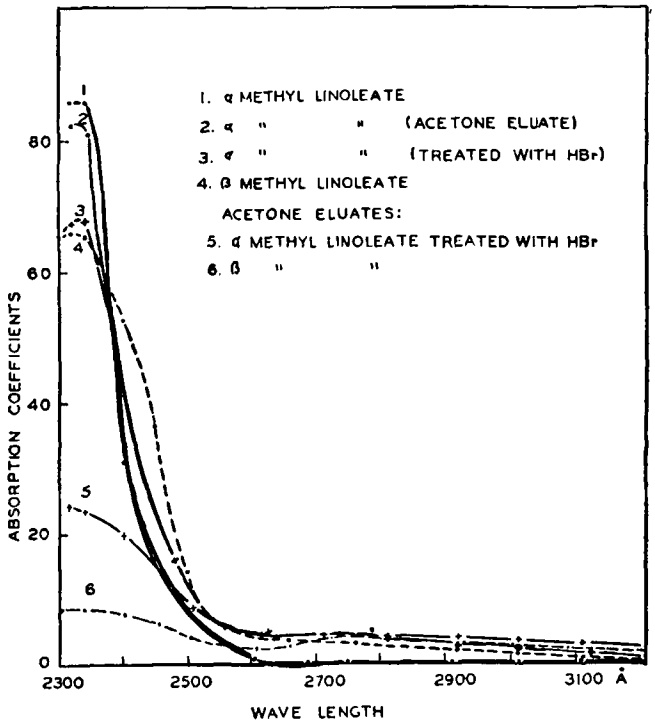

FIG. 2. Relationship between wave lengths and absorption coefficients of the α- and β-methyl esters.

Summary

Methyl esters of α- and β-linoleic acid were chromatographed on activated alumina and separated into fractions eluted with Skellysolve F and acetone, respectively. Methyl α-linoleate was found to be chromatographically homogeneous in character while methyl β-linoleate was not. The latter contained a small amount of yellow viscous material which appeared to be monobromooleic acid; this bromine could not be removed by treatment with zinc in absolute alcohol. A similar substance could be isolated from methyl α-linoleate through which bromine-free, hydrogen bromide had been bubbled for two hours at 0° C. The presence of this bromine containing substance was shown to be partly responsible for the differences in the characteristics of α- and β-linoleic acid.

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A Study of Lipids in Commercial Feeds

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Introduction

THE present paper is an investigation of the total extract, phospholipid, unsaponifiable matter, and total fatty acids of cottonseed meal, soybean meal, meat and bone scraps, wheat gray shorts with screenings, alfalfa leaf meal, whole barley, and whole oats in the anhydrous ether and 3 + 1 alcohol-ether extracts. It also includes a study of the phosphorus in the alcohol-ether extract, and a method is proposed for the determination of phospholipids in plant material. The samples were selected at random from those collected by the Feed Control Service of the Texas Agricultural Experiment Station for routine analyses.

It has long been recognized that ether alone does not completely extract lipid matter, especially phospholipid, from biological matter (1) but that alcohol and ether remove them more completely (2, 3). A number of methods of extraction have been recommended, most of which involve the use of hot alcohol plus some fat solvent. Mixtures of alcohol with ether (4), chloroform (5), or benzene (6) have been used. The function of the alcohol is two-fold. It has a higher penetrating power than a solvent not miscible with water, and it "breaks" insoluble lipid-protein complexes unaffected by unmodified fat solvents. The addition of ether, chloroform, or benzene gives added fat-solvent power to the mixture.

A 3 + 1 alcohol-ether mixture is the most commonly used solvent for the extraction of lipids from animal tissues, especially for micromethods (7), and a similar mixture was found by Guerrant (4) to be most effective for the extraction of lipid phosphorus from seeds. Bornmann (5) found that Hertwig's (8) alcohol-ether method for the extraction of "lipoids" from cereal products and eggs was incomplete. It should be pointed out, however, that in the latter method a 10-gm. sample was heated with only 30 ml. of 70% alcohol, then 55 ml. of unheated 95% alcohol, and finally 85 ml. of ether added. Thus the ratio of

sample to hot alcohol was too high (4, 9) and the ratio of alcohol to ether too low (4, 10).

No claim is made that the extraction of lipids from feeds in the present study is absolutely complete. No effort was made to find such a method.

