with half lives from 25 min to 2 1/2 hr, copper, sodium, bromine and arsenic, with half lives from 12 to 36 hr, and zinc, cobalt, iron, chromium, with half lives from about 30 days to about 5 years. In addition phosphorus should also be mentioned. The radioactive phosphorus isotope P^{32} has a half life of about 14 days and emits only β-particles.

Some important features of this analytical method relevant to the analysis of oils should be pointed out in more detail.

1. The registration of the activity from an activated element is mostly performed by recording high energy γ-photons. This reduces matrix effects. As a result, it is not necessary to prepare the standards in the same way as the sample to avoid an uneven absorption of γ-photons in the standard and in the sample. Activities in biological materials may, for instance, be compared with standards dissolved in water.

2. Especially when trace elements in the sub-microgram area (1 ppb to 0.1 ppm) are analyzed, the risk of contamination during handling and analyzing the samples is considerable. For the activation analysis this is only a problem before the activation. After activation, the addition of impurities, even foreign sources of the elements to be analyzed, will be of no consequence for the results.

3. In many cases the matrix activity and also the activity induced in trace elements is negligible or does not interfere with the registration of the induced activity in the trace element to be analyzed. In these cases it is therefore possible to perform a nondestructive analysis of one or more elements. Examples of such matrixes are organic and biological materials. This type of nondestructive analysis is an interesting way to study the level of impurities in samples taken for instance from different steps during the processing of the oil, and also to study the relation between two or more impurities and how the different steps in the process may change this relation. This can be done by comparing γ-spectra of activated samples taken from the different steps.

If the γ-radiation (the γ-photons) from the induced activity in the element to be analyzed, is covered or interfered by γ-photons from other activities, it is necessary to carry out a radiochemical separation to purify the element enough to perform a registration with the γ-spectrometer. This is done usually by adding a carrier (milligram amount of the element to be analyzed). When the carrier has exchanged with the activated isotope, ordinary chemical separation methods are used to isolate this. Often only one or a few separation steps are necessary to get the sample pure enough to secure an adequate registration.

4. A distribution pattern of trace elements in flat sections (films, polished or flat surfaces, etc.) can sometimes be obtained by using autoradiographic methods. A uniform exposure of the film reveals a homogeneous distribution whereas areas with a higher exposure indicate an inhomogeneous distribution of inorganic impurities or of particles. Due to high specific activity in these areas the corresponding exposed areas on the film may be rather large and no conclusion can be drawn as to the real size of the area. Also a variation in the background exposure may be observed when the oil is not evenly distributed. A γ-spectrum of the oil will indicate the main radioactive isotope present. This technique is especially useful for solids but also for frozen liquids or liquids with high viscosity.

TABLE I

Nondestructive Activation Analyses of
Trace Elements in Unprocessed and Processed Marine Oils

Sample	As (ppm)	Br (ppm)	Na (ppm)
Crude oil	8-14	7-10	~0.1
After alkaline treatment and washing	< 0.2	7-10	~0.3
After bleaching	< 0.2	6-10	~0.2
After hydrogenation and deodorization	< 0.2	4-7	~0.1

TABLE II

Destructive Activatonalyses of
Trace Elements in Hydrogenated Marine Oils

Sample		Cu (ppm)	Ni (ppm)	Fe (ppm)
No. 1	10 samples	0.004-0.04	0.03-0.14	< 0.5-12
No. 2	6 samples	0.006-0.012	0.10-0.72	5 -12

GENERAL INFORMATION ON ACTIVATION ANALYSIS OF OILS

The main elementary constituents of oils (carbon, hydrogen, oxygen, and in raw oils also phosphorus) will not give any detectable γ-radiation (γ-peaks) when irradiated with thermal neutrons. The β-particles from phosphorus, P^{32}, will be recorded as bremsstrahlung on the γ-spectrometer. It is therefore possible to perform a nondestructive analysis of some of the trace elements present in the oils. Other trace elements, where the induced activities are low or where the γ-spectrum is complicated by γ-photons from

