Improved Method for Quantitating and Obtaining the Unsaponifiable Matter of Fats and Oils

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A quantitative method for saponifying and isolating the unsaponifiable matter (UM) from fats and oils is detailed. The method, which is a modification of a previously described procedure, is faster, uses about half the solvent, and is easier to execute. The minimum time needed to completely saponify a large number of fats and oils is established and conditions for removing contaminating soap from the extracted UM are described. Evidence presented shows some fats and oils to be incompletely saponified using previously indicated time-temperature conditions. The method compares favorably with the AOAC method, but is not applicable to the analysis of marine oils.

Quantitation of the unsaponifiable matter (UM) of fats and oils is one of the more important analytical determinations in lipid chemistry. A significant segment of the fat and oil industry uses the value obtained (in conjunction with moisture and free fatty acid values) as the basis for the buying and selling of fats, oils and greases. Aside from the economic aspect, the quantitation and isolation of the UM has other potentially valuable analytical applications. The UM of practically all fats and oils contains sterols, fatty and triterpene alcohols and hydrocarbons as the major components. Using modern chromatographic techniques, it can be demonstrated that a more or less complex array of members exists within each class (1-4). The chromatogram of a given class or of the total UM may serve as a unique fingerprint for a fat or oil (5). This by itself and/or along with an accurate figure for total UM may serve to identify that fat or oil as well as offering a possible means of detecting adulteration. In addition to the naturally occurring classes, many other classes of chemicals may find their way into fats and oils and thus be potentially isolated from the UM. Some fat-soluble, alkali- and heat-stable drugs, pesticides, herbicides and environmental chemicals are some examples. The same is true for mutagens, carcinogens and lipid oxidation and decomposition products generated during culinary practices and during the processing and storage of fats, oils and foodstuffs.

Utilization of the UM to its full analytical potential has not been realized due to the arduous and expensive nature of all official methods. Attempts have been made to overcome some of the shortcomings of the official methods. Slover et al. (3) described a simple saponification method together with a capillary gas chromatographic system to quantitate the sterols and tocopherols present in the UM. Earlier, Schwartz et al. (6) showed that the UM of butter oil could be quantitated and isolated by simply blending the oil and alkali and heating for 20 min at 100 C, followed by extraction of the soap-Celite mixture with benzene. Maxwell and Schwartz (7) applied these conditions with minor modifications to study the UM in a variety of animal, vegetable and marine fats and oils.

The purpose of the work described below is to present a modification of the original method of Schwartz et al. (6) which considerably shortens the procedural time and reduces the volume of solvent needed to isolate the UM by about 50%. In addition, the conditions needed to completely saponify a large variety of individual fats and oils and to quantitate and isolate their UM free of soap have been established. Finally, the modified method has been compared to the official (AOAC) method.

EXPERIMENTAL

Materials. Glass mortars (16 oz), glass pestles (8 or 16 oz), 200-ml screwcap certrifuge bottles with rounded bottoms sufficiently flattened for standing, flat-bottomed aluminum foil dishes (70 mm), ASTM sand, and Alcoa grade F-20 alumina were all obtained from A.H. Thomas, Philadelphia, Pennsylvania. The alumina was purified (optional) by washing one part with 2.5 parts 2-propanol on a Buchner funnel using gravity flow, followed by removal of excess alcohol by vacuum application and drying $\geq$ 3 hr at 130 C. Celite 545 was obtained through Fisher Scientific Co., King of Prussia, Pennsylvania.

Extraction of oil from seeds. When the oil was extracted from seeds in this laboratory it was done as follows. For seeds with relatively low (< 25%) oil, four parts of seeds and one part Celite were ground for 2-3 min in a coffee mill. The powder was transferred to a chromatography tube containing about a 2-cm layer of Celite and tamped sufficiently to eliminate air spaces. The oil was extracted by passing at least 10 parts of 1:1 hexane:dichloromethane through the bed. The removal of oil from the powder usually could be followed visually as a dark or yellow zone moving with and directly behind the solvent front. The solvent was removed at 40-45 C under a stream of nitrogen until the oil was constant in weight.

