Adsorption of Soy Oil Phospholipids on Silica

HELEN G. BROWN and **HARRY E. SNYDER,** Department of Food Science, University of Arkansas, Route 11, Fayetteville, AR 72701

ABSTRACT

An experimental method for adsorption of phospholipids from soybean oil was developed based on chromatographic properties of the oil components. The traditional method for removing phospholipids involves hydrating the gums. However, when crude oil in hexane is applied to thin layers or columns of silica, the phospholipids irreversibly adsorb. The triglycerides can be eluted with non-polar solvents and phospholipids with a polar solvent system. Hence, there is a basis for a selective adsorption of phospholipids on silica.

The system involved stirring one hundred milliliters of oil in solvent (i.e., miscella) with one gram of silica for 15 min. The phosphorus content, before and after the reaction, was analyzed by wet ashing and Fiske-Subbarow colorimetric reaction. Addition of isopropanol (at least 1%) to the hexane miscella caused an increase in phosphorus adsorption, most likely due to liberating triglyceride from adsorption sites. Increased adsorption was achieved by deactivating the silica. Oil concentration did not appear to affect the adsorption. The amount of phosphorus adsorbed depended on the concentration of the phospholipid.

When phospholipid adsorbed per gram of silica is plotted vs the residual phospholipid, the plot resembles a Freundlich isotherm for reversible adsorption. Yet the adsorption is irreversible. Possible explanations for this type of adsorptive behavior are explored.

INTRODUCTION

The presence of impurities and non-triglyceride materials in freshly extracted soybean oil necessitates its refining prior to consumption. In the refining process numerous operations are involved to produce a bland, lightly colored and stable oil. The first step in processing is the removal of phospholipids. There have been several different techniques established for removing phospholipids from soy oil. A general procedure is degumming the oil; this involves mixing oil with 1-3% water with agitation for 30-60 min at 70 C to hydrate the phosphatides, followed by centrifugation to separate the degummed oil from the gums (also known as soybean lecithin) (10). Degumming reduces the phosphorus content of the oil from 500-900 ppm (1.6% to 2.8% phospholipid) to 12-170 ppm.

In our laboratory we use the chromatographic behavior of crude soybean oil as a basis for investigating the selective adsorption of phospholipids. When crude oil is applied to thin layers or columns of silica, the phospholipids are irreversibly adsorbed and thus allow the elution of the triglycerides with non-polar solvents (1,2,3).

Linteris and Handschumaker (1) conducted an extensive investigation of different methods for determining refining losses of crude oils, including a chromatographic technique that involved washing crude oil through an alumina column with ether and removal of phospholipids and free fatty acids. Lunde et al. (2) mixed silica with oil in chloroform and eluted triglycerides with chloroform. Beal et al. (4) demonstrated phospholipid removal during bleaching, which is an adsorption operation. Gutfinger and Letan (3) compared the efficiency of adsorbents for removing phospholipids and pigments from soybean oil. They found that silicic acid and florisil had poor ability to adsorb phospholipids and had low bleaching ability.

The purpose of this research was to investigate conditions under which phospholipids could be selectively removed by adsorption onto silica from a hexane miscella leaving the triglycerides in solution. The ultimate goal is to establish a new refining procedure for crude soybean oil.

MATERIALS AND METHODS

Crude soybean oils and lecithin were obtained from Riceland Foods (Stuttgart, Arkansas) and Farmland Industries (Van Buren, Arkansas). After being made into a miscella with HPLC purity solvents, the crude oil was filtered through a one micron ultrafilter to remove meal residue and other insoluble materials.

Our experimental technique involved a batch system with 100 ml miscella being stirred for 15 min at room temperature with 1 g of silica (Silicic acid 100-200 mesh, BioRad Laboratories, Richmond, California). For some of the experiments, the silica was deactivated by pipetting the appropriate amount of water into a flask containing the silica, rotating several times to break up water beads and equilibrating overnight. The miscella was analyzed for phosphorus and crude oil. After 15 min stirring, the silica was allowed to settle, and the miscella was analyzed for residual phosphorus. The amount of phosphorus adsorbed was determined by difference between the original phosphorus and the residual. Isotherm plots were made from the mg phosphorus adsorbed per gram of silica against the mg residual phosphorus per 100 ml miscella.

Phosphorus was analyzed by the method of Bartlett (6) using the Fiske-Subbarrow ammonium molybdate color reaction read at 830 nm on a double-beam spectrophotometer. Data for the adsorption isotherms represent averages of four colorimetric values (replicates in duplicate).