The methods for and results of the determination of phospholipids are as numerous as the investigators. Phospholipids have been precipitated in acetone and weighed (11, 12); they have been calculated from the phosphorus content of crude alcohol-ether (13) and ether extracts (12, 14) with (15) and without (16, 17, 11) precautions against oxidation, although it has been repeatedly shown (18, 19, 20) that the latter treatment renders phospholipids insoluble in fat solvents.

Methods

A. *Alcohol-ether extraction.*

1. Separation of lipid from sample. Twenty-five grams of cottonseed meal or 10 gms. of meat and bone scraps were transferred to 300-ml. round bottom flasks and 100 ml. of 3 + 1 alcohol-ether added. The mixture was refluxed for 1 hour and filtered through a fritted glass filter with suction. The alcohol-ether extraction was repeated once followed by 3-10-minute extractions with 100 ml. volumes of anhydrous ether. The extracts were evaporated in turn to a small volume in a 400-ml. beaker. After the last extraction the combined extracts were evaporated almost to dryness and re-extracted 5 times with boiling anhydrous ether. The re-extracts were filtered through a fritted glass filter into a tared 125-ml. Erlenmeyer flask, evaporated to dryness in an oven at 90° and weighed. The constituent lipids were then determined as will be described below.

2. Extraction in a fixed volume. Five grams of sample were transferred to a 100-ml. volumetric flask calibrated at 103.4 ml.* and 60 ml. of 95% alcohol added. The mixture was brought to about 70° in hot water and then placed on the hot water bath out of contact with steam for one-half hour. To the hot mixture 25 ml. of ethyl ether were carefully added, mixing well after each few ml. The flask was marked at the volume and then shaken in a mechanical shaker for 15 minutes. Ethyl ether was then added to the mark to replace loss by evaporation, the flask cooled to room temperature, made to the 103.4-ml. mark with 95% alcohol and well mixed by inversion. The solution was filtered or centrifuged. An aliquot was removed for phospholipid determination. Total lipid, non-saponifiable matter, and total fatty acids were determined on a large aliquot of the remainder.

B. *Phospholipid.*

An aliquot containing approximately 0.05 mg. phosphorus was transferred to a 1" x 6" pyrex test tube calibrated at 15 ml. and evaporated to dryness, it being made certain that all odor of solvent had disappeared. To the residue 0.4 ml. of 60% perchloric acid and 3 drops of nitric acid were added and the mixture heated over a micro burner, with care to avoid splattering, until the solution was water-clear. It was occasionally necessary to add more nitric acid, one or two drops at a time. After cooling, 5 ml. of water and 1 drop of phenolphthalein solution were added, the solution titrated to alkalinity with 0.5 N sodium hydroxide and 1.5 ml. of 5 N sulfuric acid added. The solution was made to volume (15 ml.) with water, 1 ml. of 2.5% ammonium molybdate and 0.5 ml. of 1,2,4-aminonaptholsulfonic acid reducing agent added (22) and the color determined after 10 minutes.

C. *Total extract.*

A large aliquot, usually about 75% of the total alcohol-ether extract, was evaporated to dryness in a beaker and re-extracted 5 or 6 times with boiling ethyl ether. The extract was cleared by centrifugation or filtered through a small cotton plug into a tared 100- or 125-ml. Erlenmeyer flask, evaporated to dryness on the hot water bath, dried in the oven at less than 100° C. and weighed.

In case of the official ether extract the aliquot was evaporated and weighed.

D. *Unsaponifiable matter.*

One ml. of 50% potassium hydroxide solution and 5 ml. of 95% alcohol were added to the weighed total extract and heated on the hot water bath, out of contact with active steam, for one hour, the solution being kept at 5 ml. Two ml. of water were added and the mixture extracted with petroleum ether. All pigments remained in the dilute alcohol layer except some slight amounts in the case of alfalfa meal. There was no trouble with emulsions.

Four 15-ml. volumes of petroleum ether, 30°-60° boiling range, were used. Each extract was evaporated in turn in a 50-ml. conical bottom centrifuge tube. After final evaporation to complete dryness the residue was extracted with petroleum ether. Appreciable quantities of soap remained undissolved, having been carried into the ether solution with alcohol. The final petroleum ether solution, cleared by centrifugation, was transferred to tared 50-ml. beakers by decantation, evaporated to dryness, and weighed.