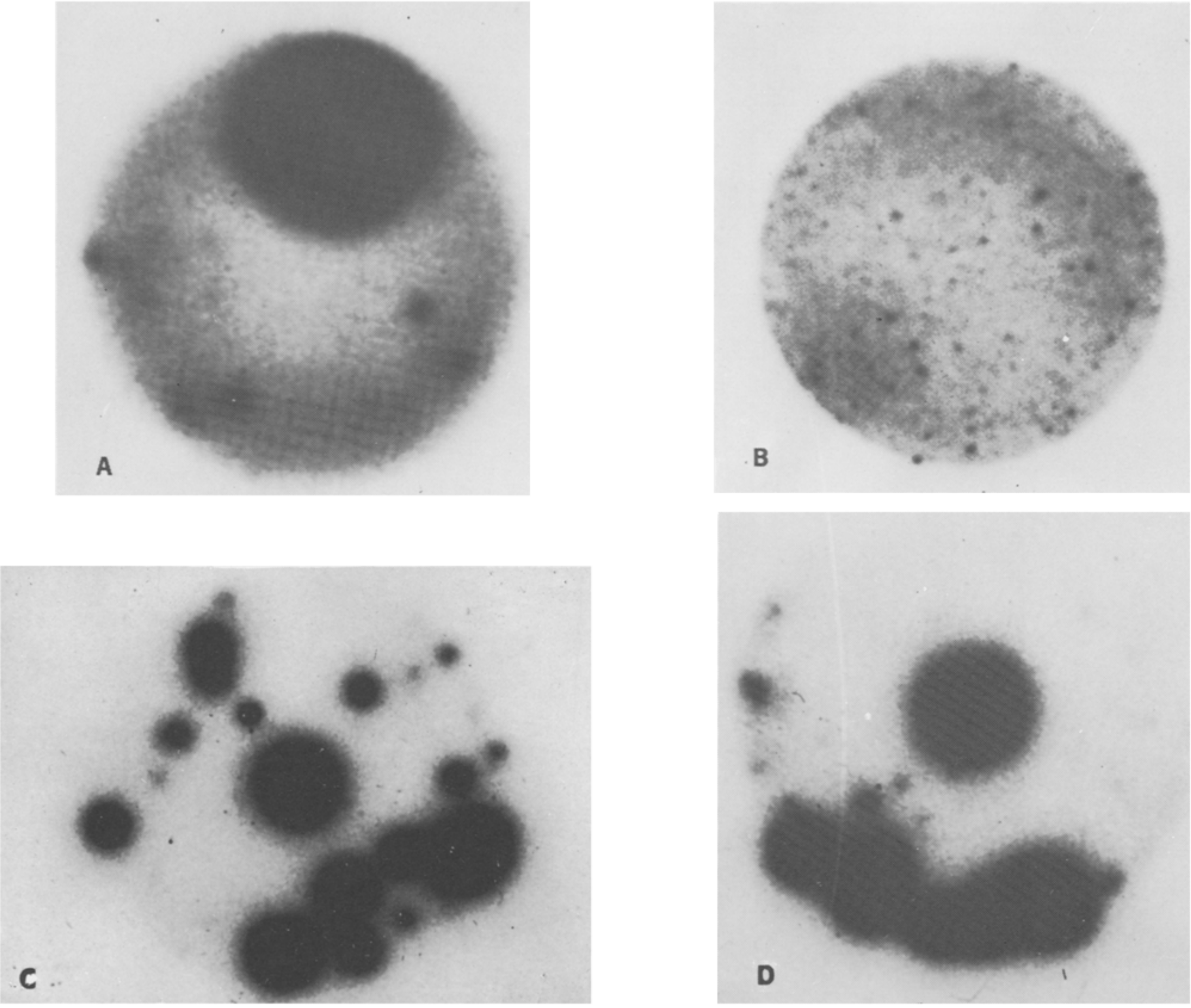

FIG. 2. Autoradiographs: A, Neutron activated refined fish oil; exposure time about 2 hr. B, Inactive fish oil where radioactive arsenic has been introduced from a water phase. C, Neutron activated refined soybean oil. The phospholipid content is low (25-50 ppm). D, Neutron activated refined peanut oil with a low phospholipid content (~25 ppm).

other activities, have to be separated by chemical methods before registration. This can be done either by wet or dry destruction of the oil or by different types of extraction methods. The extraction can be carried out by dissolving the oil after activation in a suitable solvent and extraction with water at an adjusted pH. The elements are then transferred into the water phase and subsequently separated by chemical methods. Saponification is an alternative method for bringing some of the trace elements into the water phase without destruction of the organic matter.

