in labeled glycerol activity experimentally observed. The postulate that not glycerol but a precursor, such as dihydroxyacetone (26), is involved in triglyceride and phospholipide resynthesis explains the failure of numerous observers to note the incorporation of more than traces of labeled free glycerol into glycerides when fed with fat or free fatty acids (25, 32, 33, 34, 39). Only that fraction of ingested glycerol which forms the ketone derivative would be available for glyceride synthesis. This concept also explains the failure of glycerol, hydrolyzed from fat during digestion and absorption, to be reutilized (26, 33, 34).

The formation of phospholipide as a product of resynthesized triglycerides resolves the conflict between the observation which clearly demonstrates the incorporation of ingested fatty acids and the rate of turnover studies with P^{32} which indicate that the phospholipides cannot be intermediate in triglyceride synthesis (37).

Finally, the apparent contradiction between the observations that intralumenary lipolysis proceeds only to the monoglyceride stage and the clear-cut evidence that glycerol is lost and not reused during absorption, is resolved.

It is not meant to infer that the results point unequivocally to the above mechanism. For example, all the phospholipides except sphingomyelin were analyzed as a unit, including any phosphatidic acid. Had they been partitioned, it is possible that all the labeled glycerol would have been found in one or the other and could have the same activity as in the triglycerides. This would mean that triglycerides are formed from phospholipides since that conversion involves no dilution of glycerol.

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Soybean "Lecithin" and Its Fractions as Metal-Inactivating Agents[1]

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"LECITHIN" has been used as an antioxidant in edible oils more or less widely ever since its antioxygenic properties were first described by Bollmann in 1923 (2). The phosphatides have since that time been extensively investigated both as a primary antioxidant, as a synergist, and as a metal inactivator in antioxidant mixtures (6, 7, 8, 10, 12, 14, 18, 19). Although there is much evidence showing a positive improvement in the oxidative stability of fats and oils upon phosphatide addition and although lecithin has been approved by the Food and Drug Administration for use in fats, there also have been questions raised as to its protective value (9). The concentration of lecithin employed in studying the oxidative stability of vitamins and edible oils has been varied from its saturation level, about 5% (3, 8), to levels of 0.02% (4). Within these wide concentration ranges, variations in results are to be expected. Insofar as color and flavor characteristics of soybean lecithin are concerned, results obtained are dependent upon the concentrations employed (1, 4). Above 0.02%, adverse color and flavor characteristics limit the use of soybean lecithin.

As a result of studies on the composition of soybean lecithin, a number of fractions of size suitable for oil-stability evaluations have become available. It was believed that individual components of the mixture of phosphatides known as "soybean lecithin" might vary in their color, flavor, and stabilization

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TABLE I

Analysis of Phosphatide Fractions

Preparation	Phosphorus	Nitrogen total	Choline nitrogen	Amino nitrogen	Sugar	Inositol
	%	%	%	%	%	%
Commercial lecithin	1.97	0.78	0.28	......	6.0	2.7
High-sugar fraction	0.99	0.63	0.13	0.29	53.0	1.5
Alcohol-soluble	3.39	1.58	0.92	0.47	......	....
Alcohol-soluble at 50°C.	2.71	1.24	0.13	......	......	....
Alcohol-insoluble	3.50	0.72	0.014	......	2.11 3.23[a]	9.8
Cadmium-precipitated lecithin	3.99	1.73	1.74	0.10	0.14	....
Low-partition-coefficient inositide	3.69	0.65	......	......	0.96	7.5
High-partition-coefficient inositide	3.42	0.77	......	......	......	9.1
Inositol-phosphatidic acids	3.39	0.23	......	......	1.58 2.58[a]	21.6

[a] Refluxed with 2N H_2SO_4, see reference (16).

characteristics and that individual components might have properties superior to those of the mixture. Investigation of phosphatides and their fractions as metal inactivators for soybean oil is the subject of the present communication.