E. *Total fatty acids.*

Five ml. of 1 + 1 hydrochloric acid were added to the centrifuge tube containing soap residue of the petroleum ether extract and heated in the hot water bath for several minutes The acid solution was transferred to the bulk of the soaps in the saponification flask, the tube being washed with petroleum ether. The acidified mixture was heated on the water bath several minutes and 15 to 20 ml. of petroleum ether added. In many cases there was still some insoluble material in the ether layer. This layer was, therefore, transferred by decantation to a 50-ml. conical bottom centrifuge tube. After 3 extractions of 15 ml. each the solution was centrifuged and the clear supernatant solution decanted into a tared 100- or 125-ml. Erlenmeyer flask. The centrifugate was dissolved in 2-1 ml. portions of hot alcohol and transferred back to the acidified solution. This solution was extracted twice more with 15-ml. portions of petroleum ether, which were now clear. The extracts were added to the first three, evaporated to dryness, and dried in the oven at 90° to constant weight.

As in the extraction of unsaponifiable matter the pigments remained in the dilute alcohol layer except in the case of alfalfa leaf meal. Ethyl ether, however would extract the pigment from the alcohol layer.

Notes on Methods

A. *Extraction of total lipid.*

In the earliest experiments the alcohol-ether extracts were washed from the samples, isolated, and

* It was found that 5 gms. of most samples occupied 3.4 ml.

weighed. However, because of the manipulative difficulties and attendant errors, the time required and the effect of isolation on the phospholipid, a procedure was devised to effect the extraction in a fixed volume. Within the limits of experimental error the method of making the sample to a fixed volume resulted in as complete extraction as the washing procedure. The fixed volume method, therefore, was used for all alcohol-ether extractions in the present study.

B. *Official 16-hour anhydrous ether extractions* (21).

The alcohol-ether method of extraction was compared with the official 16-hour anhydrous ether procedure routinely used in feed control laboratories. The extracted lipids were partitioned into phospholipid, unsaponifiable matter, and total fatty acid in order to determine the fractions responsible for any differences in the two methods. These are compared in Table I.

C. *Phospholipid.*

The method recently reviewed by Bloor (7) for the microdetermination of phospholipid by precipitation of the magnesium chloride salt in acetone and the subsequent determination of all or some constituent of the precipitated phospholipid is almost universally followed, at least for animal tissues. In our hands, in the analyses of commercial feeds, this method gave what appeared to be too low results. It is possible that a large portion of the phospholipid was made insoluble in petroleum ether during the evaporation of alcohol-ether. The numerous manipulations may also result in possible losses. If it could be shown, therefore, that the total alcohol-ether soluble phosphorus is lipid phosphorus, these losses in accuracy and time could be avoided.

The method used to show this was similar to that reported by Man (18). Aliquots of the alcohol-ether extracts of a number of different kinds of commercial feeds were analyzed for total phosphorus in the manner described below. Similar aliquots were evaporated almost to dryness in a 1" x 6" test tube under reduced pressure in a slow stream of illuminating gas at a temperature of less than 70° C. The residues were extracted 4 times with 30°-60° boiling petroleum ether. The clear (centrifuged) extracts were evaporated in turn and finally to dryness. Total phosphorus was determined on the residue. The results (Table II) show that, with the exception of meat and bone scraps, from 95% to 99% of the total alcohol-ether soluble phosphorus could be recovered in the petroleum ether re-extract. The small loss must be assumed to be due to some unavoidable oxidation and experimental error. There may be some question in the case of meat and bone scraps in which case the loss was 11.8%. It was concluded, therefore, that the phosphorus of the alcohol-ether extract of cottonseed meal, soybean meal, barley, oats, alfalfa leaf meal, wheat gray shorts, and possibly meat and bone scraps, is lipid phosphorus.

Phosphorus determinations were also made on the official 16-hour ether extract. The results are given in Table I, where they are contrasted to those of the alcohol-ether extract.

The determination of phosphorus by the method of Fiske and Subbarow (22) without modification was found impractical because of the large amount of lipid material containing approximately 0.05 mg. of phosphorus. The quantity of sulfuric acid was insufficient for the digestion. The method of King (23) in which perchloric acid is substituted for sulfuric offered a solution to this problem. It was found, however, that the loss of perchloric acid during the digestion varied greatly, resulting in different pH values of the resultant solution. This was finally controlled by neutralizing the residual perchloric acid, after digestion, with sodium hydroxide and adding a fixed amount of sulfuric acid to the neutralized solution.

