Sequestering and Exchange of Metal Ions in Edible Oils Containing Phospholipids

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ABSTRACT

Metal ions (Zn^{2+} and Fe^{3+}) are transferred from water into vegetable and marine oils when these oils contain phospholipids. Ions present in the oil may be exchanged with the same or with other kinds of cations in a water phase in contact with the oils. Radioactive isotopes of zinc and iron were used for measuring the exchange rate. The sequestering action by the oil strongly depends on the contents of phospholipids and reaches a maximum (ions v/s phospholipids) in the concentration range between 3 and 60 ppm of phospholipids (0.1-2 ppm of phosphorus) in the oil. The same sequestering effect is demonstrated when purified phospholipids (phosphoethanolamine and acetone-refined soybean lecithin) are added to edible oils from which the natural content of phospholipids has been removed. Free fatty acids do not appear to sequester multivalent ions nor to influence the sequestering action by the phospholipids when added to the oil.

INTRODUCTION

Traces of heavy metals, particularly copper and iron (1), catalyze oxidation of unsaturated lipids and development of rancidity in edible oils and fats. Recent investigations involving the contents of heavy metal ions in edible oils show that distribution and concentration of the metals are related to the phospholipid content in the oil (2). Also, it was found that the level of heavy metals in the oil increases with the storage time of the raw fish material before production of the oil (3). The metal ions are available naturally in the glue water during processing (4). Such prolonged storage also results in an increase in the level of phospholipids.

Based on these apparent correlations between heavy metals and phospholipids in oils, further investigation of the possible relationship between multivalent metal cations and the content of phospholipids in oils was deemed worthwhile. The experimental techniques for such an investigation should be designed in such a way that the methods used and results obtained would be helpful for practical application in the industry as well as in fundamental research. A basic step in industrial oil refining technology is the removal of unwanted ions and other components by washing with water solutions; thus, the transport of multivalent ions between a water phase and an oil phase was seen to be of prime importance. The application of radioactive tracers to follow the actions under investigation was selected since this methodology greatly simplifies quantitative measurements. The metal ions in both the water and the lipid phase may be measured by direct counting of the radionuclides. As suitable test ions, zinc (Zn^{2+}) and iron (Fe^{3+}) were chosen. Both elements have radioactive isotopes with relatively long half-lives, namely, ^{65}Zn with $T_{1/2}$ = 245 days and ^{59}Fe with $T_{1/2}$ = 45 days. They also represent di- and trivalent ions, respectively.

EXPERIMENTAL PROCEDURES

Oil Treatments

The oils used in this work were either prepared in the laboratory or were of commercial origin (Table I). The marine oils prepared in the laboratory were produced by extracting homogenized raw fish material with chloroform: methanol (2:1, v/v). After extraction (2 hr at 40 C), the mixture was allowed to settle and the chloroform-oil phase was separated. The chloroform was evaporated in a rotavapor, and the resultant oils were washed twice with distilled water and centrifuged before use.

Raw and refined grades of soybean oil were used as delivered from the refinery. In addition, samples of soybean oil with a low content of phospholipids (<0.1 ppm) were prepared by dissolving 1 part of the oil in 10 parts of chloroform and mixing with activated silica gel (Merck 0.22-0.55 mm) to form a slurry. The slurry was then transferred to a silica gel column (internal diameter 2 cm, height 30 cm), and the oil was eluted by further addition of chloroform to the column, thus leaving the phospholipids adsorbed to the activated gel. The selected oils are presented in Table I.

Purified phosphoethanolamine was obtained from Koch-Light laboratories (Colnbrook, Bucks, U.K.) and was used as received.

Soybean lecithin was refined by repeated slurrying in cold acetone to give a white, neutral smelling powder. This was separated further by thin layer chromatography (TLC) into several fractions. The TLC system employed was based on a silica gel substrate, developed with a mixture of chloroform, methanol, and water (2:2:1). Phospholipids were visualized by spraying with molybdic acid. Each of the fractions was added to phospholipid-free soybean oil for subsequent equilibration experiments.

