A STUDY OF THE INFLUENCE OF SOLVENT AND MOISTURE IN THE EXTRACTION OF LIPIDES FROM PLANT TISSUE

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A STUDY OF THE INFLUENCE OF SOLVENT AND MOISTURE IN THE EXTRACTION OF LIPIDES FROM PLANT TISSUE

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RAUL ANTONIO ZAMBRANA (HOPPE)

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CONTENTS

Page

I. Introduction 1

II. Historical 2

1. Determination of Lipides by Solvent extraction 2

2. Refractometric determination of lipides 6

3. Commercial extraction of fats and oils by the use of solvents 7

III. Experimental 11

1. Influence of moisture in the determination of Total lipides by Standard procedures 14

2. Influence of moisture in the determination of Total lipides using various solvents 18

3. Influence of moisture in the determination of Unsaponifiable matter by the Kumagawa-Suto Method 22

IV. Discussion of results 30

V. Conclusions 37

VI. Bibliography 39

INTRODUCTION

Numerous organic solvents have been used for various purposes in the extraction of lipides from biological tissue.

In the quantitative determination of lipides, up to date, no solvent has been found satisfactory for use as a standard or so called official solvent. The implication is that each solvent does not completely extract identical materials (1).

In addition to the specificity of solvent action many other factors have been studied which affect the yield of lipide extracted. In the quantitative determination as much as in the commercial extraction of lipides it is of importance to know to what extent those factors affect the gross extract or some particular components of the lipide fraction.

The purpose of the research connected with this problem was to compare the values obtained in the extraction of "Total Lipides" and "Non-Saponfiable Matter" by several solvents in materials containing different amounts of moisture, and to determine how the presence of any moisture would affect the amount of gross extract and Non-Saponifiable Matter.

The term "Total Lipides", as used in this paper, refers to the gross extract obtained by the different solvents.

The method used in the determination of Non-Saponifiable Matter is the modified Kumagawa-Suto procedure (2).

HISTORICAL

The application of solvents to the extraction or solution of lipides has been done with different aims, such as their determination; their commercial extraction and their fractionation.

1. DETERMINATION OF LIPIDES BY EXTRACTION

Biochemists have tried for many years to find a solvent, a mixture of solvents or a sequence of solvents, which could be used as a standard for the determination of lipides in bilogical tissue.

Very outstanding works were made by Schulze and Steiger (3), Maxwell (4), Rather (5), Hertwig (6), and Rask and Phelps (7), and from their studies it is now well known that the Official Method of the Association of Agricultural Chemists does not give an accurate measure of the lipide content of a material.

Many solvents have been suggested for analysis of fats and comparative studies favor special solvents, depending upon the material under consideration and the conditions under which the work was carried out.

Taufel and Standgil (8) working with flax seed used tri-chlorethylene, benzine, carbon disulfide, benzene, ethyl ether, acetone and chloroform. The unsaponifiable matter, the saponification number and iodine number were fairly constant in all portions. Trichloroethylene gave the highest yield.

Walker (9) determined fat in 30 different types of feeding stuffs. These were run according to the Official Method, and both anhydrous ethyl ether and dichloromethane were used. He found four results lower with dichlorethane; twenty five were higher, and thirteen were within 0.10 percent of each other. On the average there were 0.23 percent more fat dissolved by the chlorinated hydrocarbon.

After this work of Walker, directions were sent to 17 chemists for collaborative work. About 400 tests were made on 27 types of feeding stuffs. The results showed that ethyl ether did not remove all the fat, and dichloromethane was not recommended because of difficulty of removal and its expense (10).

Vizem and Guillot (11) compared carbon disulfide, ethyl ether and petroleum ether. Carbon disulfide was found the best solvent of the three. Ethyl ether and petroleum ether are relatively poor fat solvents, the solubility of fats being approximately the same in both. Solid glycerides are less soluble in ethyl ether than in petroleum ether.

Dustman (12) made a study on the effect of certain solvents and of sequence of extraction in the removal of fats from bones of chicks. He concluded that: 1) Acetone and ethyl ether removed approximately equivalent quantities of extractive, but the acetone extracted less ash; 2) absolute ethanol and 95 percent ethanol removed more ash than did either acetone or ethyl ether; 3) when absolute ethanol preceded acetone and ethyl ether as the first solvent the total extract was somewhat less than when the sequence was reversed; 4) ether or acetone used after 95 percent ethanol removed only very small quantities of extractives, but 95 percent ethanol used after ether or acetone removed much more additional material.

Igarashi and Joshitoyo (13) showed that some additional agents in the extraction of soybean, containing 3 to 5 percent of water, had a favorable effect. They added sodium chloride, sodium acetate, claclum acetate, acetic acid, sodium hydroxide, calcium hydroxide, calcium chloride, soybean oil fatty acids, and the sodium, calcium and magnesium soaps of the soybean fatty acids.

Extraction with carbon tetrachloride, chloroform, carbon disulfide, ethyl ether and petroleum ether of egg yolk, cheese rind and chromed leather showed that in general more extract is obtained and more real fat with carbon tetrachloride than with any of the others (14).

Harrison (15) working with fish meal used 12 different solvents, and extraction periods ranging from 4 to 32 hours. The purpose of these determinations was to find the rate at which the various solvents approached a maximum extract value, the gross extract value of the solvents on the meals as received, and the gross extract value of the solvent after the meals had been subjected to storage conditions that would lead to oxidation of the fat. All the solvents, except acetone gave very little if any increase in extraction value beyond 4 hours. In the case of acetone 16-24 hours were required to reach a maximum value. From the standpoint of gross extraction the supposedly aliphatic petroleum hydrocarbons gave the lowest values, and these in general increased with boiling temperature.

Ethyl ether, carbon disulfide, and cyclohexane gave quite similar values, slightly above petroleum ether, hexane, and heptane.

It is of interest that cyclohexane, which is an alicyclic compound gives a value intermediate between the open chain solvents and benzene, which is definitely an aromatic compound. Likewise, the chlorinated aliphatic hydrocarbon solvents were found together on basis of extraction value and give results higher than the unsubstituted chain or ring hydrocarbons. The cyclic ether, 1, 4-dioxane, gave the highest values of the solvents tested. The oxidation of the meal decreased the extract value of both ethyl ether and petroleum ether.

