Removal of Nonhydratable Phospholipids From Soybean Oil[1]

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ABSTRACT

On the basis of the literature concerning the chemical and physicochemical properties of phosphatidic acid and phosphatidates, an experimental investigation of the conditions under which nonhydratable phospholipids (Mg/Ca-phosphatidates) are removable from water degummed soybean oil was carried out. The experiments were carried out by mixing water-degummed oil at different fixed pH values with buffer solutions with and without admixture of Ca^{++}, Mg/Ca-binding reagents and surfactants. The results indicate that the nonhydratable phospholipids can be removed in a chemically nonconverted state as a component of micelles or of mixed emulsifiers. Furthermore, the nonhydratable phospholipids are removable by conversion into dissociated form, i.e., by removal of Mg and Ca from the phosphatidates, which can be accomplished by acidulation or by treatment with Mg/Ca-complexing or Mg/Ca-precipitating reagents. Alkali-refining experiments have shown that removal or chemical conversion of the nonhydratable phospholipids result in reduced emulsion formation and in improved separation of the deacidified oil from the emulsion layer and the soapstock.

INTRODUCTION

The nonhydratable phospholipids in soybean oil are that fraction of the phospholipids which, by repeated stirring of the oil with water, is not transformed into the hydrated, oil insoluble form.

Chemical Composition

Previous investigations in our laboratory (1) have indicated that the greater part of the nonhydratable phospholipids in soybean oil is magnesium and calcium salts of phosphatidic and lysophosphatidic acids. The presence in the nonhydratable phospholipids of Mg and Ca is later confirmed by other investigators (2-4). The hydratable phospholipids in soybean oil differ from the nonhydratable phospholipids by containing a strongly polar group like choline, ethanolamine, serine and inositol, which is ester-bonded to one of the acid groups in the phosphatidic acid (1,5,6).

Chemical Properties of Phosphatidic Acid

The pK_A values for micellar dispersions of phosphatidic acid in water (pK_1 = 3.8 and pK_2 = 8.6) (7,8) imply that phosphatidic acid is undissociated at pH values under 1.8, half-dissociated at pH values of about 6, and fully dissociated at pH values exceeding 10.6. The pK_A values for lysophosphatidic acid are somewhat higher than the values for phosphatidic acid (9).

Determinations of cation-binding stability constants for micelles of phosphatidic acid in water have shown (7-13) that fully dissociated phosphatidic acid is almost completely bonded with magnesium and calcium ions in a molar ratio of nearly 1:1. The binding depends on pH, on the type and on the concentration of other cations. For example, at pH 6 and pH 7, it is roughly 1000 times stronger than the binding to sodium and potassium ions (7-11).

The conditions under which phosphatidic acid and lysophosphatidic acid are undergoing hydrolysis into fatty acids, intramolecular esters and glycerophosphoric acids are described in References 13 through 16. The neutral salts, like the magnesium and calcium salts, are probably more stable than the free acids (14). Phosphatidic acid is probably more stable than lysophosphatidic acid (13).

Physicochemical Properties of Phosphatidic Acid

The literature about the solubility of phosphatidic acid and phosphatidates (7,8,10,12-14,17-19) indicates that if these phospholipids contain unsaturated, long chain fatty acids (as in soybean oil), the solubility has certain characteristics. Undissociated phosphatidic acid is insoluble in water and soluble in both polar and nonpolar organic solvents. Dissociated phosphatidic acid is slightly soluble in polar organic solvents and insoluble in nonpolar organic solvents. Mg/Ca phosphatidates are insoluble in water, soluble in nonpolar organic solvents and insoluble in polar organic solvents. The latter is in agreement with the fact that the content of nonhydratable phospholipids in soybean oil can be reduced substantially by fractionation in cold acetone (4).

Undissociated phosphatidic acid and Mg/Ca-phosphatidates are nonhydratable (1,20). Dissociated phosphatidic acid forms micelles (7,8,10) or liquid crystals (21) in contact with water. The water-binding capability increases with an increasing degree of dissociation (20).

Phosphatidic acid and especially lysophosphatidic acid show greater oil surface activity than the corresponding hydratable phospholipids. The Mg/Ca phosphatidates and the Mg/Ca lysophosphatidates apparently also show great oil surface activity (1).