EXPERIMENTAL PROCEDURES

Materials

The oils analyzed in this investigation were either commercially available or produced in our laboratory.

Irradiation

About 1 ml of each oil was sealed off in quartz ampoules and was then ready for neutron activation. P.A. Chemicals (Merck, Darmstadt) of the elements to be analyzed (NaCl, NH_4Br, NaH_2PO_4, As_2O_3, $ZnCl_2$, $FeCl_3$, $MnCl_2$, $NicCl_2$ and $CuCl_2$) were sealed in the same kind of ampoules and irradiated together with the oil samples. Especially at irradiation times up to 24 hr, it is often advisable to dissolve the standard before sealing in the ampoules. The irradiation was performed with a neutron flux of $4 \cdot 10^{12}$ n/cm^2 sec in the nuclear reactor JEEP 2 Kjeller, Norway.

Separation and Registration

After activation the following procedures were used for preparing the different trace elements in the oils for registration. Both principles and detailed separation procedures for the elements to be analyzed have been discussed elsewhere (4) and only some of the main steps of the analysis are outlined here:

Saponification. During conventional saponification copper and nickel ammonium complexes were added as carrier. The carrier will exchange with the activated copper and nickel in the oil samples. After the saponification the nickel is precipitated with dimethylglyoxime and copper as copper sulfide.

Wet Destruction. The oils were treated with concentrated warm sulfuric acid, nitric acid and hydrogen peroxide. Carriers of the elements to be analyzed were added and conventional separation methods were applied after the destruction of the organic matter.

Extraction. The oils are dissolved in hexane and mixed with hydrochloric acid at a pH of about 2. Carriers of the elements to be analyzed are added to the water phase. The activated trace elements are extracted into the water phase and will exchange with the carriers; the different elements

TABLE III

Nondestructive Activation Analyses of Trace Elements in Vegetable Oils

	(ppm)				
Sample	Mn	Zn	Cu	Na	Br
Crude oils	a	<0.05-1.1	<0.005-1.0	0.03-5	<0.01-4
Refined oils	<0.005-0.1		<0.005-0.3	<0.01-2	<0.01-4

[a]not determined.

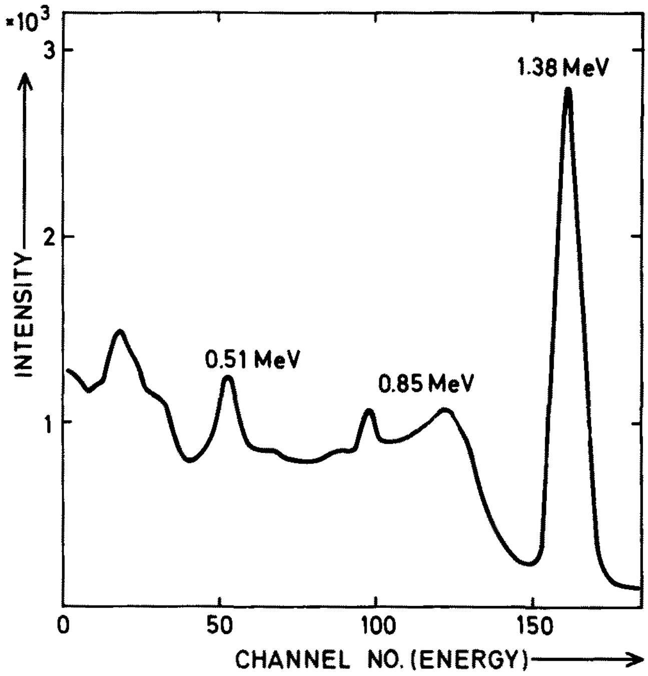

FIG. 3. Gamma spectrum of neutron activated linseed oil. The main peaks belong to Cu^{64} with 0.51 MeV, Mn^{56} with 0.85 MeV and Na^{24} with 1.38 MeV.