For seeds containing > 25% oil, three parts of seeds and one part of Celite were ground in a mortar until fairly homogenous, and the powder extracted as described above.

Saponification procedure. Potassium hydroxide pellets (6 g) were crushed to a coarse powder with the pestle in a mortar. The oil or melted fat (10 $\pm$ 1 g) was pipetted onto the powder and the exact weight of oil (to the hundredth place) determined by difference. Two ml of distilled water were added, and the ingredients were ground for 1-2 min until a smooth mix was obtained. It was important that the pestle contact any oil that may have been deposited accidentally on the sides of the mortar. After about 15 min most of the viscous or solid soap adhering to the pestle was removed with a spoon

TABLE 1

Unsaponifiable Matter (UM) of Fats and Oils Obtained by an Improved Procedure

Fat or oil	UM % ± Std. Dev. (n)	Minimum time at 130 C[a] Min	Medium Used[b]
Alfalfa seed	4.36 ± 0.03 (3)	45	Al_2O_3
Almond (R)[c]	0.37 ± 0.01 (3)	45	Celite
Almond (U)[c]	0.27 ± 0.02 (3)	45	Celite
Amaranthus cruentus seed	8.84 ± 0.14 (3)	45	Al_2O_3
Apple seed	0.91 ± 0.00 (3)	45	Al_2O_3
Apricot kernel (R)	0.44 ± 0.01 (3)	45	Celite
Avocado (R)	0.56 ± 0.01 (3)	60	Al_2O_3
Avocado (U)	4.05 ± 0.03 (4)	60	Al_2O_3
Avocado (U)	3.65 ± 0.07 (4)	60	Al_2O_3
Beef oil	0.17 ± 0.01 (3)	60	Celite
Brazil nut	0.49 ± 0.01 (3)	45	Celite
Butter oil	0.32 ± 0.01 (4)	30	Celite
Butter oil	0.29 ± 0.00 (3)	30	Celite
Candlenut (*Moluccana*)	0.30 ± 0.02 (3)	45	Al_2O_3
Canola (U)[d]	1.04 ± 0.00 (3)	45	Al_2O_3
Canola, alkali treated & bleached[d]	0.95 ± 0.01 (5)	45	Al_2O_3
Canola fully (R)[d]	0.95 ± 0.01 (4)	45	Al_2O_3
Cashew nut	0.14 ± 0.01 (3)	45	Celite
Castor (R)	0.50 ± 0.01 (3)	45	Celite
Chia seed	0.90 ± 0.03 (3)	45	Al_2O_3
Chicken (jar)	0.23 ± 0.01 (4)	45	Al_2O_3
Chicken (jar)	0.17 ± 0.01 (4)	45	Al_2O_3
Cocoa	0.33 ± 0.01 (3)	60	Celite
Coconut (R)	0.15 ± 0.00 (3)	45	Celite
Coconut (U)	0.20 ± 0.01 (4)	45	Celite
Corn (U)[d]	1.52 ± 0.04 (3)	60	Al_2O_3
Corn (bleached)[d]	1.35 ± 0.06 (3)	60	Al_2O_3
Corn (R)[d]	1.07 ± 0.02 (4)	60	Al_2O_3
Corn (R)	0.99 ± 0.02 (5)	60	Al_2O_3
Corn (R)	0.94 ± 0.01 (4)	60	Al_2O_3
Cottonseed (U)[d]	0.69 ± 0.02 (3)	45	Al_2O_3
Cottonseed (R)[d]	0.60 ± 0.02 (4)	45	Al_2O_3
Crambe	0.61 ± 0.01 (3)	60	Al_2O_3
Date pit	0.93 ± 0.02 (3)	60	Celite
Evening primrose	1.91 ± 0.02 (3)	45	Al_2O_3
Garden purslane seed	1.80 ± 0.10 (3)	45	Al_2O_3