Kaye, Leibner and Connor (16) recommended isopropyl ether for the extraction of fat from feces. It has proven more efficient than other common fat solvents.

Hieserman (17) found the Koch method to be the most efficient in the removal of the lipide material for the initial extraction. However, he concluded that a large percentage of non-lipide compounds, especially when cholophyll pigments are present, are included in the value obtained by this method.

In the extraction of cottonseed with several organic solvents, Frampton and Webber (1) found evidence that the composition of the extract obtained with a given solvent will vary from seed specimen to seed specimen, and the composition of the extract obtained with a given seed specimen is dependent on the nature of the solvent used. The quantity of material extracted with a given specimen is also dependent on the nature of the solvent, and it is suggested that the selection of any particular solvent as the standard or official solvent to be used in the assay of oil with cottonseed is arbitrary. Heat treatment of cottonseed reduced the quantity of lipids which could be extracted with petroleum ether. The influence of temperature in several extractions was found not to be of importance. It is probable, however, that the most important factor contributing to the differences may be specified as biological. Eash (18), nevertheless, working with the same material said that the amount of extract obtained increases with the temperature of extraction. He used a mixed solvent of the pentane type, containing a minimum of isopentane, isohexane and hexane, and with a boiling range between 35-40°C.

2. REFRACTOMETRIC DETERMINATION OF LIPIDES

For a rapid determination of fats the Refractometric method has been largely used.

Monochloronaphthalene (19), and (20) and Bromonaphthalene (21), (22), (23), and (24) are considered the best solvents for refractometric methods. Bromonaphthalene has the advantages over chloronaphthalene as a solvent as it is less volatile. Other solvents as bromobenzene, amyl acetate, isobutyrate, chloroform, and benzine have been also used with good results.

3. COMMERCIAL EXTRACTION OF FATS AND OILS BY THE USE OF SOLVENTS

The commercial extraction of fats and oils from plant and animal sources started at the beginning of the present century, particularly in England, France, and Germany. Its application was the natural result of the requirements of Germany and the Low Countries for supplies of edible oils and of protein feeds for their livestock.

In this country, which normally produces a surplus of edible fats and oils, the oilseed processing industry grew up around machines suitable for a decentralized system of relatively small mills located within the crop-producing areas. Only in a very recent years have American manufacturers entered the solvent extraction field.

The list of solvents used and suggested is very long. From the standpoint of commercial extraction the choice of a solvent for a particular extraction depends upon a number of factors. First, it must be a good solvent, i.e., it must penetrate into the cell structure of the solid material and dissolve out the soluble substances. It must be easily removable from both the oil and from the residue, leaving both in a marketable condition. Second, it should not corrode the equipment, thus avoiding tendencies toward contaminating the oil with traces of certain metals. Third, it should be cheap. This factor, almost alone, accounts for the rather general use of relatively low-priced hydrocarbons where at all possible, despite hazards connected with their use (25). Fourth, inflammability, and fifth, toxicity.

Carbon disulfide has been discarded as a solvent (26) for common extractions on account offire hazards and danger to health. Chlorinated hydrocarbons are good solvents but they extract undesireable resins, albuminoids, coloring matter and oxidized oils. Trichloroethylene is the most efficient chlorinated solvent but years of experience with it led to its abandonment, because of inferior products and its bad effects upon the health of the workers. Benzene is well suited for extraction of castorroil press cakes, but its vapors cause poisoning. Well purified and odorless petroleum benzine, 42-105°, is beyond doubt the best solvent for common extraction. The ordinary grades require careful purification with 66° sulfuric acid to remove aromatic hydrocarbons. When thus purified, it extracts only oils and fats, free from impurities, and it may be completely recovered.

Ehrlich (27) gave many advantages of o-dichlorobenzene such as stability, low fire hazard, easy volatility, great avidity for fats, and its ability to extract them completely even from substances containing an appreciable quantity of water.

Dichloroethane (28), and Dichloroethylene (29) and (30) have been used with success, giving an extraction residue suitable for animal feeding.

Many solvents are used in the extraction and simultaneous purification of oils. Seiyu (31), and (32) used a mixture of carbon disulfide and ethanol, or methanol and a mixture of chlorinated hydrocarbons, such as trichloreothylene or dichloroethylene, with ethanol. After extraction the solvent is separated into two layers; the lower one contains pure oils, while the upper one contains impurities.

The use of solvents to obtain from semidrying oils a fraction more highly unsaturated then the original oil has been investigated. Furfural was found to be the most suitable selective solvent of those examined (32). Aniline, polyethylene glycol, furfuryl alcohol, and monoethyl ether of ethylene glycol have also been used in this respect.

Within recent years, petroleum companies have stepped up their production of special solvent naphthas having particularly narrow boiling ranges, low evaporation residues, excellent stability, and other properties required of good solvents. In the soybean industry the commonly used solvent is essentially a normal hexane fraction boiling from 146 to 158°F. In most European plants, a gasoline boiling from 160 to 194°F is used.

Apparently there is reported only one commercial soybean extraction plant in the world using any solvent other than a petroleum cut. The exception is the Manchurian Soybean Industry Co., in Dairen. The solvent is 99.8 percent ethanol, and it is used at 80°C under pressure.

Considerable research has been directed toward the use of methanolbenzene and ethanol-benzene mixtures for soybean extraction in cases where phosphatides recovery is of importance.

Still other solvents have been considered. Benzene was used in some of the early installations. Methanol and ethanol show partial miscibility with glyceride type of fats and oils at low temperatures. The oil dissolved in the solvent-rich layer is more highly unsaturated. This has been suggested as a possible method for separating the drying and non-drying constituents present in semidrying oils, soybeans and corn oils (25).

In using solvents for extracting lipides, whatever the purpose is, it is necessary to consider factors such as selective solvent action, temperature and rate of extraction, moisture content, age and oxidation of the material to be extracted.