Counting Techniques

The level of phosphorus in the oils was determined by means of neutron activation analysis (3). Samples containing ca. 100 mg of the oils were sealed in quartz ampoules rinsed with HNO_3 and were irradiated for 1 day in a nuclear reactor at a neutron flux of 1×10^{13} n per cm^2 sec. After irradiation, the oil samples were transferred to inactive glass vials and measured in a γ spectrometer equipped with 3 x 3 in. NaI crystal detector (well type). The phosphorus content was estimated by summing up the counts from a certain number of channels of pulse height analyzer as derived from the bremsstrahlung of the phosphorus iso-

TABLE I

Selected Oils and Their Content of Phosphorus

Sample	Treatment	Content of phosphorus[a] (ppm)
Herring	Factory produced	<0.2
Herring	Laboratory produced Chloroform: methanol extracted	4
Soybean	Factory produced Hexane-extracted Bulk lecithin removed, dried	100
	Fully refined in plant	4
	Phospholipids removed by chromatographic methods (laboratory)	<0.1

[a] Determined by neutron activation.

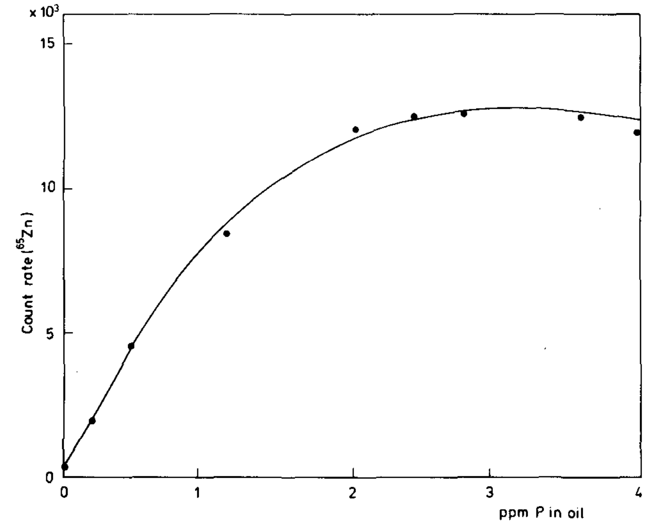

FIG. 1. Uptake of ^{65}Zn in herring oil with increasing contents of phospholipids in the oil.

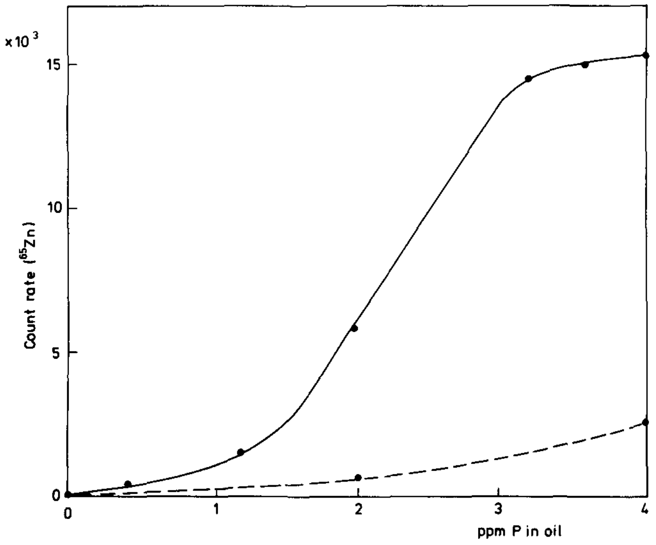

FIG. 2. Uptake of ^{65}Zn in soybean oil with increasing contents of phospholipids in the oil. —— Radioactivity in oil with pure tracer; -- radioactivity in oil with 12 ppm Zn^{2+} added to the water.

tope ^{32}P with $T_{1/2}$ = 14.1d. The counting was done after short-lived radioactive isotopes, especially of bromine and arsenic, had decayed to insignificance. The analysis was confirmed by counting the ^{32}P beta with a Geiger-Müller counter. Sodium phosphate irradiated under the same conditions was used as comparative standards throughout.