Content of Nonhydratable Phospholipids in Soybean Oil

With a fixed quality of soybean flakes a relatively low content of nonhydratable phospholipids in water-degummed oil is obtainable by extraction at a low temperature with a low moisture content in the flakes and by extraction with a solvent more polar than hexane, e.g., ethanol-containing benzene (1). From numerous patents and papers (22,23) it appears that it is possible to reduce or remove the content of nonhydratable phospholipids in water-degummed soybean oil by treatment with several reagents, e.g., with organic as well as inorganic acids, with anhydrides, bases, different salts and different adsorbents.

EXPERIMENTAL PROCEDURES

Materials

All of the used lots of water-degummed soybean oil were industrially hexane-extracted. The analytical data for these oils appear in Table I. The pH values and the compositions of the used buffer solutions appear in Table II. The buffer solutions from pH 1 to pH 13 were prepared by dilution of the ampoule standard pH buffers Fixanal from Riedel-deHaen. The cellulose fiber filteraid Solka Floc BW-40 from Brown Co. was used as filteraid for removal of residual buffer solution from the oils after treatment. All other reagents were used as supplied from commercial suppliers. The pancreatin was

[1]Presented at the ISF-AOCS Congress, Chicago, September 1970.

TABLE I

Analytical Data for Different Lots of Water-Degummed Soybean Oil

Oil lot	Production month	After first water-degumming step					After second water-degumming step[a]				
		P, mmole/kg	Mg, mmole/kg	Ca, mmole/kg	Mg/Ca	(Mg+Ca)/P	P, mmole/kg	Mg, mmole/kg	Ca, mmole/kg	Mg/Ca	(Mg+Ca)/P
A[b]	Oct. 1968	—	—	—	—	—	4.36	1.55	2.38	0.65	0.90
B	Dec. 1968	5.22	1.89	2.59	0.73	0.86	4.37	1.61	2.39	0.67	0.92
C	Feb. 1969	5.76	1.85	2.35	0.79	0.73	4.11	1.57	2.00	0.79	0.87
D	Feb. 1969	7.24	2.25	3.35	0.67	0.77	5.92	2.08	3.07	0.68	0.87
E	Mar. 1969	5.48	1.75	2.39	0.73	0.76	4.06	1.43	2.07	0.69	0.86
F	Apr. 1969	5.25	1.65	2.08	0.79	0.71	3.80	1.39	1.79	0.78	0.84
G	Apr. 1969	6.52	2.01	2.74	0.73	0.73	4.99	1.81	2.34	0.77	0.83
H	Jun. 1969	5.21	1.66	2.25	0.74	0.75	4.60	1.60	2.25	0.71	0.84
I	Jul. 1969	6.22	1.76	2.59	0.68	0.70	5.46	1.73	2.60	0.67	0.79
J	Aug. 1969	6.74	1.92	2.83	0.68	0.70	5.56	1.77	2.62	0.68	0.79
K	Sep. 1969	5.88	1.73	2.33	0.74	0.69	4.66	1.62	2.21	0.73	0.82
L	Dec. 1969	5.68	1.73	2.33	0.74	0.71	4.76	1.67	2.21	0.76	0.82
M	Mar. 1970	7.11	2.09	3.05	0.69	0.72	6.47	2.13	3.08	0.69	0.81
Average of oils B-M		6.03	1.86	2.57	0.73	0.74	4.90	1.70	2.39	0.72	0.84

[a]The oils A-G are treated more intensively with water during the second water-degumming step than the oils H-M.

[b]"Oil A" mentioned in Tables IV-IX is identical with "Oil A after the second water-degumming step." After 16 additional water-degumming steps (propeller stirring with water followed by centrifugation) in the laboratory, the P content in "Oil A" is still 4.36 mmole/kg. "Oil A" contains 0.72% free fatty acids.