Grapeseed (R)	0.55 ± 0.02 (3)	45	Celite
Hazelnut (R)	0.25 ± 0.02 (3)	45	Celite
Hazelnut (U)	0.25 ± 0.01 (3)	45	Celite
Ironwood seed	0.64 ± 0.07 (3)	45	Celite
Jojoba	44.70 ± 0.70 (3)	45	Celite
Lanolin	38.70 ± 0.80 (4)	60	Al_2O_3
Lard	0.16 ± 0.01 (4)	60	Celite
Linseed (raw)	0.90 ± 0.04 (3)	45	Al_2O_3
Linseed (boiled)	0.89 ± 0.01 (3)	45	Al_2O_3
Lettuce seed	0.94 ± 0.00 (3)	45	Celite
Macadamia nut	0.34 ± 0.02 (3)	45	Celite
Mustard seed	1.26 ± 0.02 (3)	60	Al_2O_3
Neat's foot	1.04 ± 0.04 (3)	45	Al_2O_3
Olive (Greek)	0.65 ± 0.01 (3)	45	Celite
Olive (Italian)	0.61 ± 0.02 (4)	45	Celite
Palm (R)	0.13 ± 0.02 (4)	45	Celite
Peanut (U)[d]	0.39 ± 0.01 (4)	45	Celite
Peanut (R)[d]	0.30 ± 0.01 (4)	45	Celite
Peanut (Virginia)	0.30 ± 0.03 (2)	45	Celite
Peanut (Florunner)	0.30 ± 0.03 (3)	45	Celite
Peanut (Spanish)	0.30 ± 0.00 (3)	45	Celite
Pecan nut	0.24 ± 0.01 (3)	45	Celite
Pignolia seed	0.66 ± 0.02 (3)	45	Celite
Pistachio nut	0.71 ± 0.02 (3)	45	Al_2O_3
Poppy	0.32 ± 0.00 (3)	60	Celite
Prosopis velutina	3.02 ± 0.03 (3)	45	Al_2O_3
Pumpkin seed	0.66 ± 0.02 (3)	45	Celite
Quinoa (low saponin)	5.22 ± 0.06 (3)	45	Al_2O_3
Quinoa (high saponin)	6.34 ± 0.07 (3)	45	Al_2O_3
Red clover seed	2.63 ± 0.11 (3)	45	Al_2O_3
Rice bran (raw)	5.20 ± 0.10 (3)	45	Al_2O_3
Rice bran (stabilized)	4.77 ± 0.03 (3)	45	Al_2O_3
Rice bran (U)[d]	4.22 ± 0.09 (3)	45	Al_2O_3
Rice bran minus gums, waxes[d]	3.52 ± 0.12 (3)	45	Al_2O_3
Rice bran fully (R)[d]	2.19 ± 0.07 (3)	45	Al_2O_3
Rice bran (USA)	4.20 ± 0.01 (3)	45	Al_2O_3
Rice bran (Hubei, China)	4.20 ± 0.10 (3)	45	Al_2O_3
Rice bran (China)	4.10 ± 0.04 (3)	45	Al_2O_3
Safflower (R)	0.42 ± 0.00 (3)	60	Celite
Sesame seed	1.38 ± 0.02 (3)	60	Al_2O_3
Soy (U)[d]	0.57 ± 0.00 (3)	60	Al_2O_3
Soy (R)[d]	0.49 ± 0.01 (3)	60	Al_2O_3
Soy (R)[d]	0.50 ± 0.02 (3)	60	Al_2O_3
Squash seed	0.67 ± 0.02 (3)	45	Al_2O_3
Sunflower (R)	0.65 ± 0.03 (3)	45	Celite
Tallow (edible)	0.12 ± 0.01 (3)	45	Celite
Tomato seed (Heinz)	1.22 ± 0.03 (3)	45	Al_2O_3
Tomato seed (Rutgers)	1.16 ± 0.02 (3)	45	Al_2O_3
Trisun (U)[d]	0.64 ± 0.01 (3)	45	Al_2O_3
Trisun (cooking grade)[d]	0.43 ± 0.02 (3)	45	Al_2O_3
Trisun (salad grade)[d]	0.35 ± 0.01 (3)	45	Al_2O_3
Tung	0.31 ± 0.01 (3)	45	Celite
Vernonia galamensis seed	1.55 ± 0.02 (3)	60	Al_2O_3
Vernonia galamensis (R)	1.42 ± 0.01 (3)	60	Al_2O_3
Walnut	0.53 ± 0.01 (3)	45	Celite
Watermelon seed	0.98 ± 0.05 (3)	45	Al_2O_3
Wheat germ	4.78 ± 0.05 (3)	45	Al_2O_3
Wheat germ	4.98 ± 0.16 (3)	45	Al_2O_3