Radioactive tracers of zinc and iron were obtained from The Radiochemical Center (Amersham, U.K.). The zinc tracer consisted of $^{65}Zn^{2+}$ dissolved in 0.1 M HCl (>100 mCi/mgZn) and the iron tracer of $^{59}Fe^{3+}$ dissolved in 0.1 M HCl (3-30 mCi/mgFe). The tracers were diluted so that 1 ml of an aqueous solution gave ca. 5 x 10^4 gamma counts per min with a 3 x 3 in. Na crystal detector (well type).

Sequestering and Exchange Procedures

The sequestering and exchange experiments were carried out by mixing 5 ml of oil with 5 ml of distilled water (to which the cation in question was added-radioactive or non-radioactive). The pH of the aqueous solution during the equilibration was neutral to slightly alkaline. After intimate mixing for 1 hr at 40 C, the two resultant phases were subsequently separated by centrifugation. For measuring the radioactivity, 1 ml of each separated phase was used.

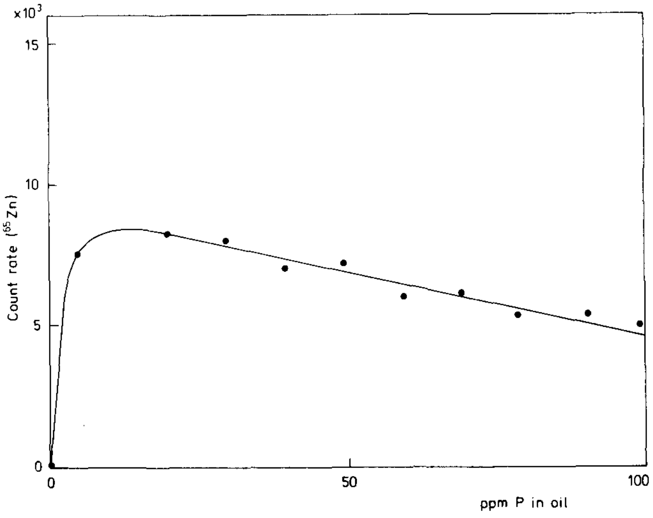

FIG. 3. Uptake of ^{65}Zn in soybean oil with higher levels of phospholipids in the oil.

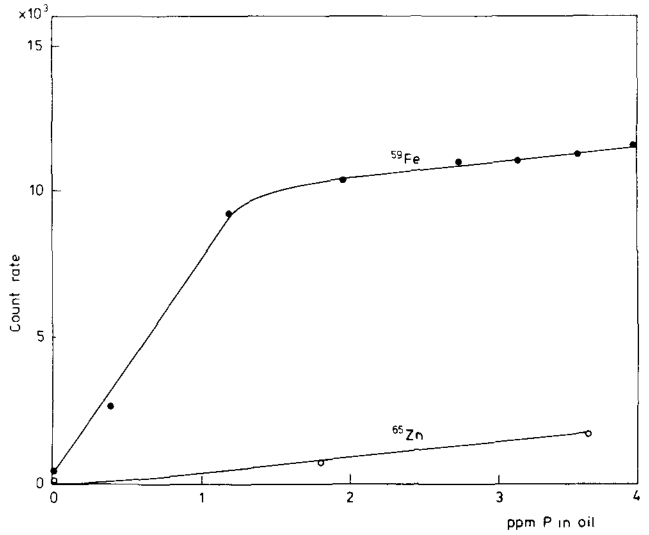

FIG. 4. Uptake of $^{59}Fe^{3+}$ (—●—) in soybean oil as a function of the content of phospholipids in the oil, and uptake of ^{65}Zn (—○—) in soybean oil with phosphatidyl ethanolamine added to the oil.

After the centrifugation, a distinct interlayer was sometimes observed between the oil and the water phase. This presumably consists of emulgated phospholipids. By freezing and thawing of the sample and repeated centrifugation, this interlayer was reduced markedly, but not completely removed.