Materials and Methods

Commercial lecithin containing 30% free oil was fractionated in a manner similar to that described by Scholfield *et al.* (16). Reference is made to the flow sheet and Craig distribution analysis in this publication. It is pertinent to note however that evaporation and concentrations were performed at low temperatures and under vacuum in order to avoid decomposition and color development. The cadmium-precipitated lecithin to be described was obtained from the alcohol-soluble fraction. The inositide fractions were obtained from the Craig countercurrent distribution of alcohol-insoluble fraction as indicated on the flow sheet. The low-partition coefficient inositide was obtained from tube 5, and the more hexane-soluble inositide (high-partition coefficient fraction) was obtained in the last tube of a 25-tube countercurrent distribution. The inositol-phosphatidic acids were prepared from a composite of tubes 7 through 13 by precipitation with methanol from a chloroform solution (15). The low-sugar inositide fraction was obtained from tube 6 in a similar distribution of another preparation. The chemical analytical data of Table I and the evaluation data of Table II are presented for the various fractions in the same order as they occur in the flow sheet.

Evaluations of color, oxidative stability, and flavor characteristics were made as follows. Weighed amounts of lecithin or its fractions were dissolved in a *minimum* of ether and added to a kilogram of soybean oil. Deodorizations were carried out in the all-glass, 4-unit deodorizer (17) at 210°C. for 3 hours under a vacuum of less than 1-mm. pressure. At a level of 0.2%, the maximum concentration employed, phosphatide did not foam in the deodorizer. Colors of deodorized oils were determined by the official spectrophotometric method (13). The oxidative stability in the deodorized oil was measured by determining the peroxide development in the oils held under A.O.M. conditions for 8 hours. Flavor evaluation was made by a taste panel of 15-18 people by the published laboratory procedure (11).

Experimental Results

The first section of Table II shows the effect of increasing concentration of commercial soybean lecithin upon color, oxidative stability, initial flavor, and flavor after storage for 4 days at 60°C. These results confirm and extend the previous observations (4). It is apparent that the oxidative stability and initial flavor is significantly improved (over the control) by the addition of 0.05% lecithin. It is also apparent however that the color of the oil has been darkened. Addition of lecithin at the 0.1% level greatly dark-

TABLE II

Effect of Soybean Lecithin and Its Fractions on Color, Oxidative Stability, and Flavor of Soybean Oil

Preparation	Concentration	Color	Peroxide-value A.O.M. conditions 8 hours	Flavor score	
				Initial	4-Day—60°C.
	%				
Control	0.00	1.4	39	7.8	3.5
Commercial lecithin	0.02	1.7	18	8.6	6.3
Commercial lecithin	0.05	3.0	9	8.7	5.8
Commercial lecithin	0.10	6.4	7	8.8	5.6
Control	0.00	1.9	40	7.8	5.9
Commercial lecithin	0.10	4.7	4	8.3	5.6
Low-sugar phosphatides	0.10	3.6	6	8.3	5.0
High-sugar fraction	0.10	2.3	42	....	....
Control	0.00	0.7	27	8.2	5.4
Fraction soluble in alcohol	0.10	5.6	3	7.3	3.5
Fraction soluble in alcohol at 50°C.	0.10	5.6	2	8.5	4.7
Fraction insoluble in alcohol	0.10	4.5	2	8.2	6.0
Low-partition-coefficient inositides	0.10	5.0	2	8.9	4.9
High-partition-coefficient inositides	0.10	5.0	8	7.5	4.9
Inositol-phosphatidic acid	0.08	1.7	6	8.3	5.3
Cadmium-precipitated lecithin	0.10	1.9	7	7.8	4.3
Control	0.00	1.3	32	6.0	4.7
Cadmium-precipitated lecithin	0.005	1.3	33	6.5	4.6
Cadmium-precipitated lecithin	0.01	1.3	32	6.6	5.4
Cadmium-precipitated lecithin	0.02	1.3	28	6.6	5.6
Cadmium-precipitated lecithin	0.05	1.7	20	7.6	6.0
Cadmium-precipitated lecithin	0.10	2.2	11	8.3	5.5
Cadmium-precipitated lecithin	0.20	3.1	10	8.1	4.2

ened the oil but made little or no further improvement in oxidative stability and initial flavor.