TABLE II

Composition and pH Values of the Buffer Solutions

Standard pH value,	Composition		Measured pH value[a]	
20 C	Constituents	Concentration, amount per liter	20 C	60 C
"-1"	HCl, concentrated	1000 ml	—	—
0	HCl, 1 N	1000 ml	0.1	—
1	HCl, 1 N KCl	134 ml 3.73 g	1.0	0.9
2	HCl, 1 N NaCl Citric acid, monohydrate	8.2 ml 3.58 g 6.43 g	2.0	1.9
3	NaOH, 1N NaCl Citric acid, monohydrate	20.6 ml 3.49 g 8.47 g	3.0	2.9
4	NaOH, 1 N NaCl Citric acid, monohydrate	68.0 ml 2.57 g 11.76 g	4.0	3.9
5	NaOH, 1 N Citric acid, monohydrate	196.4 ml 20.26 g	5.0	5.0
6	NaOH, 1 N Citric acid, monohydrate	159.6 ml 12.53 g	6.0	5.9
7	Na_2HPO_4, 2 H_2O KH_2PO_4	7.26 g 3.52 g	7.0	7.0
8	HCl, 1 N $Na_2B_4O_7$, 10 H_2O	20.5 ml 4.77 g	8.0	7.8
9	HCl, 1 N $Na_2B_4O_7$, 10 H_2O	4.6 ml 4.77 g	9.0	8.8
10	NaOH, 1N $Na_2B_4O_7$, 10 H_2O	18.3 ml 4.77 g	10.0	9.6
11	NaOH, 1 N NaCl NH_2CH_2COOH	48.9 ml 2.99 g 3.84 g	11.0	9.6
12	NaOH, 1 N NaCl NH_2CH_2COOH	54.5 ml 2.67 g 3.42 g	11.9	10.7
13	NaOH, 1 N NaCl NH_2CH_2COOH	95 ml 0.222 g 0.375 g	13.0	11.6
14	NaOH, 1 N	1000 ml	13.9	12.6

[a]The pH values are measured before mixing the buffer solutions with the oil. After separation of the buffer solutions from the treated oils pH values are found, which deviate less than 0.1 pH value from the values before mixing.

TABLE III

Content of Nonhydratable Phospholipids Before and After Industrial Water-Degumming[a]

Sample	P, mmole/kg	Mg, mmole/kg	Ca, mmole/kg	Mg/Ca	(Mg+Ca)/P	Mass balance[b]
Oil before degumming	16.90	2.84	2.64	1.08	0.32	100.0%
Oil after degumming	5.51	1.74	2.31	0.75	0.74	98.3%
"Lecithin" from the degumming[d]	681	96.3[c]	13.0[c]	7.41	0.16	1.7%

[a]The water-degumming is carried out by stirring with 2% water at 75 C followed by centrifugation.

[b]Calculated on the basis of the P contents.

[c]Determined by atomic absorption after wet incineration.

[d]Consists of the hydrated phospholipids together with entrained oil and entrained nonhydratable phospholipids (1).

a technical product from Novo, Copenhagen. The Naja Naja was purchased from Koch-Light Lab. The soy protein was an edible isolated proteinate. The oleylamine was purchased from E. Merck. The ethoxylated fatty alcohol was a technical lauric-myristic product with an average of 4 moles ethylenoxide per mole fatty alcohol.

Analyses

The content of P in the oils was determined according to AOCS Official Method Ca 12-55. The contents of Mg and Ca were determined by atomic absorption spectrophotometry (24). With a few exceptions all results are the average of at least two determinations. The standard deviations for single determinations are: for P; 0.024 mmole/kg; for Mg; 0.056 mmole/kg; and for Ca; 0.065 mmole/kg.

Mixing Procedure

The separate experiments were carried out by mixing 30 g oil with 240 ml buffer solution in a 1 liter conical flask, which was placed vertically and shaken with 150 double strokes per minute in a shaking thermostat. After shaking, the oil-water mixture was centrifuged, and the separated oil phase was filtrated with a cellulose filteraid, which is without influence on the content of P, Mg and Ca in the oil (4). The used proportion between oil and buffer solution ensures that the pH values of the separate buffer solutions after mixing do not deviate more than 0.1 pH unit from the corresponding pH values before mixing. When it was necessary to adjust the pH value before mixing, this was done by adding a solution of HCl or of NaOH. The mixing time, mixing temperature, the type and concentration of admixtured chemicals for the separate experiments are shown in Tables IV-IX.