Three series of experiments have been carried out, as listed below:

Series 1. Sequestering as a function of content of natural phospholipids: Varying levels of phospholipids were obtained by mixing the original oil with oil from which the phospholipids had been removed. However, the same amount of radioactive $^{65}Zn^{2+}$ or $^{59}Fe^{3+}$ was added to the water phase in each of the following equilibration experiments: (a) herring oil with 0-110 ppm phospholipids (0-4 ppm P) - $^{65}Zn^{2+}$; (b) soybean oil with 0-110 ppm phospholipids (0-4 ppm P) - $^{65}Zn^{2+}$; (c) soybean oil with 0-2800 ppm phospholipids (0-100 ppm P) - $^{65}Zn^{2+}$; and (c) soybean oil with 0-110 ppm phospholipids (0-4 ppm P) - $^{59}Fe^{3+}$.

Series 2. Sequestering with added isolated phospholipids and fatty acids and with the natural phospholipids removed: The same amount of $^{65}Zn^{2+}$ was added to the

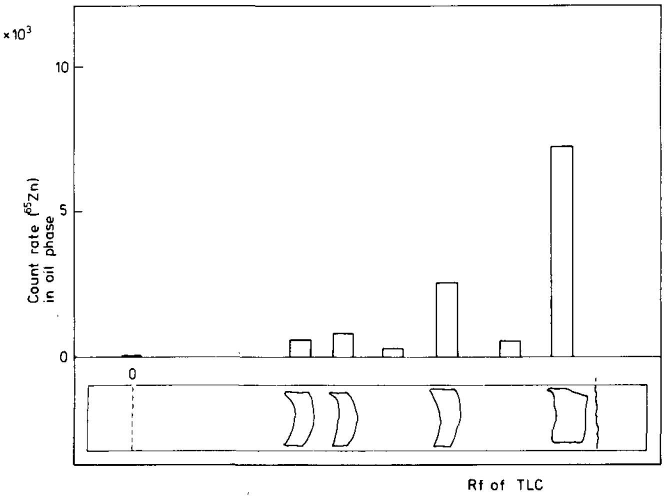

FIG. 5. Uptake of ^{65}Zn in soybean oil with acetone-refined soybean lecithin separated on thin layer chromatography (TLC) and added to the oil. TLC: Silica gel-$CHCl_3$:CH_3OH:H_2O.

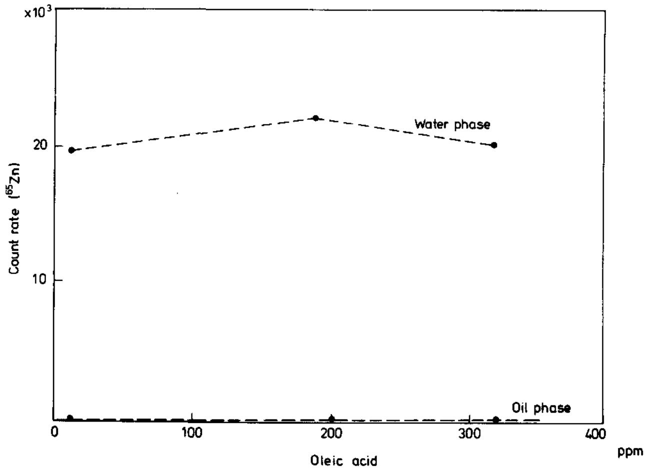

FIG. 6. Uptake of ^{65}Zn in soybean oil with oleic acid aded to the oil.

water phase in each equilibration experiment: (a) soybean oil with added phosphatidyl ethanolamine (0-4 ppm) - $^{65}Zn^{2+}$; (b) soybean oil fractions from TLC-fractionated acetone-refined lecithin - $^{65}Zn^{2+}$; and (c) soybean oil with added oleic acid (0-400 ppm) - $^{65}Zn^{2+}$.

Series 3. Exchangeability of sequestered zinc: (a) Soybean oils with 1400 ppm phospholipids (50 ppm P), previously equilibrated to contain radioactive $^{65}Zn^{2+}$. Addition of varying levels (0-4 ppm) of nonradioactive Zn^{2+} from aqueous phase. (b) Soybean oils with varying content of phospholipids, previously contacted with a constant amount of radioactive $^{65}Zn^{2+}$ as in series (1b), followed by equilibration with 0.001 N nonradioactive Fe^{3+}. Equilibration repeated once. (c) Same as (3b), except with nonradioactive 0.001 N Zn^{2+} substituted for Fe^{3+}.