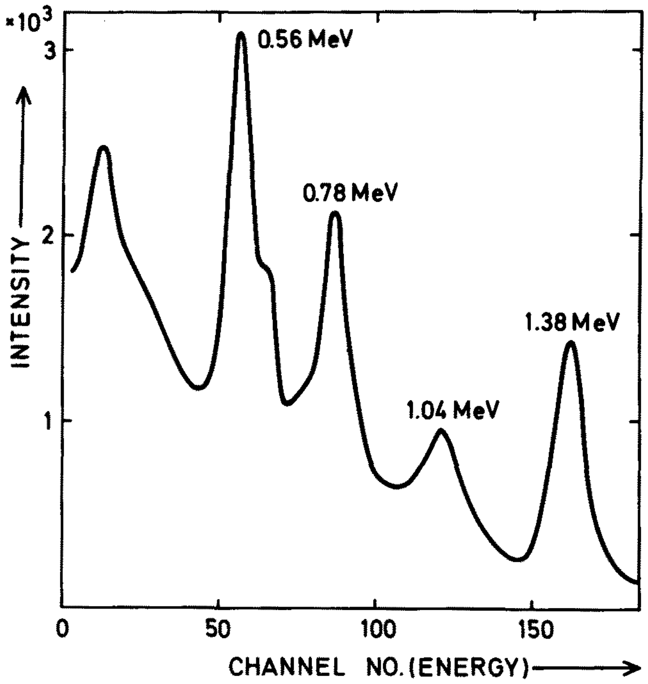

FIG. 4. Gamma spectrum of neutron activated coconut fat. The main photopeaks belong to Br^{82} with 0.56 MeV, 0.78 MeV, 1.04 MeV and Na^{24} with 1.38 MeV.

are then separated to the degree of purity that enable a recording with a γ-spectrometer.

Direct Registration. The oils are poured into inactive glass vials and are recorded directly with a γ-spectrometer.

Distribution Studies. The neutron activated oil is poured onto a thin plastic film and placed in contact with a photographic emulsion. An ordinary x-ray film can be used. After exposure the film is developed and handled as an ordinary x-ray film. The exposure is about 14 hr on a fine grain x-ray emulsion or 2 hr with a medium grained film. Gamma spectra of the oils are recorded after the exposure. The procedure should be repeated after a few days to see how the shortlived isotopes influence the distribution pattern on the film and correlate this with the γ-spectrum.