RESULTS

The Proportion Between P, Mg and Ca

The proportion Mg/Ca for the examined soybean oils varies after first water-degumming step between 0.67 and 0.79 (Table I). This proportion remains approximately constant for each of the oil lots by additional treatment of the oil with water (Table I) or with different acids and bases (Table IV), salts, complexing agents and adsorbents (4). The proportion is found to be greater in the hydrated phospholipids than in the corresponding nondegummed oil and greater in the nondegummed oil than in the corresponding degummed oil (Table III). The proportion (Mg+Ca):P is theoretically equal to 1 in the phosphatidates and the lysophosphatidates, and equal to 0 in that part of the hydratable phospholipids which contains less than two dissociable acid groups per molecule. Accordingly we have found that (Mg+Ca)/P is very low in the trade product "Soy Lecithin" (Table III), is greater after than before industrial water-degumming (Table III), and is greater after the second than after the first water-degumming step (Table I). After the second water-degumming step the proportion varies between 0.79 and 0.92 (Table I). The proportion depends greatly on the pH value of the water phase (Table IV). Treatment with concentrated hydrochloric acid results in a considerable P content in spite of complete elimination of the Mg and the Ca. With increasing pH value up to pH 10, the proportion increases from 0.0 to 1.0. From pH 10 the proportion shows no significant deviation from 1.0.

Dependence on pH and on Water-Washing

The P content decreases in the alkaline range with increasing alkalinity and in the acid range, except for the treatment with concentrated hydrochloric acid, with in-

TABLE IV

Dependence of Contents of P, Mg and Ca on pH of the Buffer Solution

pH	P,[a] mmole/kg	Mg,[a] mmole/kg	Ca,[a] mmole/kg	Mg/Ca	(Mg+Ca)/P
"-1"	1.88	0.00	0.00	---	0.00
0	0.30	0.01	0.00	---	0.03
1	0.42	0.09	0.11	---	0.48
2	1.02	0.27	0.43	0.63	0.69
3	1.19	0.29	0.48	0.60	0.65
4	2.99	0.85	1.41	0.60	0.76
5	3.87	1.17	1.83	0.64	0.78
6	4.15	1.26	1.96	0.64	0.78
7	4.16	1.26	2.06	0.61	0.80
8	4.18	1.25	2.08	0.60	0.80
9	3.83	1.33	2.16	0.62	0.91
10	2.55	1.02	1.54	0.66	1.00
11	2.00	0.79	1.37	0.58	1.08
12	1.83	0.71	1.07	0.66	0.97
13	1.11	0.42	0.66	0.64	0.97
14	0.00	0.00	0.00	---	---

[a]Determined after mixing of "Oil A" (Table I) with the buffer solutions for 2 hr at 20 C.

TABLE V

Dependence of P Content on pH, on Mixing Time, on Mixing Temperature and on Additional Water Washing

pH	P,[a] mmole/kg			
	No additional water washing			Additional water washing
	20 C 2 hr	20 C 24 hr	60 C 2 hr	20 C 2 hr
"-1"	1.88	1.63	---	0.00
0	0.30	0.39	1.05	0.31
1	0.42	0.40	2.11	---
2	1.02	0.21	2.46	0.93
3	1.19	0.36	2.15	---
4	2.99	0.19	3.48	2.85
5	3.87	0.93	4.00	---
6	4.15	2.85	4.16	3.96
7	4.16	3.55	3.96	---
8	4.18	3.87	3.96	4.12
9	3.83	3.80	3.63	---
10	2.55	2.26	2.72	2.15
11	2.00	1.48[b]	1.98	---
12	1.83[b]	1.06[c]	1.94	1.60
13	1.11[d]	0.14[e]	1.21	---
14	0.00[f]	0.00[g]	0.00	0.00

[a]Determined after mixing of "Oil A" (Table I) with the buffer solutions.

[b]0.00% free fatty acids formed (by saponification liberated as Na soaps).

[c]0.74% free fatty acids formed.

[d]0.07% free fatty acids formed.

[e]2.21% free fatty acids formed.

[f]0.50% free fatty acids formed.

[g]29.08% free fatty acids formed.

creasing acidity (4). The P is eliminated completely after the treatment at pH 14 and almost completely after the treatments at pH 0 and at pH 1 (Table IV).

The P is eliminated completely after treatment with concentrated hydrochloric acid followed by water-washing (4). At the other pH values water-washing does not lead to appreciable diminishing of the P values (Table V).