EXPERIMENTAL

The experimental work was divided into three parts. First, the determination of total lipides in several soybean meal varieties, containing different amounts of moisture, by a) the Official Method of the Association of Agricultural Chemists, b) A.O.A.C. methods followed by the Koch procedure, and c) the Modified Koch procedure. Second, the study of various single solvents in comparison to the two above standard procedures. Third, the determination of the unsaponifiable matter by the Kumagawa-Suto method, using the modified Koch procedure and various single solvents in the initial extraction of the same material.

The materials used for the experimental work were Mandarin and Manloxi soybean varieties, generic name Soja Max, and the common or Navy beans, Phaseolus vulgaris.

The soybean meal was prepared from the above soybeans by finely grinding in a Hobart electric mill. Using a number 20 sieve, the above meal was sieved and the residue ground again. This ame operation was repeated four times and then the total mixed meal was ground together. This procedure gave an homogenous sample. The meal was allowed to remain in contact with the atmosphere for two days and then was placed in an air tight bottle to be stored until needed during the course of the experiment.

Moisture determinations were first run on each material by weighing out duplicate 5.0 g. samples in calibrated aluminum dishes in a Brabender's balance and drying the weighed samples in a Brabender's Moisture Tester set at 100°C. To avoid deterioration the samples which were to contain some moisture were dried at 80°C. The rate of drying at 80°C. was first determined by plotting the time of drying against the percentage moisture read on the scale of the instrument. The curves shown in Figure I were obtained. As may be observed those curves gave an idea regarding the approximate time necessary to remove a desired amount of moisture.

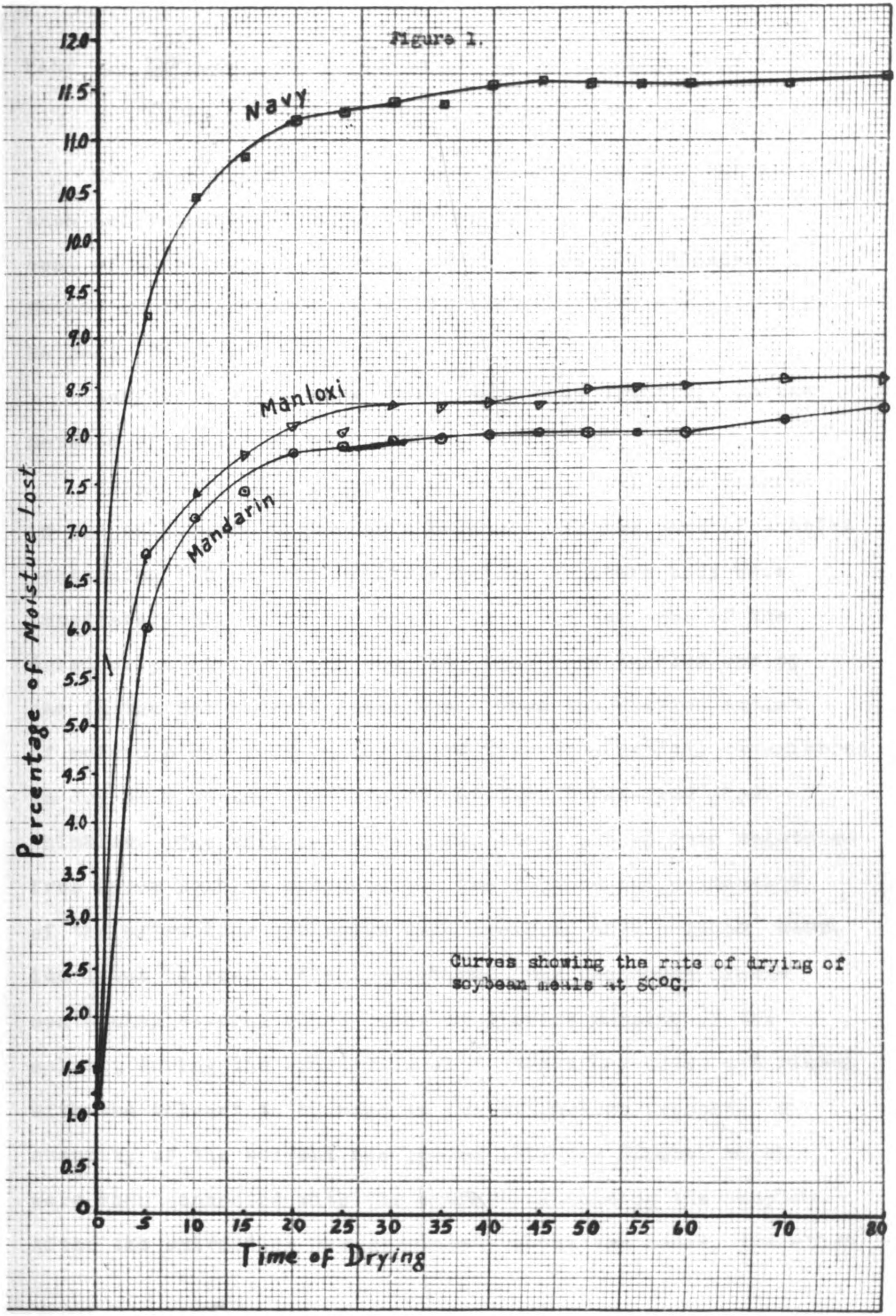
The rate of water absorption from the atmosphere by the dried and partially dried meals was observed by leaving the material in contact with the atmosphere for several minutes and redetermining its percentage moisture. The data is shown in Table I.

TABLE I

Rate of moisture absorption of soybean meal in contact with the atmosphere.

Material	Time in contact with the atmosphere. (minutes)	Percentage of moisture removed (air-dry basis)		
		100	75	25
Mandarin	0	7.88	1.97	5.91
"	1	7.87	1.96	5.90
"	2	7.86	1.96	5.90
"	5	7.79	1.94	5.87
"	10	7.67	1.92	5.76
Manloxi	0	8.25	2.06	6.19
"	2	8.13	1.99	
"	5	8.11	1.96	6.16
"	10	8.10	1.95	6.10
Navy	0	11.35	2.84	8.50
"	1	11.34	2.84	8.50
"	2	11.30	2.83	8.49
"	5	11.21	2.79	8.43
"	10	11.18	2.78	8.40

Figure 1.