TABLE I.

The Lipids of Commercial Feeds [1] as Obtained by the 16-Hour Anhydrous Ether Extraction and by Alcohol-Ether Extraction in a Fixed Volume.

	Total Extract [2]		Lipid Phosphorus		Unsaponifiable		Total Fatty Acids		Non-Phospholipid [2]	
	Ether	Alc.-Ether	Ether	Alc.-Ether [2]	Ether	Alc.-Ether [2]	Ether	Alc.-Ether [2]	Ether	Alc.-Ether
Cottonseed meal	%	%	*p.p.m.*	*p.p.m.*	%	%	%	%	%	%
Average	6.37	8.97	54	722	0.134	0.127	5.87	7.41	5.76	6.19
Minimum	5.42	8.05	43	710	0.105	0.097	4.97	6.76	4.93	5.58
Maximum	7.37	9.42	65	740	0.195	0.163	6.64	7.70	6.49	6.47
Soybean meal										
Average	2.88	6.72	81	685	0.119	0.115	2.67	3.49	2.42	2.26
Minimum	1.00	3.11	64	562	0.062	0.073	0.52	0.61	0.34	0.56
Maximum	6.36	10.25	127	787	0.165	0.200	4.36	6.23	4.25	4.89
Meat and bone scraps										
Average	11.1	13.5	131	518	0.222	0.234	10.2	11.2	9.7	10.1
Minimum	4.5	6.7	14	327	0.145	0.178	4.0	5.2	3.49	4.18
Maximum	13.5	15.9	300	606	0.324	0.300	12.5	13.4	12.1	12.8
Wheat gray shorts										
Average	3.64	4.39	36	261	0.361	0.244	3.13	3.96	3.06	3.49
Minimum	2.98	3.90	36	241	0.280	0.174	2.56	3.50	2.85	2.24
Maximum	4.13	5.10	37	298	0.384	0.299	3.63	4.34	3.57	3.86
Alfalfa leaf meal										
Average	4.89	5.75	146	259	0.908	0.811	2.31	2.40	2.05	1.93
Minimum	2.64	3.30	12	41	0.585	0.683	1.34	1.47	2.12	1.39
Maximum	6.68	7.92	262	510	0.090	0.830	2.81	3.04	2.61	2.19
Whole barley, ground										
Average	2.20	2.29	10	94	0.156	0.199	1.65	1.66	1.63	1.49
Minimum	2.13	1.93	8	53	0.106	0.182	1.52	1.39	1.52	1.30
Maximum	2.45	2.57	12	177	0.213	0.233	1.73	2.27	1.73	1.97
Whole oats, ground										
Average	5.05	5.61	49	88	0.290	0.029	3.70	4.43	3.61	4.27
Minimum	4.33	5.07	37	63	0.202	0.020	2.86	3.69	2.80	3.49
Maximum	5.53	5.87	69	119	0.460	0.035	4.35	4.98	4.23	4.87

[1] Six samples of each type of feed were analyzed except five of barley.
[2] These figures represent the ethyl ether soluble fraction of the alcohol-ether extract.

TABLE II.

The Lipid Phosphorus Content of Commercial Feeds as Determined in the Total Alcohol-Ether Extract and Its Petroleum Ether Soluble Fraction.[1]

	Lipid Phosphorus	
	Alcohol-Ether Extract	Petroleum Ether Soluble Fraction
	p.p.m.	*p.p.m.*
Cottonseed meal	722	700
Soybean meal	685	665
Meat and bone scraps	384	339
Wheat gray shorts	261	257
Alfalfa leaf meal	259	248
Whole barley	94	96
Whole oats	88	84

[1] The values are the average of six samples except five for barley.

D. *Unsaponifiable matter.*

In early determinations the alcohol was evaporated almost to dryness, about 2 ml. of water added and the mixture extracted with petroleum ether. Difficulties were encountered in separating the layers because of emulsions. It was also noted that some pigment material was partially extracted. The use of ethyl ether was no better, the only difference being that somewhat larger portions of the pigments appeared in the ether layer. To circumvent these difficulties the saponification mixture was kept at 5 ml. with 95% alcohol during saponification.