RESULTS

Typical results from sequestering experiments are shown in Figures 1-4. As can be seen, there is a marked increase in radioactivity in the oil phase as the level of phosphorus increases. A higher degree of sequestering with respect to phosphorus appears to occur in herring oil containing even less than 1 ppm, whereas this occurs in soybean oil containing 2-4 ppm of phosphorus. With soybean oil, a "lag" in uptake appears to exist for ^{65}Zn at low phosphorus levels (ca. 1 ppm P) (Fig. 2), while at higher levels the radioactivity in the oil is sometimes reduced. A similar lag phase in the complexing of trivalent iron was not observed.

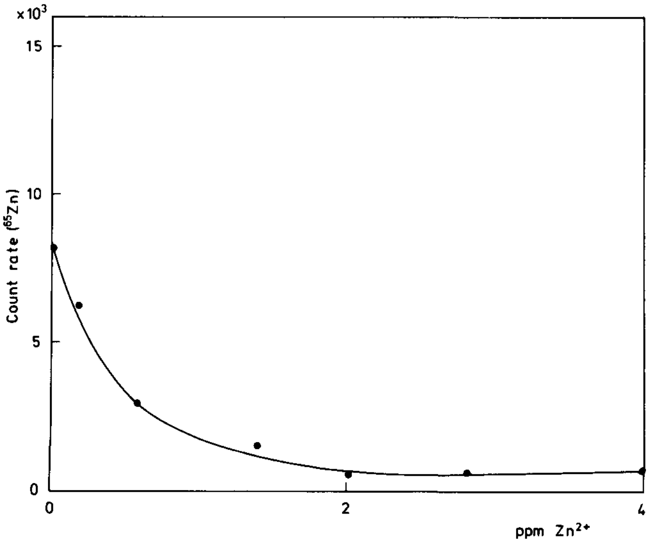

FIG. 7. Soybean oils (with 1400 ppm phospholipids–50 ppm P–previously equilibrated to contain ^{65}Zn) contacted with water containing varying levels of nonradioactive Zn^{2+}. Resultant radioactivity in the oil phase.

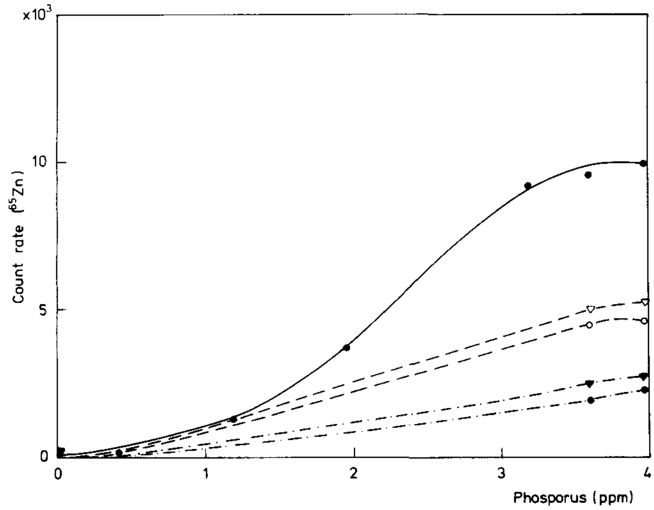

FIG. 8. Soybean oils with varying levels of phospholipids previously equilibrated to contain ^{65}Zn and subsequently contacted twice with aqueous 0.001 N ferric sulphate. ^{65}Zn activity: ———●——— in oil at start; ---△--- in water phase after first contact; ---○--- in oil after first contact; ---▲--- in water phase after second contact; ----●--- in oil after second contact.

Most of the radioactivity that was not transferred to the oil remained in the water phase. With increasing levels of phospholipids, however, an interlayer was formed between the water and the oil phase, in which some radioactivity was present.

With undiluted tracer, there is an almost total transfer of radioactivity to the oil phase when enough phospholipids are present. If nonradioactive zinc ions are added to the water, however, a much larger portion of the overall activity remains in the water phase (Fig. 2).