Curves showing the rate of drying of soybean meals at 80°C.

PART I. INFLUENCE OF MOISTURE IN THE DETERMINATION OF TOTAL LIPIDES BY STANDARD PROCEDURES.

For each method used, triplicate samples were run in each determination. Meals with zero, 25, 75 and 100 percent of theiroriginal moisture, were chosen. a) Official Method of the Association of Agricultural Chemists (A.O.A.C. Method) (34).

Anhydrous ethyl ether was prepared as described in the Methods of Analysis, page 408.

Triplicate 10 g. samples of each material were weighed in the calibrated Brabender's balance. In the case of samples containing 100 percent of its original moisture they were immediately placed in the extractors. In the case of the dried or partially dried samples, they were handled one by one in the Brabender's apparatus. When the desired amount of moisture was left in the meals they were rapidly transferred to dry 33 x 94 mm. Schleicher and Schull fat-free paper thimbles. The thimbles were immediately placed into moisture-free Pyrex Soxhlet extractors. The tops of the condensors of the extracting apparatus were equipped with calcium chloride tubes to keep out all moisture. The receiving flasks containing several glass beads to prevent bumping of the solvent during the extraction were thoroughly dried and rinsed with the solvent to be used in the extraction process. A quantity of the solvent was poured into the flasks, which were then connected with the extractors holding the samples. After the extraction was completed, about ten hours, the ether extracts containing the extracted lipides were transferred quantitatively to tared beakers. The ether was evaporated on the steam bath with the aid of an electric fan to avoid overheating and to prevent creeping. The residue was dried in the vacuumooven at 60°C. for 5 hours, cooled in a dessicoater and weighed. The drying was continued until the weight remained constant.

b) A.O.A.C. followed by Koch procedure.

In this method the A.O.A.C. procedure was followed until a constant weight of the dried extract was obtained. Then the dry residue was redissolved in anhydrous ethyl ether, filtered through quantitative (ash-free) filter paper into a weighed beaker. The ether was evaporated on the steam bath with the aid of an electric fan and the residue dried to constant weight in the vacuum oven for five hours at 60°C. This last part of the procedure corresponds to the Koch method after the last alcohol extraction has been completed and the combined ether and alcohol extracted are evaporated.

c) Modified Koch Procedure (2).

Triplicate 10 g. samples of the same materials were weighed in the calibrated balance and its moisture adjusted as noted above. The material was then quickly transferred, one by one, to 33 x 94 mm. Schleicher and Schull fat-free paper thimbles. The thimbles containing the materials were temporarily placed in a desiccator and then rapidly removed to individual continuous Pyrex Soxhlet extractors and extracted for 10 hours with 95 percent ethanol. The alcoholic extraction was followed with an ether extraction for a period of 8 hours. Following the ethyl ether extraction the residue was freed of ether by placing it in the vacuum oven at 60° for 6 hours. The ether-free material was then replaced in the apparatus and extracted further for a period of 12 to 14 hours with 95 percent ethanol. The several ethanol and ether extracts were then combined. Two ml. of a hydroquinone solution in ether containing 0.2 mg. hydroquinone were added, and the combined extracts were then evaporated on a steam bath with the aid of an electric fan, to avoid overheating and creeping,until all the liquid was driven off. The residue was finally dried in a vacuum oven for 3-4 hours at a temperature of 60°C.

The drying of the lipide was performed to facilitate a subsequent extraction with ether of the dry residue. This extraction was carried out by adding successive quantities of anhydrous ethyl ether to the sesidue, heating just to boiling on an electric hot-plate with stirring, and quickly filtering the separate portions of ether through an ashless filter-paper into a weighed 150 cc. beaker. The filtered extract was evaporated to dryness on the steam bath and the residue dried for five hours in a vacuum oven at 60°C. The dry residue was weighed until a constant weight was obtained.

The results are shown in Tables II, III, and IV.

TABLE II

Effect of moisture content on Total lipides extracted by the A.O.A.C. Method

Material	Percentage of Total Lipides (air-dried weight basis)			
	All moisture removed	Three fourths of moisture removed	One fourth of moisture removed	No moisture removed
Mandarin	19.16	18.99	19.11	19.38
(% Moist.=8.26)	19.29	19.08	19.19	19.30
	19.32		19.15	19.46
Average	19.26	19.04	19.15	19.38
Manloxi	19.16	19.41	19.54	19.54
(% Moist =8.53)	19.16	19.31	19.47	19.57
	19.09	19.41	19.46	19.50
Average	19.14	19.38	19.49	19.54
Navy	2.61	2.30	2.31	2.43
(% Moist.=11.91)	2.61	2.30	2.25	2.39
	2.60		2.28	2.41
Average	2.61	2.30	2.28	2.41

TABLE III

Effect of moisture content on Total Lipides extracted by the A.O.A.C. method followed by Koch procedure.

Material	Percentage of Total Lipides (air-dried weight basis)			
	All moisture removed	Three fourths of moisture removed	One fourth of moisture removed	No moisture removed
Mandarin	19.14	18.29	18.48	19.07
(% Moist.=8.26)	19.39	19.21	18.74	18.95
	18.74		18.88	18.88
Average	19.09	18.50	18.70	18.97
Manloxi	18.94	12.47	17.61	17.36
(%Moist=8.26)	18.59	9.70	17.79	17.43
	18.67	10.12	18.39	17.41
Average	18.73	10.76	17.93	17.40
Navy	1.35	0.86	1.27	1.28
% Moist.-11.91	1.00	0.90	1.52	1.24
	1.32		1.35	1.30
Average	1.22	0.88	1.38	1.27

TABLE IV

Effect of moisture content on Total lipides extracted by the modified Koch procedure.