E. *Total fatty acids.*

In the original procedure more difficulties were encountered in extracting the fatty acids from the aqueous acidified saponification mixture than in extracting the unsaponifiable matter. There was no trouble with emulsions, but in almost every case there was considerable amorphous material insoluble in either the aqueous or ether layers but which floated in the latter or settled at the interphase. This material did not appear to be soap since heating and standing on the acid solution did not affect it.

It was found that this material was partially soluble in ethyl ether, but if alcohol was added to the aqueous solution to 50% or more it completely dissolved in that layer. If the alcoholic concentration appreciably exceeded 50%, potassium sulfate precipitated. The latter was partially corrected by decomposing the soaps with hydrochloric acid, potassium chloride being considerably more soluble than potassium sulfate in the dilute alcohol.

Discussion

The procedure of making a sample to a fixed volume and removing aliquots for determination of constituents is common for liquid or soluble samples. Its use for such determinations as concern us here may require some experimental justification. The average total extracts of six samples of cottonseed meal were 8.9% and 9.0%, respectively, for the fixed volume and washing procedures. For six samples of meat and bone scraps the corresponding values were 12.2% and 12.7%. The total fatty acids of the cottonseed meals were 7.0% and 7.4% and of meat and bone scraps 9.8% and 11.2% by the two methods, respectively. The fixed volume method was used, therefore, to obtain a comparison of the total extract, phospholipid, unsaponifiable matter, and total fatty acids in the anhydrous ether and the alcohol-ether extracts (Table I). The nonphospholipid fatty acids also given in this table are calculated from the total fatty acid and the lipid phosphorus. Phospholipid fatty acids were considered as 17 times the lipid phosphorus, their approximate relation in lecithin. The average values of each constituent as obtained by the two methods were calculated. From these average values it is seen that the percentage of total alcohol-ether extract is consistently higher than that of the ether extract. (This "alcohol-ether extract" is, in reality, a clear ethyl ether re-extract of the alcohol-ether extract.)

The amount of ether soluble material extracted by the alcohol-ether in excess of that extracted by the anhydrous ether varies. Thus in soybean meal it is 73% greater while in oats and barley it is questionable whether the differences are significant, being 11% and 4%, respectively.

In order to determine which fractions were extracted in larger quantities by the alcohol-ether solution the different lipid fractions are compared in Table III. Phospholipid was calculated from the lipid phosphorus by multiplying by 25. Triglycerides were calculated by multiplying the non-phospholipid fatty acids by 1.04. The difference between the total extract and the sum of phospholipids, triglycerides, and unsaponifiable matter is called the undetermined fraction. This table shows that phospholipids and the undetermined fraction are the ones extracted in greater amounts.

The total extracts of wheat gray shorts, both ether or alcohol-ether, were completely accounted for by the constituents. This is also true of the ether extract of cottonseed meal and meat and bone scraps although the alcohol-ether extracts of these two feeds contained about 10% unaccountable material. In soybean meal and alfalfa leaf meal, however, approximately one-third of the total extracts cannot be accounted for in the determined fractions. The unaccountable fraction in barley and oats is about 13% and 15%, respectively, of the total extracts.

The term "unsaponifiable matter," as used here, requires some elucidation. It is the petroleum ether soluble material extractable from the alcoholic saponification mixture. Some materials such as pigments which, in other methods of determination (32), might be extracted from more dilute alcohol, remain in the more concentrated alcohol of this procedure. Ethyl alcohol extracts the pigments from the acidified mixture after the extraction of fatty acids by petroleum ether. However, it was not considered profitable to make that additional determination.

It is probable that in the case of soybean meal some of the difference between total extract and the sum of the constituents is due to the fact (24) that a fraction of the phospholipid is lipositol rather than lecithin, the inositol remaining in the dilute alcohol layer.

If it be considered that the most important reasons for determining the percentage of lipids in a feed is to obtain a measure of its calorie value, the official anhydrous ether extraction is more valuable than the alcohol-ether extraction since it gives a closer approximation to the fatty acid content. But this may also be misleading as, for example, in alfalfa leaf meal in which case the ether extract is almost 2½ times the amount of fatty acids.

The determination of phospholipid in these materials deserves some special discussion. There are comparatively few reports on the phospholipid content of plant materials, and each report is the product of a different procedure. Thus Rewald (25) found 0.58%

TABLE III.