The interpretation of flavor scores after 4 days of storage at 60°C. is rather involved in part because the addition of soybean lecithin introduces melony-cucumber off-flavors into the stored oils. Thus a qualitative as well as quantitative difference exists between the control and the lecithin-addition samples. The resulting divergence in assessment of flavor score by the panel members was large on the storage samples, and no statistically significant differences were obtained. Without the assurance of statistical evidence it is hazardous to draw conclusions; however it does appear that an optimal level of action may exist and that this level is in the region of 0.02 to 0.05%.

A survey of the effect of adding various fractions of soybean lecithin upon color, oxidative stability, initial flavor, and flavor after storage is given in the second and third sections of Table II. Their isolation and identification follow closely the flow sheet previously described (16). Chemical analyses of the fractions are recorded in Table I. In Table II, second column, it may be seen that removal of free sugars from commercial soybean lecithin by washing with 55% ethanol improved but slightly the color-darkening defect of soybean lecithin while the oxidative and flavor characteristics are substantially unchanged. The high-sugar fraction, thus removed, by itself had a reduced discoloration tendency; but also this fraction had little or no stabilization characteristics. This finding is in agreement with earlier findings on pure sugars in which neither metal inactivation characteristics nor discoloration effects were observed.

Of the seven additional fractions tested and listed in the third section of Table II, only the last two showed improved color properties. A chemical characteristic in common between cadmium-precipitated lecithin and the inositol-phosphatidic acid, and exclusive to these two samples, is the absence or near absence of amino-nitrogen groups. It therefore appears possible that the discoloration reaction involves amine groups, as was found in the case of the browning of egg oil (5). The evidence presented here as to whether sugar is or is not involved in the discoloration, *e.g.*, the Millard-type, aldehyde-amine browning reaction, is not sufficiently critical to permit a conclusion. Unfortunately, insofar as oxidative stability is concerned, cadmium-precipitated lecithin and the inositol-phosphatidic acid fractions are among the three fractions of lowest activity.

All phosphatide fractions tested to date have the common property of improving the initial flavor to give bland oils, but they also cause the development of cucumber-melony flavors on storage. It would appear therefore that phosphatides constitute precursors of these flavors. Phosphoric acid-treated oils also develop these same flavors on storage, and therefore the phosphate radical appears to be the flavor-producing moiety of phosphatides.

Since the cadmium-precipitated lecithin can be produced in comparatively large amounts and appeared to constitute a promising fat-soluble metal deactivator of natural origin, a more detailed study of the influence of concentration was made. As shown in the last column of Table II, the discoloration tendencies of cadmium-precipitated lecithin were detected at 0.05% and higher levels compared to 0.02% for the unfractionated phosphatides. At the 0.05% level oxidative stability and initial flavor were improved, and the flavor after storage appeared to reach a maximum.

Summary

The addition prior to deodorization of 0.1% of either crude phosphatides, or the alcohol-soluble, or the alcohol-insoluble fraction all improved the oxidative stability and the initial flavor of soybean salad oil. However all three additives caused significant darkening of the oils and the introduction of undesirable storage flavors when added at levels which improved the oxidative stability.

High-sugar fractions from the crude phosphatides did not darken the oil nor did they confer improved oxidative or flavor characteristics.

Cadmium-precipitated lecithin and inositol-phosphatidic acids containing no amino nitrogen gave lower color to salad oils upon deodorization than did the amino-nitrogen-containing phosphatides.

Purified cadmium-precipitated lecithin had little effect upon the oxidative stability when added at levels below 0.02%. A significant improvement results from the addition of 0.05%, and oxidative stability shows further improvement by raising the level to 0.1%; however no increase in stability was obtained by raising of the concentration above this level.

At concentrations of 0.01 and 0.05%, cadmium-precipitated lecithin had little effect on the color of the oil. At levels of 0.1 and 0.2%, significant darkening of the oils occurred though much less than with the amino-nitrogen-containing phosphatides.

Based on the flavor responses of oils to which these phosphatides were added, it appears that phosphatides constitute the precursors for the melony, bitter, cucumber flavors frequently encountered in aged soybean salad oils. These flavor responses are the same as those obtained from added phosphoric acid.

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