Material	Percentage of Total Lipides (air-dried weight basis) All moisture removed	Three fourths of moisture removed	One fourth of moisture removed	No moisture removed
Mandarin	20.22	23.92	19.44	19.34
% Moist.-8.26	20.94	21.18	21.14	19.23
	21.78	20.73	19.99	19.29
Average	20.98	21.94	20.19	19.30
Manloxi	20.75	20.22	20.54	19.34
% Moist.-8.53	20.27	19.99	20.88	19.41
	20.86	20.04	19.44	
Average	20.63	20.08	20.29	19.37
Navy	3.23	3.08	3.24	2.67
% Moist.-11.91	3.23	3.15	3.28	3.00
	3.20	3.37	3.50	3.43
Average	3.22	3.20	3.34	3.03

PART II. THE STUDY OF VARIOUS SINGLE SOLVENTS IN COMPARISON TO THE STANDARD PROCEDURES

Determination of Total Lipides using Trans-Dichloroethylene, Skellysolve B, and trichloroethylene as solvents.

All the above solvents were distilled and dried. Their boiling points were checked. Skellysolve B used in this work showed a boiling point range between 60-71°C.

The procedure for the extraction was the general procedure used by the A.O.A.C. method.

Triplicate 10 g. samples of each material were weighed in the Brabender's balance. The moisture content of each sample was controlled as above and done before. The results are shown in Tables V, VI, and VII.

TABLE V

Effect of moisture content on Total Lipides extracted by *trans*-Dichlorethylene

Material	Percentage of Total Lipides (air-dried weight basis)			
	All moisture removed	Three fourths of moisture removed	One fourth of moisture removed	No moisture removed
Mandarin	20.41	20.57	20.60	20.56
% Moist.-8.26	20.45	20.36	20.62	20.66
	20.33	20.27	20.57	20.37
Average	20.40	20.40	20.60	20.53
Manloxi	19.80	20.10	20.06	20.55
% Moist.-8.53	19.83	21.18	20.09	20.49
	19.85	20.52	20.07	20.26
Average	19.83	20.60	20.07	20.43
Navy	2.55	2.69	2.95	2.93
% Moist.-11.91	2.65	2.09	2.85	2.90
	2.66	2.69	2.90	2.88
Average	2.62	2.49	2.90	2.90

TABLE VI

Effect of moisture content on Total Lipides extracted by Skellysolve B (b.p. 60-71°C)

Material	Percentage of Total Lipides (air-dried weight basis)			
	All moisture removed	Three fourths of moisture removed	One fourth of moisture removed	No moisture removed
Mandarin	19.10	19.15	19.15	19.37
% Moist.-8.26	19.05	19.10	18.64	19.37
	19.10	19.11	18.73	19.45
Average	19.08	19.12	18.84	19.40
Manloxi	19.39	19.77		19.48
% Moist.-8.53	19.38	19.66		19.46
		19.65		19.53
Average	19.38	19.69		19.49
Navy	2.29	2.29	2.36	2.30
% Moist.-11.91	2.30	2.30	2.08	2.28
	2.30	2.32	1.98	2.30
Average	2.30	2.30	2.14	2.29

TABLE VII

Effect of moisture content on Total Lipides extracted by Trichloroethylene

Material	Percentage of Total Lipides (air-dried weight basis)			
	All moisture removed	Three fourths of moisture removed	One fourth of moisture removed	No moisture removed
Mandarin	20.34	20.62	20.18	21.00
% Moist.-8.26	20.36	20.38	20.19	20.61
	20.04		19.96	20.57
Average	20.25	20.50	20.11	20.73
Navy	2.22	2.40	2.69	2.55
% Moist.-11.91	2.57	2.57	2.65	2.34
		2.91	2.20	2.45
Average	2.39	2.63	2.51	2.45

TABLE VIII

Comparison of different Methods and Solvents in the Determination of Total Lipides

(calculated on air-dry weight basis)

Method or solvent	MANDARIN				MANLOXI				NAVY			
	% of original moist.				% of original moist.				% of original moist.			
	0	25	75	100	0	25	75	100	0	25	75	100
Koch (modified)	20.98	21.94	20.19	19.30	20.63	20.08	20.29	19.37	3.22	3.20	3.34	3.03
A.O.A.C.	19.26	19.04	19.15	19.38	19.14	19.38	19.49	19.54	2.61	2.30	2.28	2.41
A.O.A.C. followed by Koch	19.09	18.50	18.70	18.97	18.73	10.76	17.93	17.40	1.22	0.88	1.38	1.27
trans-Dichloroethylene	20.40	20.40	20.60	20.53	19.83	20.60	20.07	20.43	2.62	2.49	2.90	2.90
Skellysolve B (b.p. 60°-70°C.)	19.08	19.12	18.84	19.40	19.38	19.69		19.49	2.30	2.30	2.14	2.29
Trichloroethylene	20.25	20.50	20.11	20.73					2.39	2.63	2.51	2.45

PART III. INFLUENCE OF MOISTURE CONTENT ON THE DETERMINATION OF UNSAPONIFIABLE MATTER.

The procedure used in this work was done according to the modifications made by Hierserman (17) to the original Kumagawa-Suto Method.

The samples, after being weighed and their moisture adjusted as desired, were extracted for 10 hours with 95 percent ethanol. The alcohol extraction was followed with an ethyl ether extraction of a period of eight hours. Following the ether extraction the sample was freed of ether, by placing it in a vacuum oven at 60°C. for a period of six hours. The dried residue was then replaced in the apparatus and extracted further for a period of 12 to 14 hours with 95 percent ethanol. The several alcohol and ether extracts were combined. The combined extracts were evaporated to a volume of about 100 ml.

The above procedure was followed in the case of determining unsaponifiable matter in samples extracted by 95 percent ethanol-ether and 95 percent ethanol. When single solvents, ethyl ether, trans-dichloroethylene, Skellysolve B and Trichloroethylene were used, the extraction was carried out as in the Official Method; 16 hours extraction with the single solvent. After evaporation of the solvent, 80 to 100 ml. of 95 percent ethanol were added with stirring. The alcoholic liquid was heated on the steam bath and then the Kumagawa-Suto Method was applied.