Results from experiments with more defined phospholipids are shown in Figures 4-6. Purified phosphatidyl ethanolamine shows a much reduced, but still distinct, sequestering action (Fig. 4). The various TLC fractions of acetone-refined soybean lecithin show sequestering of $^{65}Zn^{2+}$ corresponding to phospholipids as detected by molybdic acid (Fig. 5). Oleic acid does not show any

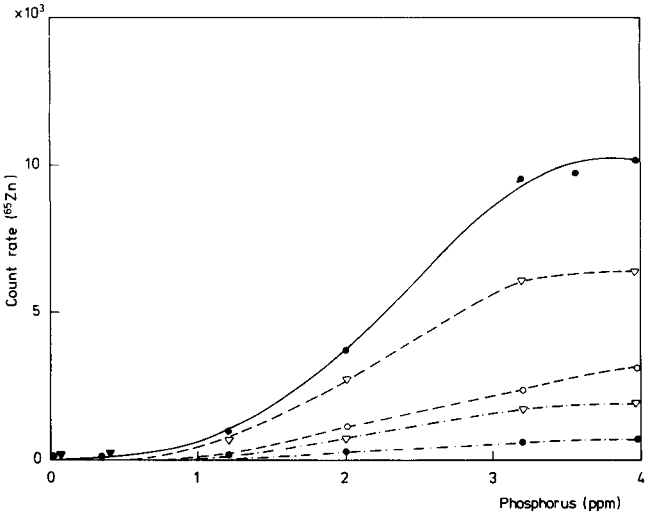

FIG. 9. Soybean oils with varying levels of phospholipids previously equilibrated to contain ^{65}Zn and subsequently contacted twice with water with 0.001 N zinc sulphate. ^{65}Zn activity: ——●—— in oils at start; ---△--- in water phase after first contact; ---○--- in oil after first contact; ---▲--- in water phase after second contact; ---●--- in oil after second contact.

sequestering action under the experimental conditions employed here (Fig. 6).

In Figure 7 are given results from contacting soybean oil with a fairly high content of phospholipids and equilibrated with radioactive zinc, with water containing increasing amounts of nonradioactive zinc ions. As the concentration of nonradioactive zinc is increased in the water phase, radioactivity in the oil phase is drastically reduced, indicating both an exchange of radioactive zinc in the oil with nonradioactive zinc in the water and a fairly limited total capacity for the complexing action.

A similar result is seen when trivalent iron ions are substituted for the divalent zinc ions. This effect is confirmed with oils with varying levels of phospholipids, equilibrated with a constant amount of $^{65}Zn^{2+}$ and subsequently contacted twice with 0.001 M solutions of either nonradioactive ferric sulphate (Fig. 8) or zinc sulphate (Fig. 9). Comparing these figures, one observes that radioactivity is brought to a lower level both when iron is present and when the ratio of activity in water to activity in oil is higher. This shows that zinc is exchanged with iron and that iron is preferably transferred to the oil phase compared with zinc.

DISCUSSION

The results obtained in these experiments show a distinct correlation between the contents of phospholipids in oils and the sequestering action on metals. This occurs particularly at concentration levels of 10-100 ppm of phospholipids. From the work carried out so far, everything points to the phospholipis as the active sequestering agent. It is possible, however, that other components present in the oil may have similar effects. One possibility is that water dissolved in the oil influences the sequestering action. With higher levels of phospholipids and in the presence of water, the formation of micelles and emulgated water is quite possible; thus, the complexing ability of the phospholipids may be modified.

The "lag" effect observed in Figure 2, where the sequestering effect for ^{65}Zn is somewhat hindered at lower phospholipid contents, points to other possible compounds with a higher affinity for the phospholipids. These compounds, however, would seem to be present in rather low concentration. They may be trivalent ions such as Fe^{3+}.

The results seem to show that trivalent ferric ions are complexed stronger than divalent zinc ions, and indicate that ions with a higher valence are bound preferably in the oil. The exchangeability, indicated by these experiments, of metal cations between an oil and a water phase appears to open new possibilities directed towards the removal of heavy metal ions by proper dimensioning of appropriate exchange processes. In particular, it appears to be practical to exchange catalytically active elements such as copper and iron for ions of less catalytic activity associated with the oxidation of edible oils.

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