employed in the spectrophotometric assays of the margarine oils at the two stages of interest in controlling vitamin A fortification. From the data presented in this report the figures obtained in Column B are the true values for the fortified margarine oil prior to churning. If the margarine control oil containing no lecithin were used in assaying such fortified oils, the estimates of vitamin A content would be erroneously high by 600 USP units per pound of margarine. The proper blank oil to be used in assays of the separated margarine oil is that containing no lecithin; the true estimates of the vitamin A content of the margarines are given in Column C of Table VII. If the margarine blank oil containing lecithin were used in assays of the separated margarine oils, the estimates of vitamin A content would have been erroneously low by 650 USP units per pound of margarine.

The difference between apparent and true vitamin A content in either instance is small, equivalent to 3.5% of the true vitamin A content. This 3.5% figure is of real significance economically and can be of very great importance should estimates of vitamin A content border on the minimal declared potency of 15,000 USP units of vitamin A per pound.

The difference between apparent and true vitamin A content of the oils listed in Table VII is obviously a real one. Statistical analyses are unnecessary since the figures for A-B and C-D are positive in every case.

The figures in Table VII are characteristic of those obtained in control assays of products made by one manufacturer and undoubtedly represent the smallest magnitude of discrepancy between apparent and true vitamin A content following spectrophotometric assays. Other manufacturers not aware of the significance of the lecithin supplement in spectrophotometric assays may find much larger differences between apparent and true vitamin A content. Discrepancies as large as 1,500-2,400 USP units of vitamin A per pound of margarine have been noted in similar tests conducted on oils kindly furnished by another manufacturer. On the basis of the results obtained with the three lecithin preparations used in the present study, large variations between apparent and true vitamin A content are attributable more to excessive heating of the lecithin-supplemented oils rather than to differences in the light absorbancy of the lecithin preparations themselves. Calculations indicate that assays of the margarines made with the oils supplemented with any one of the three lecithin preparations and held for 60 hours at 140°F. would yield vitamin A figures in error by 1,600 USP units per pound.

Summary

On the basis of light absorption measurements at 328 mμ, fluorometric tests, stability of oils to oxidative deterioration, and phosphorus analyses it has been shown that the oil separated from margarine contains no lecithin. Phosphorus analyses demonstrated that this migration of added lecithin from the oil to the aqueous phase occurs during the churning operation and prior to chilling the emulsion.

For the spectrophotometric vitamin A assay of the fortified margarine oil prior to margarine manufacture, the control oils used to set the spectrophotometer at 100% transmission must contain the added lecithin. For the assay of the oil separated from the margarine the proper blank oil should contain no lecithin.

Use of improper control oils would be responsible for estimates of vitamin A content which are either erroneously high or low by about 3.5%. Abusive handling (storage at elevated temperatures) of lecithin-supplemented oils and use of improper control oils can be responsible for differences as much as 10% between apparent and true vitamin A content.

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Wesson Loss as a Measure of the Degree of Refining

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THE time-honored Wesson method (1) of estimating the neutral oil content of crude oils or, strictly speaking, of estimating the constituents responsible for loss during alkali refining, had until several years ago a more theoretical than practical importance since refining losses in practice were usually a multiple of the Wesson loss. Only after the introduction of the Clayton (2) and other continuous refining processes did plant losses become sufficiently low to approach the Wesson or "ideal" loss within a few tenths of a percent (3). This greatly enhanced the applicability of the method in the refining industry.

Recently however the value of the Wesson method has been subjected to criticism from two independent sources, but for diametrically different reasons. Linteris and Handschumaker (4) expressed the opinion that the method, apart from being tedious and time-consuming, tends to overestimate the neutral oil content, probably owing to decomposition of phosphatides by alcoholic potassium hydroxide used in the first step of the Wesson loss determination. They suggested a chromatographic technique based on the

[1] The removal of non-glycerides by the Wesson method requires the presence of soap and therefore reacidification of the neutralized oils is essential.