In this method 7 to 8 ml. of 15M sodium hydroxide solution were added to the alcoholic solution. The ethanol was evaporated on a steam bath with the aid of an electric fan, the saponification continuing meanwhile. When the ethanol had been removed, the residue was taken up with a little warm water and quantitatively transferred to a separatory funnel. To the mixture in the separatory funnel, 30 ml. of a 20 percent hydrochloric acid solution was slowly added, with cooling. When thoroughly cool, the acid liquid was vigorously shaken with successive portions of ether. For the first shaking, 85 ml., of ethyl ether were used, while the second and third required only 10 to 15 ml. The aqueous layer was drawn off each time and the ether layer collected in a beaker. During the foregoing shaking operations, a precipitate formed in the funnel at the zone of partition of the two layers. This precipitate was dissolved in 5 ml. of N. sodium hydroxide solution; the alkaline solution was shaken with 40 ml. of ether. Then the first aqueous acid solution was added and the whole vigorously shaken. The separated ether layer was added to the ether portion and the united extracts evaporated. The residue was dried in a vacuum oven for four hours and the dry residue was then redissolved in absolute ether. The ethereal solution was filtered and evaporated to dryness. The residue was dried at 60° for four more hours in a vacuum oven at 60°C. To the residue while still warm 35 ml. of petroleum ether was added and gently rotated. The beaker was covered with a watch glass and set aside for 30 minutes to permit the settling of resinous matter. The solution was filtered through asbestos, previously washed with petroleum ether, and well washed with petroleum ether. The filtrate and washings, containing the fatty acids and unsaponifiable matter were collected into a 250 ml. separatory funnel.. To the funnel were added 30 to 40 ml. of N/5 nearly absolute alcoholic potassium hydroxide and the contents well shaken. A clear solution resulted. A quantity of water was added to this equal to the volume of alcoholic potash used, whereby a separation occurred with the 50 percent alcoholic liquid as the bottom layer and the petroleum ether solution as the top layer. The unsaponifiable substances remained in the petroleum ether whereas the soaps went to the dilute alcoholic layer. After separation of the solution, the dilute alcoholic layer was again extracted with 40 ml. of fresh petroleum ether. After evaporation of the petroleum ether from the combined extractions the residue was freed from the small traces of fatty acids. This separation was accomplished by dissolving the residue in a little absolute ethanol, adding 0.5 to 1.0 ml. of nearly absolute alcoholic sodium hydroxide N/10 solution, evaporating on the steam bath, drying the residue 1 to 2 hours at 80°C., and then extracting with hot petroleum ether. The petroleum ether extract was filtered through asbestos into a weighed 100 ml. beaker, the petroleum ether was evaporated on the steam bath and the residue dried to constant weight at 60°C. in a vacuum oven. This weight represented total unsaponifiable matter. The results obtained by the use of the different methods and solvents are shown in Tables IX to XIII.

TABLE IX

Determination of Unsaponifiable Matter in meals containing different amounts of moisture by the modified Kumagawa-Suto Methods (Solvent for initial extraction, anhydrous ethyl ether).

Material	Percentage Unsaponifiable Matter (calculated on air-dry weight basis)			
	All Moisture removed	Three fourths of moisture removed	One fourth of moisture removed	No moisture removed
Mandarin	0.26	0.22	0.21	0.42
% Moist.-8.26	0.30	0.19	0.24	0.41
	0.25		0.18	0.40
Average	0.27	0.20	0.21	0.41
Manloxi	0.15	0.13	0.05	0.48
% Moist.-8.53	0.16	0.12	0.06	0.59
	0.24	0.10	0.07	0.43
Average	0.18	0.12	0.06	0.50
Navy	0.06	0.09	0.05	0.07
% Moist.-	0.07	0.09	0.05	0.08
11.91	0.05		0.05	0.11
Average	0.06	0.09	0.05	0.09

TABLE X

Determination of Unsaponifiable Matter in meals containing different amounts of moisture by the modified Kumagawa-Suto Method. (Solvent for initial extraction, ethanol-ethyl ether-ethanol)

Material	Percentage Unsaponifiable Matter (calculated on air-dry weight basis)			
	All moisture removed	Three fourths of moisture removed	One fourth of moisture removed	No moisture removed
Mandarin	0.99	2.57	2.48	0.09
% Moist.-8.26	0.88	2.49	2.52	0.11
	1.35	2.32	2.66	0.11
Average	1.07	2.46	2.55	0.10
Manloxi	0.99	2.10	3.03	0.08
% Moist.-8.53	0.88	1.95	2.16	0.07
	1.35	1.91	1.83	0.07
Average	1.07	1.99	2.34	0.07
Navy	0.15	0.19	0.27	0.08
% Moist.-11.91	0.14	0.28	0.21	0.07
	0.15	0.22	0.13	0.06
Average	0.15	0.23	0.29	0.07

TABLE XI

Determination of Unsaponifiable Matter in meals containing different amounts of moisture by the Modified Kumagawa-Suto Method. (Solvent for initial extraction, *trans*-dichloroethylene)

Material	Percentage Unsaponifiable Matter (calculated on air-dry weight basis)			
	All moisture removed	Three fourths of moisture removed	One fourth of moisture removed	No moisture removed
Mandarin	0.36	0.33	0.31	0.18
% Moist.-8.26	0.36	0.34	0.31	0.19
	0.37	0.33	0.30	0.17
Average	0.36	0.33	0.31	0.18
Manloxi	0.32	0.32	0.32	0.35
% Moist.-8.53	0.36	0.35	0.31	0.36
	0.33	0.36	0.31	0.36
Average	0.34	0.34	0.31	0.36
Navy	0.08	0.13	0.10	0.11
% Moist.-11.91	0.07	0.09	0.08	0.10
	0.06	0.12	0.08	0.10
Average	0.07	0.11	0.09	0.10

TABLE XII

Determination of Unsaponifiable Matter in meals containing different accounts of moisture by the modified Kumagawa-Suto Method. (Solvent for initial extraction, Skellysolve B)