TABLE VI

Dependence of P Content on Admixture of Na^+ or of Ca^{++}

pH	P,[a] mmole/kg		
	Without additive	Na^+[b]	Ca^{++}[c]
"-1"	1.88	---	---
0	0.30	0.54	0.33
1	0.42	---	0.30
2	1.02	0.51	0.82
3	1.19	---	1.00
4	2.99	3.94	2.13
5	3.87	---	3.92
6	4.15	4.03	4.08
7	4.16	---	3.99
8	4.18	3.21	3.86
9	3.83	---	3.88
10	2.55	2.01	3.85
11	2.00	---	3.74
12	1.83	1.80	3.19
13	1.11	0.77	2.99
14	0.00	---	0.17[d]

[a]Determined after mixing of "Oil A" (Table I) with the buffer solutions for 2 hr at 20 C.

[b]Admixture of 300 g NaCl/liter buffer solution.

[c]Admixture of 1.5 g $CaCl_2$/liter buffer solution.

[d]If the water phase is saturated with $CaCl_2$ instead of containing 0.15% $CaCl_2$, the P content decreases only to 1.67 mmole/kg oil.

Dependence on Mixing Time and on Mixing Temperature

Extension of the mixing time from 2 to 24 hr results, at several pH values, in a pronounced lowering of the P level. After the extended mixing time, the P is eliminated completely at pH 14 and almost completely at pH 13 and at pH values from 0 to 4 (Table V).

Increasing the temperature from 20 to 60 C results in somewhat increased P values in the acid range. In the alkaline range the P contents are nearly unchanged if the pH values are determined at 20 C (Table V). This means that identical buffer solutions result in nearly the same P values at 20 C as at 60 C. Consequently the P values in the

TABLE VII

Dependence of P Content on Admixture of Mg/Ca-Binding Reagents

Admixtured Mg/Ca-binding reagent			Mixing conditions[a]		P,[b] mmole/kg	
Type	Reagent	Concentration	pH	Time, hr	Without additive	With additive
Mg/Ca-complexing reagent	EDTA	Saturated	7	24	3.55	0.80
	EDTA	10%	10[c]	2	2.55	0.00
	EDTA	3.8%	12	24	1.06	0.00
	EDTA	10%	13[c]	2	1.11	0.00
Mg/Ca-precipitating reagents	NaF	Saturated	7	24	3.55	0.25
	Na_2SO_4	Saturated	7	24	3.55	0.38
	Na-citrate	Saturated	7	24	3.55	0.00
	Na-oxalate	Saturated	7	24	3.55	1.33
	Na-tartrate	Saturated	7	24	3.55	0.02
	Na_2CO_3	Saturated	12	2	1.83	0.43
	Na_3PO_4	Saturated	12	2	1.83	0.42
	Na-citrate	0.1%	12	2	1.83	1.71
	Na-oxalate	0.1%	12	2	1.83	1.80
	NaF	Saturated	13	2	1.11	0.04
	Na_2SO_4	20%	13	2	1.11	0.14
	Na_2CO_3	Saturated	13	2	1.11	0.09
	Na_3PO_4	Saturated	13	2	1.11	0.13
	$Na_4P_2O_7$	Saturated	13	2	1.11	0.09
	Na-citrate	Saturated	13	2	1.11	0.04
	Na-oxalate	Saturated	13	2	1.11	0.18
	Na-tartrate	25%	13	2	1.11	0.25

[a]Determined after mixing of "Oil A" (Table I) with the buffer solutions.

[b]Mixing temperature: 20 C.

[c]NH_4^+ -buffer.

alkaline range are lower at 60 C than at 20 C, if the pH values are determined at the mixing temperature.

Dependence on Admixture of Na^+ or of Ca^{++}

Admixture of Na^+ in large concentrations does not lead to substantial diminishing of the P values. Admixture of Ca^{++} results in highly impeded P removal in the alkaline range (Table VI). This influence of Ca^{++} also reveals itself in that the P content in an oil, which is deacidified during a preceding treatment with $Ca(OH)_2$ solution (Table VIII, footnote i), cannot be reduced to the comparative P values (Table IV) by treatment with the corresponding buffer solutions at the same conditions (Table VIII). The effect of Ca^{++} may be partly attributable to the reduced contact between the oil and the water phase, which is the consequence of the formation of fatty acid Ca soaps instead of Na soaps.