[a]Minimum time needed to reach constancy.

[b]Medium needed to pass aliquot of extract of UM over.

[c]R, refined; U, crude oil.

[d]Indicates same oil.

and the material sticking to the spoon was removed by smearing it onto uncoated portions of the mortar. The pestle and spoon were set upright in a 4-oz jar, and the mortar was covered by stretching a piece of plastic wrap over it. Saponification was completed by heating the mortar and jar at 130 C ± 2° in an oven for a minimum of 45 min. (30 min for butterfat).

Extraction of UM. The mortar was cooled at room temperature (520 min) and 3.5 g Celite was ground with the soap. Any soap adhering to the mortar and pestle was scraped off with the spoon, and any soap adhering to the spoon was scraped off with a flat (knife) spatula. The powder was reground until it appeared homogeneously fine. Most of the powder adhering to the pestle was removed, and the powder was transferred with the spoon through a 3-inch powder funnel into a centrifuge bottle. The pestle, spoon and spatula were rinsed into the mortar using 25 ml of extracting solvent (hexane:dichloromethane, 1:1) delivered from a pipet. The sides of the mortar were rinsed with 25 ml more of solvent by circling the pipet around the upper periphery. The rinsings were decanted into a 50 ml volumetric flask, made to the mark, and poured carefully into the centrifuge bottle while washing down any powder adhering to the funnel. The bottle was capped tightly, swirled gently to wet all of the powder and let stand for 30 min. The contents were then swirled and centrifuged for 5 min at 3,000 rpm. The supernatant was decanted into a graduated cylinder or other suitable recptacle. The volume of extract obtained varied from slightly more than 20 ml to slightly less than 40 ml, depending on the fat or oil being studied, but usually was in the 30–40 ml range. The volume of extract that is obtained is unimportant, as the calculation is based on the original 50 ml of solvent. A convenient aliquot, usually 25 ml or, in the case of fats and oils relatively low in UM, 35 ml, was pipetted down the side of a 30-ml coarse-sintered glass funnel containing either (i) 2 g of Celite covered with about 0.5 cm of sand, or (ii) 3 g of alumina covered with 0.5 cm of sand. In either case the effluent was collected in a tared (analytical balance) aluminum dish. After draining, if (i) was used the sides of the funnel were washed with 5 ml of solvent followed by an additional 5 ml of solvent. If (ii) was used the sides were washed with 5 ml of 20% methanol in ethyl acetate, followed by 20 ml more of this solvent. The effluent was evaporated to constant weight on an explosion-proof hot plate at 40–45 C under a stream of nitrogen. The percentage of UM in the sample was calculated as follows:

$$\% \text{ UM} = 100 \times \frac{\text{wt of UM} \times 50}{\text{wt of sample} \times \text{vol of aliquot}}$$

RESULTS AND DISCUSSION

A number of variables essential to the development of a quantitative method for measuring and obtaining the UM from fats and oils were studied thoroughly. These included completeness of saponification of ester bonds, completeness of extraction of the UM from the soaps, and removal of, or correction for, any soap co-extracting with the UM.

Completeness of saponification of ester bonds. This was determined by saponifying all fats and oils at least three times at 130 C at 15-min intervals, for example, 45, 60 and 75 min or 45, 60, 75 and 90 min. Constancy in the value obtained for UM was taken to mean that saponification was complete. The approximate minimum time needed to completely saponify each fat or oil at 130 C was thus also established, as was the precision of the method. These data are presented in Table 1 for ca. 100 fats and oils listed alphabetically. All fats and oils examined were saponified completely in 60 min, the majority in 45 min, and only butter oil in 30 min.