Material	Percentage of Total Lipides (air-dried weight basis)			
	All moisture removed	Three fourths of moisture removed	One fourth of moisture removed	No moisture removed
Mandarin % Moist.-8.26	0.17 0.17 0.16	0.29 0.29 0.26	0.60 0.68 0.77	0.28 0.28 0.27
Average	0.17	0.28	0.68	0.28
Manloxi % Moist.-8.53	0.26 0.25 0.26	0.16 0.20 0.18		0.15 0.16 0.14
Average	0.26	0.18		0.15
Navy % Moist.-11.91	0.10 0.16 0.12	0.09 0.09 0.10	0.10 0.11 0.12	0.10 0.08 0.09
Average	0.13	0.09	0.11	0.09

TABLE XIII

Determination of Unsaponifiable Matter in meals containing different amounts of Moisture by the modified Kumagawa-Suto Method. (Solvent for initial extraction, Trichloroethylene)

Material	Percentage of Total Lipides (air-dried weight basis)			
	All moisture removed	Three fourths of moisture removed	One fourth moisture removed	No moisture removed
Mandarin % Moist.-8.26	0.06 0.05 0.04	0.12 0.13 [illegible]	0.07 0.06 0.07	0.14 0.15 0.14
Average	0.05	0.13	0.07	0.14
Navy % Moist.-11.91	0.05 0.05	0.02 0.03 0.03	0.04 0.04 0.05	0.06 0.06 0.06
Average	0.05	[illegible]	0.04	0.06

TABLE XIV

Comparison of solvents and methods in the initial extraction of Unsaponifiable Matter in meals containing different amounts of moisture, (calculated on air-dry basis)

Method or solvent used in the initial extraction	MANDARIN % Moisture-8.26				MANLOX I % Moisture - 8.53				NAVY % Moisture - 11.91			
	0	1/4	3/4	100	0	1/4	3/4	100	0	1/4	3/4	100
Alcohol-ether-alc. (modified Koch)	1.07	2.46	2.55	0.10	1.07	1.99	2.34	0.07	0.15	0.23	0.20	0.07
Anh. Ethyl Ether (A.O.A.C.)	0.27	0.20	0.21	0.41	0.18	0.12	0.06	0.50	0.06	0.09	0.05	0.09
trans-Dichloroethylene	0.36	0.33	0.31	0.18	0.34	0.34	0.31	0.36	0.07	0.11	0.09	0.10
Skellysolve B.	0.17	0.28	0.68	0.28	0.26	0.18		0.15	0.13	0.09	0.11	0.09
Trichloroethylene	0.05	0.13	0.07	0.14					0.05	0.03	0.04	0.06

DISCUSSION OF RESULTS

If regardless of the moisture content of the material we compare the results from Tables I to VI, we can make the following observations:
In the Mandarin soybean meals the Modified Koch procedure gave the highest value. In single solvent extraction *trans*-Dichloroethylene and trichloroethylene gave very close values, 99.4 and 99.0 percent respectively of that value obtained by the Koch procedure.

Anhydrous ethyl ether and Skellysolve B are found together with lower values, 93.2 and 92.7 percent of the modified Koch method. In Manloxi soybean meals, where trichloroethylene was not used, the highest values were obtained by *trans*-dichloroethylene and the Koch method. The latter in this case gave an average value slightly lower than the chlorinated solvent. Skellysolve B and anhydrous ethyl ether, however, gave average values 96.4 and 95.8 percent respectively of the higher *trans*-dichloroethylene value.

In the Navy soybean meals the effectiveness of the solvents is in the same order as in the Mandarin soybean meals, with greater difference between one solvent and another.
The Koch method gave the highest value whereas *trans*-dichloroethylene and trichloroethylene gave respectively 85.3 and 77.8 percent extraction. Anhydrous ethyl ether and Skellysolve B are 75.0 and 70.6 percent respectively of the result obtained by the modified Koch procedure.

EFFECT OF MOISTURE CONTENT

The comparison of the results obtained in samples containing different amounts of moisture will be made assuming that the single solvents used were completely dried and that they acted under the same extraction conditions, excepting the moisture content of the materials. We shall consider each solvent as a separate case, but we must remember the selective solubility action toward specific substances contained in the meals, which might increase apparently the lipide part in the gross extract.

From reference to Tables I to VI we may note the following results.

1. Anhydrous Ethyl ether (A.O.A.C.). It has been taught that the drying of the material to be extracted with this solvent decreases the yield of extraction. Favorable results to this general idea was found in the Mandarin and Manloxi soybean meals, where the samples with less moisture than the original gave the lowest values. In the Navy beans, where the moisture content is about 12 percent, the highest value was found in the dried samples. The meal with its original moisture; that with 75 percent; and that with 25 percent remaining gave values 7.7, 11.9, and 12.7 percent respectively. Apparently the higher values obtained in the material with its original moisture in the Mandarin and Manloxi soybean meals were due to the extraction of some substances soluble in moist ethyl ether but not in anhydrous ether. This is especially noted in the Manloxi soybean

meals. When the extract obtained with anhydrous ether was redissolved in anhydrous ether, filtered and was weighed, the new values were higher in the moisture free samples.

2. Modified Koch Procedure.---Although it is very difficult to ascertain the effect of the moisture by this procedure, since 95 percent ethanol was used as solvent, from observation of Table III we can see that in the three soybean varieties, higher values were obtained in the samples containing from 0 to three fourths of its original moisture. The samples with its original moisture gave, in all cases, the lowest values.

3. Trans-dichloroethylene;---The moisture did not seem to have an appreciable influence in the extraction by means of this solvent. With the various moistures the difference was insignificant. The range being from 20.40 to 20.60 percent Total Lipides.

In the Manloxi soybean meal the highest value was given by the material with one fourth of its original moisture. The completely dried meal and that containing three fourths and all their original moisture gave lower values 3.8, 2.6, and 0.9 percent respectively.

4. Skellysolve B.---With this solvent the results vary from one material to another. In the Mandarin meal the highest value was found in the samples with their original moisture. The material with three fourths of its moisture content remaining and the totally dried samples gave the lowest values. The highest result was obtained in the samples with one fourth

of their original moisture. The lowest value is in the dried samples, namely 1.6 percent lower. The meal with all its original moisture gave a 1.1 percent lower value.