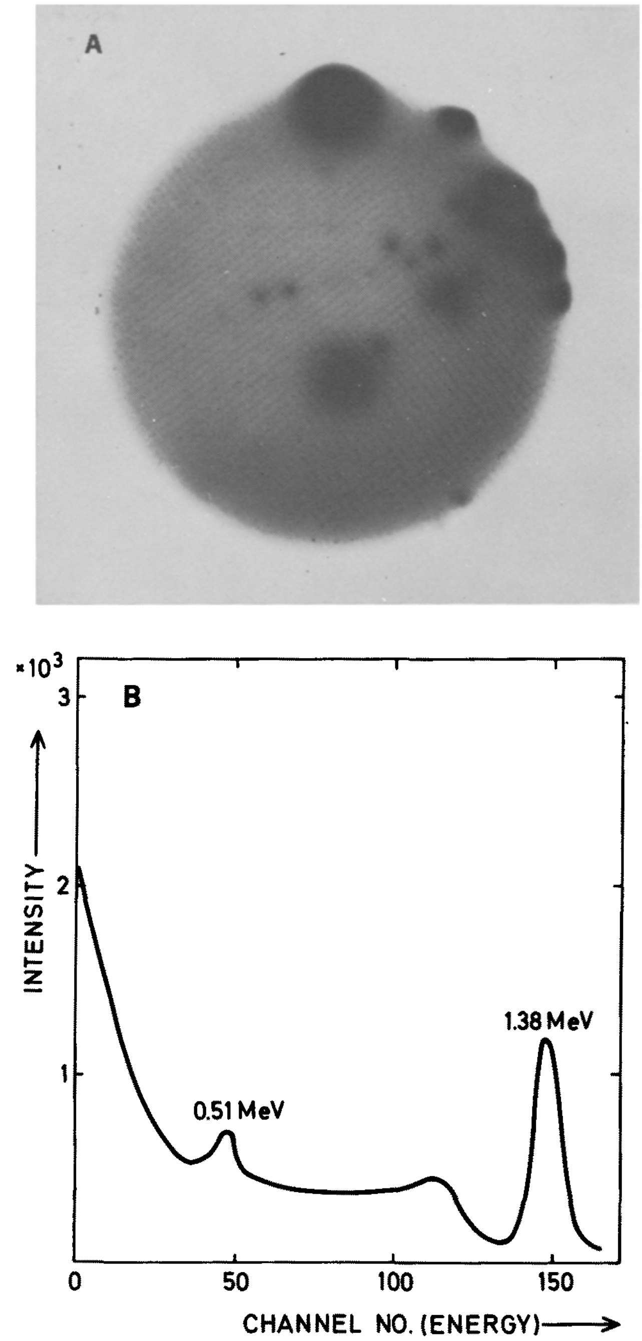

FIG. 5. Neutron activated linseed oil. A, Autoradiograph, exposure time about 2 hr. The background exposure is from P^{32} (phospholipid content ~2500 ppm). B, Gamma-spectrum recorded immediately after the exposure. Copper, Cu^{64} with 0.51 MeV and sodium Na^{24} with 1.38 MeV are the main γ-emitting isotopes. The bremsstrahlung from P^{32} is also recognized.

RESULTS AND DISCUSSION

Marine Oils

Crude oils manufactured from marine fishes (mackerel, herring, capelin and others) by conventional methods are relatively low in inorganics. Nondestructive analysis indicates that these oils contain usually less than 0.2 ppm sodium and less than 0.05 ppm copper. This is probably due to the way these oils are produced. The oil is in contact with relatively large amounts of water at 100 C followed by centrifugation. This insures an effective washing procedure and an efficient removal of the water from the oil.

Marine oils however contain some trace elements present as organic compounds (5,6). Among these arsenic and bromine have been studied in more detail. Figure 1 shows the γ-spectrum of capelin oil recorded about one day after activation and without any chemical treatment after the activation. The γ-spectrum consists mainly of the radioactive arsenic isotope As^{76} and the radioactive bromine isotope Br^{82}. When the oil is treated with alkali in the refining process the arsenic will follow the alkaline phase together with most of the phospholipids left in the oil. The bromine content will be reduced during the hydrogenation to about half of the original value. See Table I.

Due to the high content of bromine and arsenic a nondestructive analysis cannot be performed when the amount of inorganics in marine oils are low. In this case other methods have to be used to carry out an analysis. The contents of copper, nickel and iron have been analyzed in hydrogenated marine oils partly by the saponification and partly by the destruction procedure. Some of the results are shown in Table II. The two series of oil samples, No. 1 and No. 2 are from different productions.

The distribution of trace elements in a refined fish oil with a low phosphorus content is shown in Figure 2A. This oil however has a high background exposure and contains some inhomogeneities. The corresponding γ-spectrum indicates that sodium-24, arsenic-76 and bromine-82 are the main activities. As the sodium-24 with a half life of about 15 hr disappears, the inhomogeneities disappear. This indicates that the main radioactive isotopes left, bromine-82 and arsenic-76 with half-lives of 36 hr and 24 hr respectively, are homogeneously distributed and are therefore probably present as organic compounds soluble in the oil. These results were also confirmed when some experiments were carried out where radioactive arsenic was introduced into inactive oil from a water phase. Figure 2B shows an autoradiograph of radioactive arsenic introduced into a fish oil.

Vegetable Oils

There is no evidence so far that organic bound trace elements are present in vegetable oils. Nondestructive analysis of inorganics present in the oils with a high sensitivity for activation analysis is therefore possible. In vegetable oils one or more of the following trace elements can usually be analyzed without any chemical treatment: copper, manganese, zinc, sodium, chlorine, bromine and iron. Other trace elements have to be analyzed by radiochemical separation methods based on chemical treatment of the oils after the acvitation. Table III shows some results of nondestructive analysis of vegetable oils. Among the oils snalyzed are coconut fat, soybean oil, olive oil, peanut oil and linseed oil. Figure 3 and 4 show the γ-spectra of linseed oil and coconut fat. Copper, manganese and sodium are observed in the linseed oil whereas coconut fat contains mostly bromine and sodium. The content of inorganic ions in the different types of oils depends greatly on the possibility of contamination during the refining and hydrogenation steps and also on how the processed oils are stored afterwards. A simple washing procedure of the finished product may often reduce the level of impurities (parts per million) by a factor of 5-10.