RESULTS AND DISCUSSION

Solvent

Crude soybean oil is extracted with commercial hexane. Although hexane is a good solvent for extracting triglycerides, phospholipids are not very soluble in non-polar solvents. When crude oil is chromatographed on silica, triglycerides moved slightly in hexane while the phospholipids were completely adsorbed. Thus, to enhance this selective adsorption of phosphorus compounds (i.e. phospholipids) on the silica, it was necessary to increase the polarity of the hexane (such as mixing with isopropanol) so that triglycerides would be eluted, leaving the phospholipids adsorbed to the silica.

Figure 1 shows how increasing isopropanol (IPA) in the miscella affected residual phosphorus. With no isopropanol

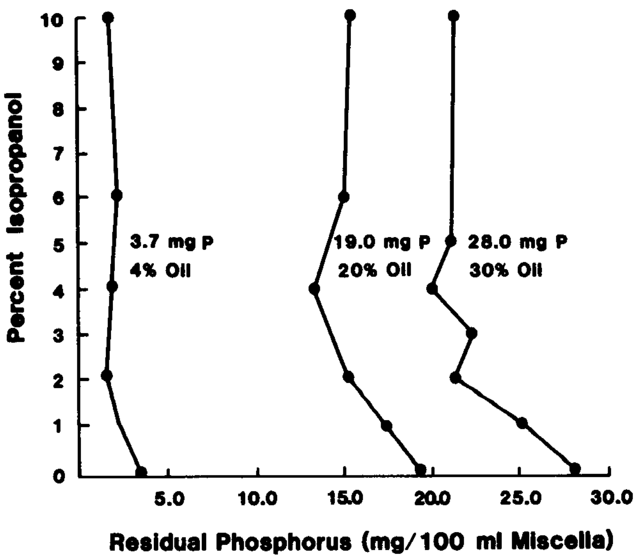

FIG. 1. Effect of increasing IPA level on residual phosphorus of miscellas containing 3 levels of oil and phosphorus.

in the solvent system only a small amount of phosphorus was adsorbed. Addition of isopropanol caused a decrease in residual phosphorus; as the phosphorus adsorbed increases, there is a corresponding decrease in residual phosphorus. Above 2% IPA there was little change in phosphorus adsorption. By testing different concentrations of oil (with 900 ppm phosphorus) in the 100 ml miscella, the values of the 3.7 mg phosphorus with 4% oil, 19.0 mg with 20% oil and 28.0 mg with 30% oil were obtained.

One phenomena consistently observed was that yellow pigment and oil appeared to adsorb to the silica to a greater degree when IPA was not in the solvent (evidenced by more yellow silica after decanting the miscella). Carotene has a shorter retention time than neutral triglycerides on HPLC silica columns eluted with 0.75% (v/v) IPA in hexane (7). We think that the presence of the IPA in the solvent system may prevent the adsorption of the pigment and neutral triglycerides on the silica, thus freeing adsorption sites for phosphorus compounds. This then means more phosphorus being adsorbed. Consequently, in subsequent experiments we used 1% IPA in the hexane miscella.

We found no effect of temperature on phosphorus adsorption on silica. This was in accordance with Freundlich's findings on adsorption isotherms (5). There was about ±10% variability in the amount of phosphorus adsorbed during the reaction time.

Deactivation

The effect of deactivation of silica on the adsorption of phosphorus is shown in Table I. The results show that the amount of phosphorus adsorbed on silica is increased upon the addition of water to the silica. In the first experiment the active silica had adsorbed 3.2 mg phosphorus only, while the 5% deactivated silica adsorbed 60% more (5.5 mg). The amount adsorbed per gram would be even higher when based on the dry weight of the silica. The 15% deactivated silica adsorbed only 1.0 mg more than the amount adsorbed in the 5% deactivated silica. The results obtained in the first experiment were observed in the second experiment using the same level of phosphorus in the miscella. Deactivation of the silica is known to increase the strength of adsorption of polar compounds and to improve chromatography by increasing linear capacity (8). Bleaching earths also are more effective when deactivated (9). On the basis of these results, 5% deactivation was used in our subsequent experiments.

Pattern of Adsorption of Phospholipids on Silica

The adsorption isotherm for phosphorus on 5% deactivated silica is shown in Figure 2. The data were obtained by reacting a constant amount of adsorbent (1 g) with miscellas of different oil concentrations for 15 min. Preliminary experiments showed that adsorption was essentially completed in this time. The adsorption seemed to follow Freundlich's isotherm for affinity between solute and specific adsorbents:

$$\frac{X}{m} = Kc^n$$

where X = amount adsorbed, m = amount of adsorbent, K,n = constants for the adsorbent and c = residual concentration.