[2] This probably explains the results claimed for the De Laval Shortmix process according to which inter alia a coconut oil showing a Wesson loss of 7.20% has been alkali refined with an overall plant loss of 6.74% (7).

method of the International Commission of Fats and Oils, Supplement II_{14} (5), which, according to them, gives lower results for neutral oil and is easier to carry out.

On the other hand, Desnuelle and Micaelli in an interesting communication (6) questioned the belief that the Wesson loss represents a minimum value below which the actual refining loss cannot be reduced. The French authors maintain that in the factory the neutralization of oils with alkalies does not remove non-glycerides to such an extent as the Wesson technique, with the result that factory refined oils after reacidification with pure fatty acids[1] show still an appreciable Wesson loss after deducting the amount of fatty acids added. There is therefore a possibility of obtaining in the large scale refining a higher yield than that indicated by the Wesson loss determination if the entrainment of neutral oils is eliminated by efficient mechanical means.[2] Furthermore the Wesson loss conception, according to Desnuelle and Micaelli, is misleading not only in attributing an "inevitable" character to the loss of non-glycerides (which as mentioned above is partly avoided in practice) but also in assuming that losses of triglycerides are "evitable." A certain amount of glycerides might enter the structure of the soapstock and thus not be recoverable by any mechanical process.

This last point is of a rather speculative nature and has not been pursued in the present investigation. On the other hand, the point regarding the Wesson loss of oils after alkali refining is real, and some refiners would probably not expect the neutralization process as carried out in practice to be less rigorous than the Wesson technique. The question seemed to be important enough to warrant scrutiny and prompted the present investigation. In adjunction the chromatographic method of estimating loss constituents as recommended by Linteris and Handschumaker has been examined.

Experimental

Desnuelle and Micaelli used in their experiments peanut oil exclusively. It appeared desirable to extend the investigation to other oils, and beside peanut oil, crude soya bean, cottonseed, rapeseed, sunflower, and coconut oils were examined. The oils were refined on a laboratory scale which permitted alterations in refining methods more readily than would be feasible in large scale refining.

The amount of non-glycerides was established by estimating the Wesson loss on carefully dried oils, according to the Jamieson procedure (8), and deducting from this loss the free fatty acid content found by titration and calculated as oleic acid for all oils except coconut and rapeseed oil. For these the molecular weight of free fatty acids was assumed as 208 and 300, respectively. The free fatty acid content obtained in this way differs slightly from that estimated gravimetrically, but in the present work only approximate values for non-glycerides in the crude oils were needed. Crude oils with appreciable amounts of non-glycerides, preferably above 2%, were selected. The corresponding chromatographic losses were established, using a column of 18 mm. diameter and 50 cm. long, packed with alumina, and following Linteris' and Handschumaker's procedure. The amount of non-glycerides in this case was calculated from the formula:

Percentage of non-glycerides = percentage of chromatographic loss − percentage of free fatty acids in crude oils + 0.15

since the free fatty acid content of oils after passing through the column was on the average of 0.15%. Results are shown in Table I.

TABLE I
Content of Non-Glycerides in Crude Oils Calculated From the Wesson Loss and From the Chromatographic Loss

Oil	F.F.A., %	% Non-glycerides	
		Wesson loss −F.F.A.	Chromatographic loss −F.F.A. +0.15
Peanut oil	1.52	2.98	3.25
Coconut oil	1.95	2.02	2.87
Sunflower oil	3.30	2.20	2.49
Soybean oil I	1.71	2.26	2.90
Soybean oil II	2.75	5.35	5.70
Rapeseed oil	0.79	1.51	2.07
Cottonseed oil	3.40	1.90	2.30

One hundred-gram samples of crude oils were refined with aqueous alkalies employing three different techniques:

a) To the oils preheated to 40°C. a 10% solution of sodium hydroxide was added with stirring, the temperature raised to 65°C., and the stirring continued for 5 minutes. After one hour's settling at 65°C. the clear oils were siphoned off, washed three times with water at 80°C., and dried *in vacuo*. The excess of sodium hydroxide was 10% over the theory for coconut oil and 25% for the remaining oils.