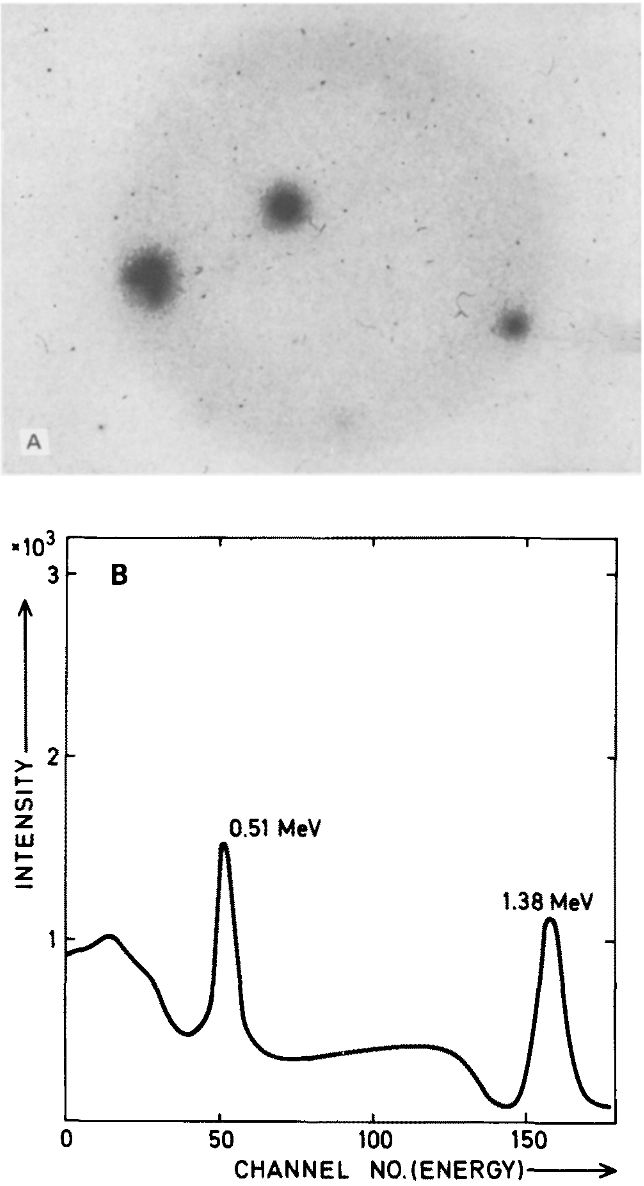

FIG. 6. Neutron activated olive oil. A, autoradiograph, exposure time about 2 hr (phospholipid content ~25 ppm). B, Gamma-spectrum recorded after the exposure. Cu^{64} and Na^{24} are the main radioactive isotopes present in the oil.

Figures 5 and 6 show two autoradiographs of activated linseed and olive oil together with corresponding γ-spectra. The linseed oil contained about 0.25% phospholipids and the olive oil about 0.003%. When the γ-spectra of the oils are compared with the corresponding autoradiographs it is evident that the inhomogeneous areas on the exposed film consist mainly of radioactive copper, sodium and bromine. The inhomogeneities on the autoradiographs disappear as the radioactive isotopes of copper, sodium and bromine disintegrate.

Although too few experiments have been performed, the results seem to indicate that oils relatively high in phospholipids have a more homogeneous distribution of trace elements than oils low in phospholipids. Figure 2C and 2D show two more autoradiographs of vegetable oils with a low phospholipid content. Oils with about the same amount of trace elements and with a high phospholipid content show an approximately homogeneous distribution of trace elements. These results are also confirmed by autoradiographing inactive oils with high and low phospholipid content where radioactive tracers (Br^{82}, Cu^{64}, and Na^{24}) have been introduced from a water phase.

Besides the content of phospholipids, small amounts of water present in the oils may also be of importance in this connection, providing small areas with a higher water concentration where the trace elements probably can be concentrated.

When studying the effect of trace elements on the autoxidation of oils more emphasis should be concentrated on the distribution of these trace elements, and on how this distribution is influenced by the content of phospholipids and probably also of the content of small amounts of water in the oils.

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