Dependence on Admixture of Mg/Ca-Binding Reagents

Complete or almost complete elimination of the P at nonextreme pH values is obtained by admixture of Mg/Ca-complexing agents like EDTA (4), and by admixture until saturation of anions, which form Mg/Ca salts of low solubility products at the concerned pH values. Nonsystematic tested examples of such anions are fluoride, sulfate, carbonate, phosphate, pyrophosphate, oxalate, citrate and tartrate (Table VII).

Dependence on Admixture of Surfactants

With cationic and anionic as well as with nonionic surfactants, the P can be eliminated at pH values where the buffer solutions without additives result in large P values. Results obtained by admixture of oleylamine, fatty acid Na soap, ethoxylated fatty alcohol and different proteins, including phospholipase A containing enzymes, are shown in Table VIII. Since the treatments with the enzymes did not result in sufficiently increased contents of free fatty acids in all the experiments, we cannot explain the demonstrated effects as being due solely to enzymatic hydrolysis. The different P contents at pH 11 (3.64, 2.00, 0.26) (Table VIII) are obtained by treating oils, containing 0.1%, 0.7% and 1.4% fatty acids, with the same buffer solution, which corresponds to treating an oil practically free of fatty acids with buffer solutions of the same pH value and containing increasing amounts of fatty acid Na soap (0.01%, 0.09%, 0.18%).

TABLE VIII

Dependence of P Content on Admixture of Surfactants

Admixtured surfactants			Mixing conditions[a]		P,[b] mmole/kg	
Type	Reagent[c]	Concentration, %	pH	Time, hr	Without additive	With additive
Nonionic	E.F.A.[d]	1.0	0	2	0.30	0.65
Nonionic	E.F.A.	1.0	1	2	0.42	0.49
Nonionic	E.F.A.	1.0	2	2	1.02	0.27
Cationic	Oleylamine	1.0	2	2	1.02	0.00
Nonionic	E.F.A.	1.0	3	2	1.19	0.08
Nonionic	E.F.A.	1.0	4	2	2.99	0.00
Cationic	Oleylamine	1.0	4	2	2.99	0.02
Protein	Casein[e]	2.0	4	2	2.99	1.11
Protein	Casein[e]	2.0	4	24	0.19	0.00
Protein	Pancreatin[f]	0.02	4	24	0.19	0.02
Nonionic	E.F.A.	1.0	5	2	3.87	0.00
Cationic	Oleylamine	1.0	5	2	3.87	0.02
Nonionic	E.F.A.	1.0	6	2	4.15	0.14
Cationic	Oleylamine	1.0	6	2	4.15	0.02
Protein	Pancreatin[g]	0.02	6	24	2.85	0.28
Protein	Naja Naja[f]	0.02	6	24	2.85	0.00
Protein	Soy protein[e]	2.0	6	24	2.85	0.00
Nonionic	E.F.A.	1.0	7	2	4.16	0.50
Nonionic	E.F.A.	1.0	8	2	4.18	2.13
Nonionic	E.F.A.	1.0	9	2	3.83	2.40
Nonionic	E.F.A.	1.0	10	2	2.55	1.96
Nonionic	E.F.A.	1.0	11	2	2.00	1.16
Anionic	Na soap[h]	0.09	11	2	2.00	0.26
Anionic	-Na soap[i]	-0.08[i]	11	2	2.00	3.64
Nonionic	E.F.A.	1.0	12	2	1.83	1.22
Anionic	-Na soap[i]	-0.08[i]	12	2	1.83	3.57
Nonionic	E.F.A.	1.0	13	2	1.11	0.53
Anionic	-Na soap[i]	-0.08[i]	13	2	1.11	3.49
Nonionic	E.F.A.	1.0	14	2	0.00	0.01
Anionic	-Na soap[i]	-0.08[i]	14	2	0.00	2.24

[a]Mixing temperature: 20 C.

[b]Determined after mixing of "Oil A" (Table I) with the buffer solutions.

[c]At pH values exceeding 7 the buffer solution contain, in addition to the stated surfactants, fatty acid Na soaps arising from the natural content of free fatty acids in the oil. At pH values exceeding 10, these Na soaps amounts to 0.009%.

[d]E.F.A., ethoxylated fatty alcohol.

[e]No free fatty acids liberated by hydrolysis.