Relation of Total Extracts to the Determined Constituents.[1]

	Phospholipid [2]	Triglycerides [3]	Unsaponifiable	Sum of Lipids	Total Extract	Undetermined
Cottonseed meal	%	%	%	%	%	%
Ether extract	0.134	6.03	0.134	6.29	6.37	0.08
Alcohol-ether extract	1.80	6.35	0.127	8.27	8.97	0.70
Soybean meal						
Ether extract	0.202	2.52	0.119	2.84	3.88	1.04
Alcohol-ether extract	1.71	2.35	0.115	4.17	6.72	2.00
Meat and bone scraps						
Ether extract	0.75	10.10	0.115	10.96	11.2	0.24
Alcohol-ether extract	1.50	10.50	0.222	12.22	13.5	1.28
Alfalfa leaf meal						
Ether extract	0.365	2.13	0.908	3.40	4.89	1.49
Alcohol-ether extract	0.648	2.00	0.811	3.46	5.75	2.29
Wheat gray shorts						
Ether extract	0.091	3.18	0.361	3.63	3.64	0.01
Alcohol-ether extract	0.652	3.62	0.244	4.52	4.39	0.0
Barley						
Ether extract	0.025	1.70	0.156	1.88	2.20	0.32
Alcohol-ether extract	0.235	1.55	0.199	1.98	2.29	0.31
Oats						
Ether extract	0.120	3.75	0.290	4.16	5.05	0.89
Alcohol-ether extract	0.220	4.45	0.293	4.96	5.61	0.65

[1] The values represent the averages of six samples except five for barley.
[2] Lipid phosphorus × 25.
[3] Non-phospholipid fatty acid × 1.04.

phospholipid as lecithin in cottonseed press cake by extracting the the sample with petroleum ether and then repeating with 20:80 alcohol-benzene mixture. The solvents were evaporated, the dry residue re-extracted with petroleum ether and the phospholipids twice precipitated with acetone and weighed. Rewald found 6 to 8% phospholipid in the oil expressed from the seeds and very little in the oil left in the cakes.

Hayward (33) found 1.67 and 1.62% phospholipids in solvent and "old process" soybean oil meals respectively by extracting the meals with a 85:15 gasoline-96% alcohol mixture. Kraybill (34) has reported more phospholipid in solvent than in press extracted soybean oil meals. Conversely this may be compared to the work of Goldovski and Lischkevitsch (26) who concluded that expressed sunflower seed, cottonseed, and ground nut seed oils contain 0.031 to 0.06% phosphatide, but the same oils obtained by extraction with light petroleum contain 0.2 to 0.4%. The latter statement is more in agreement with the finding of Guillemot and Reiss (27) that the lipids of peanut press cake contain a much higher percentage of phospholipids than the expressed oil.

Diemar, Bleyer, and Schmidt (15) extracted barley, wheat, and oats with acetone. After drying the residue, it was extracted with alcohol, benzene, alcohol-benzene 4:1, and alcohol-benzene 1:4 in turn. The solvents were evaporated in a vacuum in a stream of nitrogen, redissolved in peroxide free ether, precipitated with acetone, and weighed. They found 0.16, 0.12, and 0.14% phospholipid in the barley, wheat, and oats respectively. This is low as compared to 0.235% and 0.220% for barley and oats respectively in the present work.

The most ambitious attempt to determine the phospholipid content of seeds is that of Guerrant (13). This author, after trials to determine the best proportions, extracted a series of seeds with 4 + 1 alcohol-ether at 30° C. for 2 hours, shook, allowed to settle or centrifugated, and analyzed the clear supernatant for phosphorus. Among many other results he obtained 0.0442, 0.0388, and 0.0348% lipid phosphorus for barley and 2 samples of oats respectively. Upon multiplying by 25, these figures become 1.10, 0.97, and 0.87% phospholipid.

This method of analyses is strikingly similar to that used in the present investigation except that the extraction is at the low temperature of 30° C. Although it would appear that the method of extraction is too mild, the results are approximately 5 times that of the present method and 8 to 10 times that of Diemar *et al.* Guerrant's analyses, however, were of fresh seeds while the present analyses are of commercial feeds ground and stored for an unknown period.

Rewald (11) found that about 0.5% phospholipid in grass as the sum of the phosphorus content of the acetone, petroleum ether, and alcohol-benzene extracts evaporated and re-extracted. Shorland (12) found about 0.2% in dried cocksfoot grass and 1.5 to 1.7% in fresh.