When the Freundlich equation was expressed in logarithmic form ($\log x/m = k + n \log c$), a linear plot of the adsorption isotherm was obtained (Fig. 3). The values for K and n are 0.33 and 0.53 respectively, with a correlation coefficient of 0.95. The results show that the Freundlich equation is valid only up to about 17 mg/100 ml original phosphorus; beyond this point the amount adsorbed (about

TABLE I

Effect of Deactivation of Silica on Phosphorus Adsorbed[a]

	Active	Deactivation[b]	
	0	5%	15%
Experiment 1			
Original P (mg)	22.3	21.0	20.1
Adsorbed (mg)	3.2	5.5	6.5
Adsorbed (mg) (per g dry silica)	3.2	5.8	7.6
Experiment 2			
Original P (mg)	14.5	14.5	14.5
Adsorbed (mg)	4.2	4.8	5.2
Adsorbed (mg) (per g dry silica)	4.2	5.1	6.2

[a] 100 ml miscella composed of crude soybean oil in hexane with 1% IPA; reaction time of 15 min; 1 g silica.

[b] Deactivation is expressed as percent moisture added to the silica.

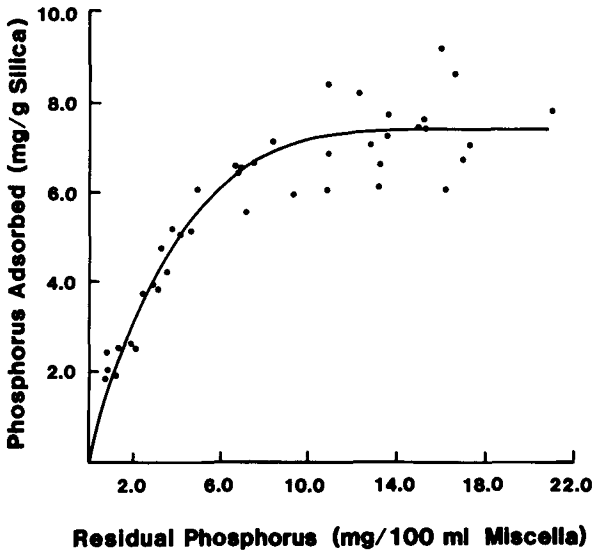

FIG. 2. Isotherm of phosphorus adsorbed on 5% deactivated silica from 1% IPA: hexane miscellas.

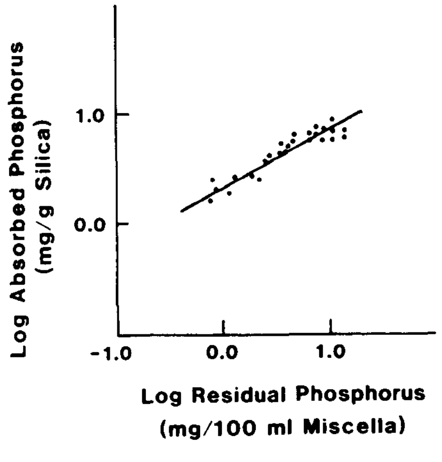

FIG. 3. Adsorption isotherm for phosphorus plotted as log adsorbed vs log residual ($\log x/m = 0.33 + 0.53 \log c; r = 0.95$).

TABLE II

Phosphorus Adsorbed in Sequential Reactions on Silica

	Reaction 1[a]		Reaction 2[b]	
Experiment	P in miscella (mg)	Adsorbed P (mg)	P in miscella (mg)	Additional adsorbed P (mg)
1	18.4	4.3	2.6	1.3
2	11.4	2.1	11.4	2.0
3	25.0	4.1	25.0	.6

[a] Reaction 1: 1 g deactivated silica stirred in 100 ml miscella for 15 min.

[b] Reaction 2: silica from reaction 1 was rinsed with hexane and stirred in 100 ml second miscella for another 15 min.

7 mg/g silica) no longer increases as indicated by the leveling off in the curves (Fig. 2), and by a steady increase in residual phosphorus above 8 mg/100 ml miscella. Subsequently, K increases and n decreases starting in this region of the curve.