[f]0.06 Per cent free fatty acids liberated by hydrolysis.

[g]22.4 Per cent free fatty acids liberated by hydrolysis.

[h]Na soaps of destilled soybean fatty acids.

[i]Before mixing with the buffer solutions the content of free fatty acids in the oil is reduced from 0.72% to 0.10% (corresponding to a reduction of the content of Na soap in the water phase from 0.09% to 0.01%, cf. footnote c) by treatment with a saturated $Ca(OH)_2$-solution.

TABLE IX

Behavior of the Nonhydratable Phospholipids, Some Supplementary Experiments

Experiment No.	Treatment of the oil[a]	Analyses, after the treatment
1	Refining in laboratory refining apparatus by stirring for 30 min at 50 C with NaOH solution (pH 13 and pH 14). Washing by sprinkling with water. Separation by standing.	Large emulsion layer. Poor separation of the oil layer from the emulsion layer. Cloudy oil.
2	Shaking 2 hr at 20 C and pH 1. Separation by centrifugation.[b] The oil phase is afterwards treated as described in No. 1.	Small emulsion layer. Distinct separation of the oil layer from the emulsion layer. The oil is less cloudy than in No. 1.
3	Shaking 2 hr at 20 C and pH 1.[c] No separation. The oil-water mixture is afterwards treated as described in No. 1.	The separation is better than in No. 1 and poorer than in No. 2.
4	Shaking 2 hr at 20 C with 1.0 N NaOH saturated with $CaCl_2$. Separation by centrifugation.	1.67 mmole P/kg oil.
5	Shaking 30 min at 20 C with 1.0 N NaOH. No separation. Saturation with $CaCl_2$. Afterwards as described in No. 4[d].	0.18 mmole P/kg oil.
6	Shaking 2 hr at 20 C with 1.0 N NaOH. No separation. Saturation with $CaCl_2$. Afterwards as described in No. 4[d].	0.02 mmole P/kg oil.
7	Shaking 2 hr at 20 C with 1.0 N NaOH. Separation by centrifugation.	0.00 mmole P/kg oil. 0.32 mmole P/kg water phase[e], which corresponds to 2.56 mmole P/kg initial oil[f].
8	Shaking 4 hr at 20 C with 1.0 N NaOH. Separation by centrifugation.	0.00 mmole P/kg oil. 0.50 mmole P/kg water phase[e] which corresponds to 4.00 mmole P/kg initial oil[f].
9	Shaking at 20 C with saturated $Ca(OH)_2$ solution. pH 12.5. Separation by centrifugation.[g] The oil phase is afterwards shaked for 24 hr at pH 11 with NaF-saturated buffer solution. Separation by centrifugation.	0.29 mmole P/kg oil.
10	Shaking 2 hr at 20 C and pH 13 with Na citrate saturated buffer solution[c]. Separation by centrifugation.	0.04 mmole P/kg oil. 0.00 mmole P/kg water phase[e]. Smaller emulsion layer than by the same treatment without admixtured Na citrate.
11	Shaking with the different buffer solutions without additives (Table V) for 2 hr or 24 hr at 20 C. Separation by centrifugation.	The emulsion layer is usually an oil in water layer and it is larger in neutral, weak acidic and weak alkaline buffer solution than in strong acidic and strong alkaline buffer solution.

[a]"Oil A" (Table I).

[b]The nonhydratable phospholipids are thereby almost completely removed from the oil. The content of free fatty acids is unchanged. A fully refined soybean oil to which 0.7% destilled soybean fatty acids are added behaves in a similar way.

[c]The nonhydratable phospholipids are thereby converted into dissociable form.

[d]The aim is to force the unsaponified/unhydrolysed nonhydratable phospholipids back into the oil.

[e]The water phase is clear.

[f]Equal to that amount of the nonhydratable phospholipids in "Oil A before treatment" (4.36 mmole/kg), which occurs as moleculardisperse or micellar soluble P-compounds in the water phase.

[g]The free fatty acids are thereby almost completely removed from the oil. The content of nonhydratable phospholipids is only slightly diminished.