The results of phospholipid determinations in plant materials are thus as numerous as the authors, each of whom used a different procedure. The present author agrees with others (18, 19) that any method that involves the drying in air and re-extraction of phospholipid from the extracted material will give low results. Thus, in early experiments in the present work the alcohol-ether extracts of six samples of cottonseed meal were evaporated just to dryness, re-extracted with petroleum ether, the phospholipids precipitated as the magnesium chloride salt, and phosphorus analyses made of the entire precipitates. The average phospholipid value was 0.73% as compared to 1.8% by the method finally adopted.

The gravimetric determination of the isolated phospholipid would appear to be in error in the other direction. While the quantitative precipitation of phospholipid in acetone is possible as the magnesium or strontium chloride salt (28, 29), it probably is not otherwise. Furthermore, the precipitated material is highly hygroscopic, and very special technic would have to be adopted to obtain and weigh it dry. Finally, it is doubtful if the precipitated material is pure.

The practice of precipitating phospholipid as the magnesium chloride salt before its determination is due to the influence of the accepted methods by which the isolated material is oxidized and the products of the oxidation measured (30) or the quantity of di-

chromate used for the oxidation determined (31). Another reason, however, is the difficulty of digesting the comparatively large fat samples containing sufficient phosphorus for a reliable determination. This is especially true of most plant extracts which contain a lower phospholipid content than animal extracts. The author has found the use of sulfuric acid impractical and finally devised the perchloric acid procedure described above. Contrary to King (23), a high acidity did not obviate the necessity of controlling pH, nor could this be accomplished by using fixed amounts of acid (perchloric) before digestion. Consistent results were obtained and maximum color developed after 5 minutes by neutralizing the digestion mixture and adding sulfuric acid to a final normality of about 0.5 as described.

Summary and Conclusions

The total extract, phospholipid, unsaponifiable matter, and total fatty acids were determined on 6 samples each of soybean oil meal, cottonseed oil meal, meat and bone scraps, alfalfa leaf meal, wheat gray shorts with screenings, and whole oats and on 5 samples of barley, using both the 16-hour anhydrous ether method and a 3 + 1 alcohol-ether method of extraction. With the exception of whole oats and barley the total ether soluble extract was considerably greater by alcohol-ether extraction than by ether extraction, especially so in the case of soybean meal and cottonseed meal. The non-phospholipid fatty acids, however, showed no significant difference between the two methods of extraction, the differences in total fatty acids being accounted for by the different amounts of phospholipid fatty acids.

The total extract is a poor criterion of the lipid feed value of some feeds, especially alfalfa leaf meal and soybean meal. However, the ethyl ether method more nearly approximates the total fatty acid content than does the alcohol ether method of extraction.

Much of the total extract cannot be accounted for in the sum of the phopholipid, unsaponifiable matter and fatty acid contents as determined.

A method is presented for the determination of phospholipid in plant tissue.

The significance of the lipid analysis of the feeds investigated and the relative values of the anhydrous ether and a 3 + 1 alcohol ether methods of analyses are discussed.

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Studies on the Heat Polymerization and Solvent Segregation of Vegetable Oils[1,2]

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THERMAL treatment of vegetable oils is an old and commonly used industrial practice, and the nature of the polymerization reaction has been quite extensively investigated. Since the unsaturated acids alone are involved, most of these studies have been made with monohydric alcohol esters of the unsaturated acids, thus avoiding the formation of highly complex molecules (1, 2, 4, 7, 10, 11). It is generally conceded that the first products of the polymerization contain six-membered hydro-aromatic carbon rings, formed essentially by the mechanism proposed by Kappelmeier (7) and in conformity with Schreiber's isomerization theory (10).

There is not, however, the same unanimity of opinion as to the constitution of the products formed in the polymerization of vegetable oils. Such studies are complicated by the fact that mixed triglycerides undergo intramolecular as well as intermolecular reactions to form complex linear and three dimensional polymers. The elucidation of the constitution of these polymers is rendered difficult by the inherent limitations in the available analytical procedures. Furthermore, the conditions of thermal treatment heretofore employed, promote side reactions and permit the accumulation of decomposition products which are also subject to polymerization.

In a previous communication (9) describing the optimum conditions for the heat polymerization and solvent segregation of linseed oil to produce a "non-reverting" shortening and an improved drying oil we emphasized the importance of the conditions of polymerization in relation to the rate of the reactions

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