The only oils listed in Table 1 that could not be handled by the procedure described above were mustard seed and crambe oils. The soap-Celite mixtures from both of these gelled and/or did not wet with the prescribed hexane:CH_2Cl_2 extraction solvent. The UM from both powders could be successfully extracted and subsequently quantitated by packing the powder in a chromatography tube and eluting with 125 ml of CH_2Cl_2. The effluent was concentrated to about 25 ml and then treated in the usual manner.

The effect of saponifying some fats and oils at a lower temperature was also studied. The conditions described by Schwartz et al. (5) for butter oil, and later applied by Maxwell and Schwartz (6) to other fats and oils, were used. These conditions were: porcelain mortar and pestle, heating at 100 C for 20–30 min, extraction of the soap-Celite powder in a column with CH_2Cl_2 and passage of the extract through a $CaCl_2$-Celite bed. The results are in Table 2.

Although several of the values obtained at 100 C were fairly close to those obtained at 130 C, some, notably soy, linseed, Canola and tall oils, were not. We studied the saponification of soy and linseed at 100 C for periods of up to 120 min and Canola for 90 min. At these times the value for the UM of soy was down to 0.57%, that of linseed oil was 0.96% and that of Canola was 1.19%, all of these values now approaching the 130 C figures. The data in Table 2 clearly indicate that some of the values reported previously (6) were too high due to incomplete saponification.

TABLE 2

Comparison of Saponifying Fats and Oils at 100 C and 130 C

Fat or oil	UM found at	
	100 C[a] %	130 C[b] %
Beef oil	0.24	0.17
Butter oil	0.33	0.30
Canola	3.28	1.17
Chicken	0.31	0.23
Cocoa	0.47	0.33
Linseed (boiled)	1.34	0.91
Peanut (U)	0.38	0.39
Soy (R)	1.06	0.49
Tall	3.11	2.20

[a]For 20–30 min.

[b]Average of ≥3 closely agreeing values.

TABLE 3

Comparison of Saponifying Some Fats and Oils at Room Temperature and at 130 C

Fat or oil	Unsaponifiable matter	
	R.T.[a] %	130 C[b] %
Almond	0.45	0.37
Apricot kernel (R)	0.53	0.44
Butter oil	0.33	0.30
Castor (R)	0.42	0.42
Cocoa butter	0.47	0.33
Corn (R)	1.70	1.07
Cottonseed (U)	0.80	0.61
Cottonseed (R)	0.68	0.60
Grapeseed (R)	0.58	0.55
Olive	0.84	0.61
Palm (R)	0.17	0.13
Peanut (U)	0.54	0.39
Sesame (U)	1.65[c]	1.38
Soy (R)	0.58	0.49
Sunflower (R)	0.92	0.65

[a]Value obtained at room temperature in 18-24 hr.

[b]Average of ≥3 closely agreeing values.

[c]Value obtained at 65 hr.

Early in the investigation, limited attempts were made to carry out the saponification step overnight (usually 18-24 hr) at room temperature. If successful, complete saponification under these conditions would not only have a definite monetary (energy-saving) advantage in routine analyses for UM, but would also enhance the potential for isolating otherwise heat-sensitive constituents in the UM. Results are in Table 3.

The data in Table 3 indicate that only four (butter, castor, grapeseed and palm) of the 13 oils subjected to room temperature saponification approach the ostensibly true value obtained at 130 C. However, room temperature saponification overnight as a general condition in an overall method for quantitating the UM of fats and oils would thus appear to be inadequate. Nevertheless, these conditions might lend themselves to the isolation of the UM from some fats and oils with only a relatively small amount of contamination with glycerides. Thus, subsequent fractionation of the UM for otherwise thermally-labile compounds could be rendered simpler than a method which completely circumvents a saponification step.