This adsorption isotherm pattern, according to Freundlich (5), indicates that an equilibrium has been achieved between the solute in solution and solute absorbed. Such equilibrium is considered reversible, and to test this reversibility of phospholipid adsorption, we reacted one gram of silica with 2 miscellas sequentially. The results of the experiment are shown in Table II. The first experiment involved reacting the silica with a miscella containing originally 18 mg P; 4.3 mg were adsorbed. This silica was then washed with hexane and re-equilibrated with a low phosphorus miscella. We expected a new equilibrium point to be reached with the phosphorus originally adsorbed (4.3 mg) desorbing into the miscella. However, this was not the case. Instead, an additional amount of phosphorus was adsorbed in the second reaction. Other experiments equilibrating the silica with adsorbed phosphorus for the second time with the original miscella resulted in a similar pattern. Regardless of the phosphorus level, the 1 g of silica had the capacity to adsorb more phosphorus. The results shown in Table II indicate that phosphorus adsorption on silica under the conditions of our experiments is irreversible. It is reasonable that the phosphorus does not desorb in 1% IPA-hexane because this solvent will not move phospholipid on thin layer chromatography.

Total amount of phosphorus adsorbed was near 5 mg per gram of silica in these experiments. The isotherm from Figure 2 suggested that the maximum adsorption of phosphorus was near 7.0 mg/g silica, and this was found using miscella with high levels of phosphorus. However, at lower phosphorus levels only a portion of the total P is adsorbed.

Other Solvents

The adsorption of phosphorus on silica from chloroform miscellas was investigated. Figure 4 shows an isotherm with 1% IPA:hexane represented by the line (Fig. 2) and the adsorption pattern of P from chloroform represented by the points (▲). The adsorption of phosphorus from chloroform again followed Freundlich's equilibrium isotherm. It appears that silica adsorbs phosphorus in the same manner from oil in either of the solvents used in this study. Lunde et al. (2) used silica columns to adsorb phospholipids from oil dissolved in chloroform.

Adsorption Isotherms Using Variable Amounts of Adsorbent

Isotherms, especially for oil bleaching, typically are obtained by reacting undiluted oil with varying amounts of adsorbent. Figure 5 compares adsorption patterns of phosphorus on silica obtained in two different ways. Isotherm A was achieved by reacting 1 g of silica with miscellas differing in phosphorus content (same as Fig. 2). The two straight lines (B and C) represent the adsorption patterns of two miscellas reacted with 1, 2 and 3 grams of silica. Miscella B contained 11% oil and 11.0 mg phosphorus, and miscella C contained 20% oil and 20.0 mg P. The result

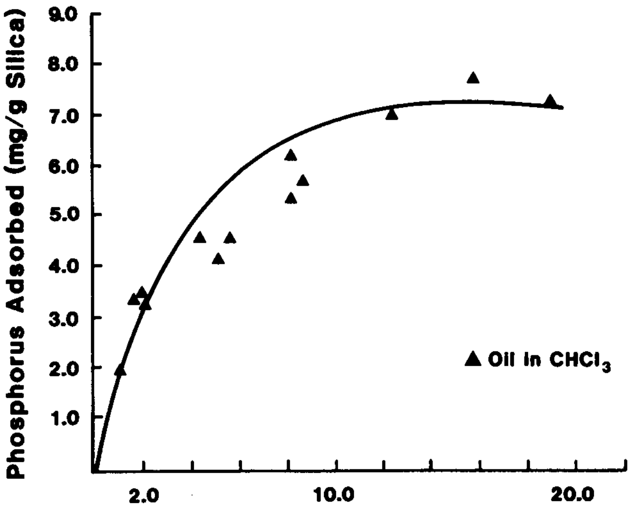

FIG. 4. Adsorption isotherm of phosphorus adsorbed from 1% IPA in hexane miscella (from Fig. 3) (line) and from a chloroform miscella ▲.

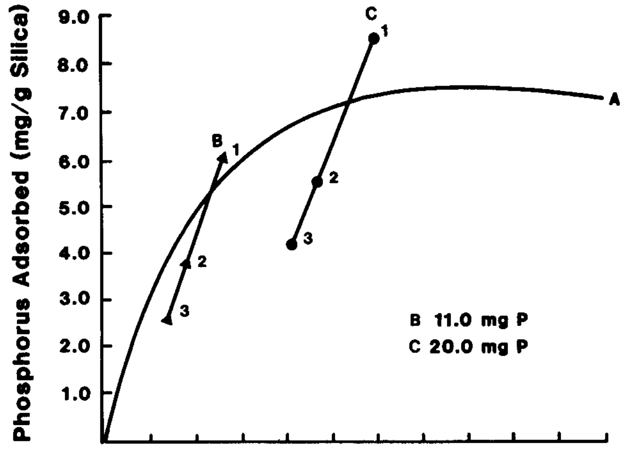

FIG. 5. Adsorption isotherm of P on silica using 1 g of silica (A) and adsorption of P using 1, 2 and 3 g of silica/100 ml miscella (B & C).