Behavior of the Nonhydratable Phospholipids During the Treatments

After the treatments, the nonhydratable phospholipids are chemically found in a nonconverted state, in an acidulated state, in a dissociated state or in a decomposed (hydrolysed) state. Physicochemically, the P compounds are found either in the oil phase (molecular disperse or micellar), in the "emulsion phase" (as emulsifier or in mesomorphous state) or in the water phase (molecular disperse or micellar). The supplementary experiments carried out to elucidate some of these chemical and physicochemical details are described in Table IX. The conclusions which can be drawn from these experiments appear in the Discussion.

DISCUSSION

Our results are explainable by considering, at the various pH values and for each component of the reaction mixture (i.e., glycerides, phosphatidic acid, lysophosphatidic acid, fatty acids, added salts, added surfactants, Na/Mg/Ca salts of present anions) the effect of the following physicochemical factors: the oil-water distribution coefficient, the degree of dissociation, the solubility product, the rate of hydrolysis and of saponification, and the interface activity, which reveals itself in formation of micelles, solubilizates, mesomorphous phases and emulsions stabilized by simple or mixed emulsifiers.

The corresponding chemical and physicochemical processes proceed with a rate highly dependent on the intensity of the contact between the oil and the water phase, i.e., dependent on the mixing equipment, on the oil-water phase-volume ratio, on the oil-water density difference, on the mixing time, on the mixing temperature and on the presence of interface active agents.

If the nonhydratable phospholipids are considered solely as Mg/Ca phosphatidates, a tentative explanation for the demonstrated effects could be offered: by treatment with

concentrated hydrochloric acid the Mg/Ca phosphatidates are acidulated and thereby converted into undissociated phosphatidic acid, the lipophility of which is so large that a great part of the acid remains in solution in the oil phase. By water-washing, the undissociated phosphatidic acid is converted into dissociated phosphatidic acid, which disappears from the oil phase in the form of micelles in the water phase or in hydrated form as liquid crystals.

By treatment with diluted acid (pH 0-6) the Mg/Ca phosphatidates are acidulated and thereby converted into dissociated phosphatidic acid, which disappears from the oil phase in the form of micelles in the water phase or in hydrated form as liquid crystals. The degree of acidulation increases with increasing contact between the oil and the water phase, i.e., with extended mixing time and with intensified mixing—the latter obtainable by admixture of proteins, of cationic surfactants or of nonionic surfactants. Mixed emulsifiers resulting in formation of oil in water emulsions (25) are possibly formed in the neutral and in the weak acid range.

By treatment with diluted base (pH 10-13) the Mg/Ca phosphatidates are probably able to disappear from the oil phase in the form of a constituent (together with the fatty acid Na soaps) of mixed emulsifiers resulting in formation of oil in water emulsions (25). Admixture of fatty acid Na soaps (Table VIII) or formation of Na soaps by saponification (Table V, footnotes b-g) result in decreased P values. Also, reduction of the content of Na soaps originating from the natural content of free fatty acids in the oil by pretreatment with $Ca(OH)_2$ solution (Table VIII, footnote i), or by admixture of Ca^{++} to the buffer solutions (Table VI) result in less decreased P values.

By treatment with strong base (1 N NaOH) the Mg/Ca-phosphatidates are probably first forming mixed emulsifiers together with the fatty acid Na-soaps and subsequently saponified to Mg/Ca-lysophosphatidate and further on to Mg/Ca-glycerophosphate (Table IX, Expt. 4-8).

The Mg and Ca are removed from the phosphatidates by treatment with Mg/Ca-complexing reagents or with Mg/Ca-precipitating reagents under circumstances (regarding pH and concentration) where the binding between Mg/Ca and the added anions is stronger than the binding of Mg/Ca in the phosphatidates. The Mg/Ca phosphatidates are converted into dissociated phosphatidic acid, which disappears from the oil phase in the form of micelles in the water phase or in hydrated form as liquid crystals.

By NaOH-refining of a water-degummed oil and a corresponding oil containing the same amount of free fatty acids but after removal or chemical conversion of the nonhydratable phospholipids, it is demonstrated that the presence of the nonhydratable phospholipids results in a highly increased amount of emulsion layer and a poorer separation of the oil phase from the emulsion layer and the soapstock (Table IX, Experiments 1-3, and 10).

By treatment with $Ca(OH)_2$ solution, the free fatty acids can be removed without eliminating the nonhydratable phospholipids (Tables VI, VIII, IX) (4).

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