b) The oils were neutralized, using sodium hydroxide solutions in quantities and strength as specified in the A.O.C.S. official method for estimating the refining loss (cup method). Temperature and times of stirring given by this method were also observed but instead of remelting the soapstock, etc., the oils were settled, washed, and dried as in a).

c) On several samples an approximation of Clayton's sodium carbonate refining was used. The oil was mixed with a 15% aqueous sodium carbonate solution in the proportion of 1 mol of sodium carbonate for each mol of free fatty acid + 20% excess and the mixture dried *in vacuo* at 75°C. The soapstock was rehydrated with an amount of 15% sodium carbonate solution equal to that used for neutralization, the temperature raised to 90°C., the oil separated by centrifuging and washed and dried as in a). The re-refining (if found advisable) was carried out on the centrifuged, unwashed oil using 1 ml. of a 10% sodium hydroxide solution for 100 g. of oil and stirring for 5 minutes at 65°C. The oil was then centrifuged, washed, and dried.

The neutralized oils were reacidified to their previous free fatty acid content with pure oleic acid, or lauric acid in the case of coconut oil, and the Wesson loss was again determined. The chromatographic loss of refined oils was also established but without previous reacidification. Results are shown in Table II.

To determine the loss of neutral oil caused by the Wesson and chromatographic method, a few samples of crude oils were subjected to two consecutive treatments. Here again oils were reacidified prior to the second Wesson treatment. Results of these tests are shown in Table III.

Discussion

The amounts of non-glycerides which remained in the oils after refining showed significant variations. They were quite appreciable in a few samples examined (cf. Table II) but on the whole lower than those

TABLE III

Loss of "Neutral" Oil After a Second Wesson and Chromatographic Treatment, Respectively

Oil	% "Neutral" oil lost after a second treatment	
	Wesson	Chromatographic
Coconut oil	nil	0.39
Sunflower oil	nil	0.18
Soybean oil I	nil	0.20
Rapeseed oil	nil	0.57
Cottonseed oil	0.02	0.25

found by Desnuelle and Micaelli in factory-refined peanut oils which were 0.50, 0.70, and 0.95%, respectively. In each case refining with sodium carbonate, according to the Clayton procedure, proved to be more efficient than treatment with sodium hydroxide in which a moderate excess of sodium hydroxide was used. Caustic refining, when following the A.O.C.S. "cup" method which specifies a large excess of alkali, and sodium carbonate treatment with caustic re-refining removed practically all Wesson loss constituents.

From these results it would appear that the amount of non-glycerides left in refined oils depends, as could be reasonably expected, on the kind of oil and on the method of refining. This would explain the results obtained by the French authors and dispose, at least partially, of their claim that actual refining does not bear comparison with the Wesson technique regarding the removal of non-glycerides. The peanut oils examined by them were probably refined in conformity with the prevalent European practice according to which an excess of 0.5-30% of sodium hydroxide over the theory is considered sufficient. While there are no official rules in European countries specifying excess of alkali, British (9a, b), German (10), Italian (11), and Russian (12) sources bear out the above figures. As a result, a certain amount of non-glycerides is left in the oil, but refining losses are kept low as might be gathered from the following British statement (9a): "The normal neutral oil losses will vary from 20 to 100% of the weight of the fatty acids depending on the oil used, the percentage of fatty acids in the crude, and on the skill of the refiner." In the U.S.A. a neutral oil loss of 20-100% of the weight of free fatty acids would be deemed much below the average when employing the batch refining system. Statistical data of Brodie (13), Bailey (14), and others show this loss to be at least 200%. The difference between the U.S.A. and the European practice has historic and commercial reasons which need not be discussed here. The introduction of continuous refining processes in the U.S.A. has reduced the refining losses considerably, but even when employing these processes a more complete removal of non-glycerides is probably achieved than in the European practice, and it appears that the Wesson technique is equal, or superior, in this respect to the U.S.A. refining methods. It is doubtful whether "refining should eliminate as completely as possible the non-glyceridic constituents from the oil" as postulated by Desnuelle and Micaelli. Fortunately it does not, otherwise all antioxidants (and vitamins in the case of fish liver oils) would be removed, thus defeating the very aim of refining. It is even debatable whether such non-glyceridic constituents as coloring matter and phosphatides could not be profitably left in oils obtained from high quality seeds as advocated by Kaufmann (15), which would mean dispensing with the neutralization altogether. However, having once adopted alkali refining, one has to accept its good and evil features, and since debris of phosphatic and nitrogenous compounds would probably affect the stability of oils more than these compounds themselves, their removal should be as thorough as possible.