TABLE III

Effect of Oil Concentration on Adsorption of Phosphorus on Silica[a]

Oil ppm P	Miscella % oil	Original P (mg/100 ml)	Adsorbed P (mg)
500	20	10.0	3.2
	40	20.0	5.0
1000	10	10.0	3.0
	20	20.0	5.1
	40	40.0	6.9

[a] 1 g of silica reacted for 15 min with 100 ml miscella.

shows that the miscella containing less phosphorus exhibited a pattern (B) similar to the early portion of the isotherm. However, the adsorption pattern of the miscella containing 20 mg P differed widely (C). Although more absolute amounts of phosphorus were being removed from the miscella with increasing amounts of adsorbent, less phosphorus was adsorbed per gram of adsorbent. Thus, as the amount of adsorbent in the miscella increases, the adsorption capacity of a unit of adsorbent decreases. The reason for this behavior is not known. It does appear, however, that there are three isotherms, one for each level of adsorbent.

Effect of Concentration of Oil on Adsorption

Experiments were conducted to see if oil concentration influenced the adsorption of phosphorus. Table III shows how much phosphorus was adsorbed from oils containing 500 and 1000 ppm P. Although it takes two times the amount of the 500 ppm oil to achieve the same phosphorus level as with the 1000 ppm oil, there was no difference in the amount of phosphorus adsorbed based on P content up to 40% oil.

It was concluded that oil concentration in the miscella did not affect phosphorus adsorption below 40% oil. In our preliminary experiments, it was observed that phosphorus adsorption did not occur at around 45% oil. Also, concentrated levels of oil are difficult to pipette for proper sampling and analysis. Consequently, the conclusion by Gutfinger and Letan (3) on the poor adsorptive characteristics of silica for P may be incorrect because their adsorption experiment was conducted on pure oil instead of a dilute oil.

The observed adsorption isotherm pattern conceivably could result from competition by either IPA or triglyceride with phospholipids for sites on the silica. Data on changing the concentration of IPA (Fig. 1) or changing the concentration of oil (Table III) do not support the concept that either is competing with phosphorus for adsorption. Currently, we are studying the rates of P adsorption on silica for further insight into the observation that an equilibrium isotherm pattern exists and yet adsorption of the phosphorus is irreversible.

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[Received June 5, 1984]

Polymorphism of Hydrogenated Canola Oil

AZZA NAGUIB-MOSTAFA and **JOHN M. deMAN**, Department of Food Science,
University of Guelph, Guelph, Ont. Canada N1G 2W1

ABSTRACT

The polymorphism of hydrogenated Canola oil was investigated using X-ray diffraction. The effects of hydrogenation conditions (selective: 200 C; 48 kPa hydrogen pressure, and nonselective: 160 C; 303 kPa) and degree of unsaturation on the transformation $\beta' \rightarrow \beta$ are discussed. A densitometer was used to follow the changes in the relative density of the characteristic short spacings, in an attempt to present a semiquantitative measure of $\beta' \rightarrow \beta$ transformation during storage. The samples studied were selectively and nonselectively hydrogenated Canola oils of iodine values (IV) 70 and 60, respectively. Among the 4 samples, the selectively hydrogenated sample with IV 70 was the most stable and the nonselectively hydrogenated sample with IV 60 the least stable.

INTRODUCTION

As a result of selective breeding, most of the rapeseed grown in Canada now consists of cultivars which are low in erucic acid and glucosinolates. These cultivars and the oil produced from them are designated as Canola. Canola oil differs from high erucic acid rapeseed oil in that the erucic acid has been replaced mainly by oleic acid, thus raising the level of C18 acids to about 95%. This has affected the melting characteristics and polymorphic behavior of hydrogenated Canola oil. Hydrogenated low erucic acid rapeseed oil has been shown to exist mainly in the (β) modification (1). This results in the formation of large crystals, in the range of 5-25 μm, which may impart a grainy texture and cause products to be hard and brittle. A commonly used procedure to prevent the formation of β crystals is the addition of sorbitan esters (2,3). Recently (4), it has been suggested that diglycerides also may be useful in preventing β crystallization.

One of the factors which may influence polymorphic stability is the *trans* content of the hydrogenated oil. It is well known (5) that selective hydrogenation conditions favor the formation of high *trans* levels. In the present study, selectively and nonselectively hydrogenated Canola oils were examined for polymorphic stability by X-ray diffraction analysis.