In the Navy meal the highest values were given by the samples with one fourth of their original moisture and the fully dried ones. The material with three fourths of its original moisture and the one with all its original moisture gave 7.0 and 0.5 percent lower values respectively.

5. Trichloroethylene.---In the Mandarin meal the highest value was obtained from the samples containing all its original moisture. The one fourth, zero, and three fourths percent moisture samples gave values 1.2, 2.4, and 3.0 percent lower respectively.

The effect of the moisture is more significant in the Navy beans, where the meal with one fourth of its original moisture gave the highest value, whereas the sample with three fourths of its moisture content and the samples with all their original moisture follow in order with 4.6 and 6.9 percent lower values respectively. In the other hand the totally dried samples gave the lowest value, namely 9.2 percent less than the higher one.

The above results show that the values in gross extract vary from one solvent to another, and from one variety to another. The influence of the moisture content of the material is not constant, but varies depending upon the solvent and the variety under consideration.

In the determination of Unsaponifiable Matter regardless of the moisture content of the material, the average values obtained by the Kumagawa-Suto Method, using various solvents in the initial extraction, vary from 0.10 to 1.54 percent in the Mandarin soybean variety, from 0.20 to 1.37 percent in the Manloxi variety and from 0.04 to 0.16 percent in the Navy beans. The highest values in the three soybean specimens were obtained by the use of the modified Koch procedure in the initial extraction. In the Mandarin soybean the order was as follows: Skellysolve B, 22.7, and *trans*-dichloroethylene, 18.8, anhydrous ethyl ether, 17.5; and trichloroethylene 6.5 percent lower than the higher Koch procedure. In the Manloxi soybean variety the order was thus: *trans*-dichloroethylene, 24.8 percent, ethyl ether, 15.3 percent and Skellysolve B, 14.6 percent of the higher Koch values. In the Navy beans the order was as in the Mandarin: Skellysolve B, 81.2, *trans*-dichloroethylene, 56.2, anhydrous ethyl ether, 43.7, and Trichlorethylene 25.0 percent of the higher Koch procedure.

Among the single solvents used in the initial extraction, Skellysolve B gave the highest value with the Mandarin and Navy beans, following in decreasing order. In the Manloxi variety the highest value was obtained with the use of *trans*-dichloroethylene. In comparison of the effectiveness of each solvent in materials with the same amount of moisture content, the modified Koch procedure gave the highest values in all the cases where the moisture content is zero, one fourth, and three fourths of its original moisture. In the samples with all their original moisture, when the initial extraction was made by the modified Koch method, the unsaponifiable matters suffers an appreciable decrease. The highest results by the use of the modified Koch procedure were found in the samples with a moisture content of one fourth and three fourths of their original moisture.

In the Mandarin soybean meals extracted by anhydrous ethyl ether, Skellysolve B and trichloroethylene the drying of the material resulted in a decrease in the yield of unsaponifiable matter. The reverse occurred in the case of <u>trans</u>-Dichloroethylene.

In the Manloxi soybean meals a decrease was noticed in the amount of unsaponifiable matter obtained when extracted with anhydrous ethyl ether; no appreciable variation when these samples were with <u>trans</u>-dichloroethylene, whereas an increase was noted when they were extracted with Skellysolve B.

In the Navy bean meals the results do not show a definite influence of the moisture but show very wide variability. Anhydrous ethyl ether, <u>trans</u>-dichloroethylene and Trichloroethylene showed a decrease in the dried samples, whereas Skellysolve B gave the highest values in the dried samples.

From the above results we may any that the single solvents show a selective solvent action. Some of this solvents are more influenced than others by the amount of moisture present in the material; consequently, no general conclusions may be drawn.

It is possible that besides specific solubility, absorption may play an important roll. It is known that in protein-rich materials, fats are absorbed upon protein surface. This might be important in the dase of soybeans, which contain a high percent protein, namely 34.1 to 46.9 percent.

In the specific case of anhydrous ethyl ether, this solvent forms a water-ether mixture with the water content of the material. This mixture extracts some substances which are insoluble in water and in anhydrous ether. Water, in all probability, when in an equilibrium-concentration with the solvent in question, will favor the extraction of substances insoluble in the single solvents.

CONCLUSIONS

1. Regardless of the moisture content of the material, the modified Koch procedure gave the highest average value for total lipides.
2. The Modified Koch procedure shows a higher percentage of lipide extracted in material with 0 to 75 percent of its original moisture. An appreciable decrease of the yield was found in the material with all its original moisture.
3. *Trans*-Dichloroethylene and trichloroethylene had the same solvent power in the case of Mandarin soybean meal. *Trans*-Dichloroethylene gave slightly higher values in the extraction of Navy bean meal, especially in the samples with 75 and 100 percent of its original moisture.
4. Anhydrous ethyl ether and Skellysolve B showed more or less the same efficiency in the extraction of Total Lipides.
5. Withdrawal of all the moisture from the material at 80°C. decreasedthe gross extract in most of the cases.
6. A definite amount of water present in the material to be extracted favored the solubility of water-soluble substances or particularly, those which are soluble in the moist solvent.
7. The initial extraction by the modified Koch procedure in the determination of unsaponifiable matter by the Kumagawa-Suto method, gave the highest values in all the materials containing from 0 to 75 percent of their original moisture.

8. The lowest values encountered in the extraction of Total Lipides by the modified Koch method in material with all of their original moisture present, was probably due to an incomplete extraction of the unsaponifiable matter.
9. Of the chlorinated solvents used in the initial extraction, trans-dichloroethylene gave the highest unsaponifiable matter. Trichloroethylene gave the lowest values for unsaponifiable matter of the solvents used.
10. Partial or total drying of the material decreased the unsaponfiable matter when anhydrous ethyl ether was used in the initial extraction.
11. The values for unsaponifiable matter obtained by the use of the different solvents in the initial extraction varied from one solvent to another.
12. The effect of the moisture content of the material also varied from one solvent to another.
13. No general rule may be drawn, from the results obtained in this work, to predict the behavoir of a particular solvent on different materials with varied amounts of moisture.

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