The saponification of both a cholesterol and a wax ester also was investigated. Cholesteryl stearate (60-64 mg) when spiked into coconut fat was saponified 90, 98, 97 and 94% respectively at 45, 60, 75 and 90 min at 130 C. Stearyl stearate (40-45 mg) was 92% saponified in 45 min and 99% in 60 min. Although the data in Table 1 indicate that most fats and oils appear to be saponified completely in 45 min, it is likely that some steryl and wax esters survive until heated for 60 min. As steryl and wax esters normally are minor components of most oils and fats, their incomplete saponification would not be reflected significantly in the weight of the UM obtained at 45 min.

Completeness of extraction of UM from the soaps. This was checked in three ways: By spiking of known components of UM into fats and oils and checking recovery by difference; by reducing the amount of lipid saponified while keeping the volume of extracting solvent constant [done on fats and oils relatively (>3%) high in UM]; and by extracting the UM from the soap in a column technique using a larger volume of, and also a more polar, solvent.

TABLE 4

Recovery of Lipids Added to Fats and Oils Prior to Saponification at 130 C

Lipid	Amount added (mg)	Fat or oil used	Recovery %
Docosane	25-32	Lard	96.0
Behenyl alcohol	29-33	Lard, palm	97.2
Cholesterol	40-57	Lard, chicken	95.0
Lanosterol[b]	26-39	Lard	96.4
Squalene	40-42	Lard	100.0
2-Nonadecanol	44-48	Palm	103.9
18-Pentatricontanol	43-47	Palm	96.7
1-Octadecanol	38-40	Coconut	96.6
b-Sitosterol	41-48	Coconut, palm	99.1
n-Octadecane	59	Palm	80.2[c]
Avocado UM	30	Coconut	99.0[c]
Soybean UM	22	Coconut	104.0[c]
2-Nonadecanone	47	Lard	82.2[c]
Eicosane	33	Lard	82.8[c]

[a]Average of 2-4 determinations.

[b]60% lanosterol + 40% dihydrolanosterol.

[c]Single determination.

Representative members of classes that occur in UM were spiked into fats and oils (all of which were naturally low in UM) and recoveries checked by difference following saponification. Results are in Table 4.

The data in Table 4 indicate the following: sterols, long-chain (≥ 18) primary and secondary alcohols, triterpene alcohols and long-chain (≥ C_{22}) hydrocarbons were recovered in near quantitative yield, but hydrocarbons (≤ C_{20}) and the C_{19} methyl ketone were not. The losses for both of these occurred during the saponification step, as no loss was observed when solutions of the compounds were evaporated under the recommended evaporation conditions. Shorter chain hydrocarbons (C_{16}) and methyl ketones (C_{13}) incurred losses during evaporation of the solvent.

The second technique for checking completeness of extraction of UM was to reduce the amount of oil saponified while keeping the volume of extracting solvent at the specified 50 ml. Those oils exceptionally high (> 3%) in UM were subjected to this technique. These were wheat germ, rice bran, alfalfa seed, jojoba and *Amaranthus cruentus* oils. Completeness of extraction was demonstrated in each case when essentially the same value was obtained for 1–2 g of saponified oil as was obtained for 10 g.

The third check for completeness of extraction of UM from the soap was to transfer the Celite-soap powder to a chromatography tube (33 × 3.8 cm) and to extract it with a total of 150 ml (50 ml rinsings + 100 ml methylene chloride). Following evaporation of solvent the residue was put over either Celite or alumina as described. No significant differences in the amount of UM were found between this technique and the recommended method, indicating complete extraction by the recommended static extraction procedure. The column technique can be used to isolate the UM from soap if a centrifuge is unavailable. It is, however, longer and uses more solvent.