The Wesson technique which, as has been shown, removes these constituents at least as thoroughly as careful refining therefore appears to have a definite value as an indication to what extent alkali refining has fulfilled its aim. The Wesson loss determination is strictly quantitative and therefore superior to qualitative tests (16) for assessing the degree of refining, such as surface tension and foam measurements, and, in giving overall results in one operation, is less time-consuming than separate estimations of free fatty acids, ash, phosphorus, and the like. It can moreover be considerably improved by using oil sample bottles or stoppered flasks, instead of separating funnels as specified by Jamieson (8) and by siphoning off supernatant layers at each stage. This makes the estimation less tedious and possibly more accurate (17). While not a rapid routine test the method can, in the hands of an expert, provide guidance as to the adoption of a suitable refining procedure, or more specifically, as to the excess of alkali required for various kinds of oils. Thus it can supplement the directions contained in the official A.O.C.S. cup method. The quantities of sodium hydroxide specified in the latter are in excess of those needed for a thorough removal of non-glycerides. In view of the above it would seem that the Wesson technique, far from having lost its value as a measure of minimum refining losses, offers advantages as a test for the purity of refined oils.

Similar advantages cannot be claimed for the chromatographic method. It will not be denied that for the evaluation of crude oils this method may be found

TABLE II

Content of Non-Glycerides in Alkali Refined Oils Found by the Wesson and Chromatographic Method Respectively

Oil	% non-glycerides in oils refined with:							
	NaOH, 10-25% excess		NaOH, A.O.C.S. official method		Na_2CO_3, Clayton technique		Na_2CO_3, rerefined Clayton technique	
	Wesson	Chromatographic	Wesson	Chromatographic	Wesson	Chromatographic	Wesson	Chromatographic
Peanut oil	0.28		nil		0.05	0.27		
Coconut oil	0.05	0.32	nil					
Sunflower oil	0.12	0.28	nil		0.05	0.32		
Soybean oil I	0.53		nil		0.35		0.06	
Soybean oil II	0.47		nil		0.38		nil	nil
Rapeseed oil	0.41		0.02	0.35	0.30		0.02	0.30
Cottonseed oil	0.62		nil	0.28	0.36		nil	0.33

equally as or more convenient than the Wesson test. However, for the evaluation of refined oils as outlined above, the Wesson method appears preferable since it gives a better indication of the point at which further alkali refining becomes wasteful. The chromatographic method not only shows losses beyond this point (cf. Table II) but in contrast to the Wesson method seems to produce adsorption of some neutral oil in the absence of free fatty acids and non-glycerides. Thus it has been found by Desnuelle and Micaelli, and confirmed during the present work, that an oil treated according to the Wesson method and reacidified suffers on repeated Wesson treatments only a loss of weight equal to that of the added free fatty acids. The loss of neutral oil is nil or practically nil[3] even in the case of the easily saponifiable coconut oil while a repeated chromatographic treatment results in a further loss (cf. Table III). Conversely, the chromatographic treatment of crude oils, which gives a higher loss than the Wesson method, yields oils with a free acid content of about 0.15% while Wesson treated oils show an acidity of approximately 0.02%, which is more in line with the results obtained on careful refining in the factory.

Summary

Wesson loss determinations carried out on a number of alkali-refined oils have shown them to retain varying amounts of non-glycerides, depending on the type of the oil and on the method of refining. Oils refined according to the official A.O.C.S. method or with sodium carbonate followed by re-refining are practically free of non-glycerides while those refined with a moderate excess of alkali over the theory (10-25%) retain appreciable amounts of non-glycerides. The Wesson method could therefore be used as a quantitative test for the degree of refining.