Removal of soap co-extracting with the UM. The official methods for determining UM in fats and oils specify correction for soaps in the residue by titration. We found passage of the extract of UM over a small bed of alumina to remove the soaps more convenient than a titration correction. The conditions described were chosen to permit all of the common classes comprising the UM to be washed through, or eluted from, the alumina quantitatively while still retaining the soap. This was demonstrated using glyceryl ethers which were assumed to be the most polar class occurring in the UM, and stearic acid which was assumed to be adsorbed by alumina to the same extent as potassium stearate. The glyceryl ethers (tested in exaggerated high amounts) were quantitatively eluted using the conditions specified while the alumina held the stearic acid (10 mg/g, maximum) quantitatively.

The protocol followed to determine whether any soap was present in the UM of the oils and fats studied was to pass the aliquot taken from the supernatant of the centrifugate of the first saponification through the Celite bed (to remove any suspended particles), obtain the weight of UM, then quantitatively transfer the residue in the extraction solvent over a bed of alumina as described and reweigh the UM. If no loss was observed, extracts of soap from subsequent saponifications of that particular fat or oil were passed over Celite; if a loss had been observed, the Celite was omitted and alumina was used. Of course, alumina can be used for all fats and oils whether or not soap is present, but this incurs an unnecessary expense if soap should not be present. Whether Celite or alumina should be used for fats and oils studied is indicated in Table 1. For the quantitation of the UM of a lipid not listed in the table, the protocol outlined above is recommended.

TABLE 5

Comparison of Improved Method With the AOAC Method for Quantitating Unsaponifiable Matter of Fats and Oils

Fat or oil	AOAC method Before Al_2O_3 %	AOAC method After Al_2O_3 %	Improved method %
Butter	0.30	0.30	0.30
Canola	0.97	0.92	1.07
Chicken	0.47	0.17	0.17
Corn	1.40	1.27	1.09
Crambe	0.77	0.54	0.61
Neat's foot	1.09	1.01	1.04
Olive	0.99	0.72	0.62
Rice bran (China)	4.73	4.25	4.10
Rice bran (Hubei, China)	4.75	4.30	4.20
Rice bran (USA)	4.32	4.25	4.20
Safflower	0.60	0.44	0.42
Sesame	1.61	1.27	1.38
Soy	0.61	0.45	0.49
Tallow	0.89	0.89	0.90
Trisun	0.93	0.66	0.64
Trisun	0.54	0.38	0.35
Tung	0.64	0.33	0.31
V. galamensis	1.71	1.57	1.42
Walnut	.089	0.60	0.53
Wheat germ	6.20	4.97	4.98

Comparison of improved method with AOAC official method. The proposed method was compared with the official AOAC method (8) using a variety of fats and oils containing low and high concentrations of UM. In lieu of the titration step specified in the AOAC method, alumina was used as described above to remove the soaps, if present. The data are given in Table 5 and include the value for UM obtained by the official method prior to removal of the soap.

Miscellaneous observations. Marine oils did not lend themselves well to the procedure. Problems were encountered in the wetting of the soap-Celite powder with the extraction solvent. This was traced to the presence of the hexane. If methylene chloride alone was used as the extracting solvent and hexane added at the end of the 30 min static extraction period (to adjust density), gelation sometimes occurred. The use of the column technique and extraction of the bed with CH_2Cl_2 circumvented these problems, but large amounts of soap were extracted with the UM. This required large amounts of alumina (and consequently more solvent) to remove the soap, nullifying the advantages of the

method. Small (~1 g samples) of oils could be taken through the procedure as described, but the accuracy was questionable and the precision poor, as many marine oils contain < 1% UM.

Glass mortars were preferred to porcelain for conducting the saponifications. The soap was much more readily removed from glass for dispersion onto Celite. This was especially apparent with unetched glass. Etching will eventually occur, but this does not become evident until about 75 saponifications have been performed, and even etched glass was superior to porcelain in this regard. Glass also cools faster than porcelain, shortening the procedural time. Glass has the obvious advantage of facilitating visual inspection.

Saran was the preferred plastic wrap for sealing the mortar. Several other brands were tried, but these did not withstand the heating conditions and/or the vapor pressure and invariably broke. On rare occasions Saran, if stretched too tightly, also broke.

A single analyst can perform 8–10 completed saponifications in a normal working day using very little laboratory space.

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