The chromatographic method is not suitable for this purpose since it shows losses when applied both to carefully refined and to previously chromatographed oils, but in agreement with the Linteris' and Handschumaker's results it has been found applicable to the estimation of loss constituents in crude oils.

[3]King and Wharton (18), on determining the Wesson loss of neutralized and bleached, but not Wesson treated cottonseed oil, to which 2% of free fatty acids had been added, found a loss of neutral oil amounting to 0.2%. However there remains the question whether this loss was actually due to saponification of neutral oil or to the removal of rest amounts of non-glycerides.

Acknowledgment

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Annual Review of Literature on Fats, Oils, and Soaps. Part II

Report of the Literature Review Committee *

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Physiology and Biochemistry

REVIEWS. The reviews concerning the text of this division were on the following subjects: Biochemical and nutritional aspects of fat chemistry (Deuel & Greenberg—*Fortschr. Chem. org. Naturstoffe 6*, 1); fatty acids in nutrition (Holman—*Proc. 3rd Conf. on Research Am. Meat Inst., Chicago, 1951*, 1; *Fette u. Seifen 53*, 332), lipide metabolism (Gurin—*Ann. Rev. Biochem. 20*, 179); oxidation of fat by autoöxidation as well as the biological process (Täufel—*Fette u. Seifen 53*, 558); importance of unsaturated acids in dermatology (Fiedler—*Ibid. 52*, 721); serum cholesterol and phospholipide (Wallis—*Bull. Ayer Clin. Lab. Penna. Hosp. 4*, 79); formation of milk fat (Täufel—*Z. Lebensm.-Untersuch. u. -Forsch. 93*, 140); and antilipotropic activity of cystine (Tyner—*Univ. Microfilms, Ann Arbor, Mich., Pub. No. 2470*, 68 pp.). The papers given in a symposium on studies on arteriosclerosis which concerned biochemistry of lipides were on renal hypertension of dietary origin (Hartroft—*J. Gerontol. 6*, 154), lipfanogens and antilipfanogens (Sims—*Ibid.* 159), lipide metabolism (Kendall—*Ibid.* 162), and metabolism of arterial tissue (Kirk—*Ibid.* 167).

FAT NUTRITION. Of the new reports on nutritive value of butter versus margarine, no difference was found in two studies while one report maintained that summer butter contained some unknown growth stimulating factor. Euler & Euler (*Arkiv. Kemi 3*, 31) recorded no significant differences in growth rate, reproduction, or lactation through four generations of rats on the two fats. Smits (*Voeding 11*, 298), who surveyed pertinent literature on the subject, was of the same opinion. Groot's (*Mededel Lab. Univ. Amsterdam, Inst. Volksvoed 11*, No. 14, 84 pp.) work indicated that the unsaponifiable fraction of summer butter had a growth promoting effect which was not due to known vitamins. He did not obtain a growth effect from vaccenic acid or the phospholipides of the butter.

The nutritive value of mono-, di-, and triglycerides were found to be the same when compared on the basis of growth, appearance, and post mortem examinations of rats fed at 25% level of the pure materials (*Mattson et al.—J. Am. Oil Chemists' Soc. 28*, 386). A comparison of monoglycerides prepared from cottonseed oil versus cottonseed oil also showed no difference as measured by growth response, reproduction ability, and lactation performance (Ames *et al.—Ibid.* 31).

Some studies reflected the need for fat in nutrition. When rats and pigs were fed diets containing two to 20% fat, the best growths and assimilations of protein and carbohydrate were obtained at the highest fat levels (Tangl *et al.—Agartudomany 2*, 365). Gomberg (*Arch. Tiernähr. 2*, 307) recorded the reductions in daily gains of calves after reducing the fat content of the milk on which they were maintained. Later he showed that adding easily digestible carbohydrates to the low-fat maintenance milks could aid in closing the difference. Diets of fat and protein were able to prolong considerably the life