UNSAPONIFIABLES IN FATS: QUANTITATIVE DETERMINATION

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Unsponfiables in fats have been investigated and a simple apparatus for countercurrent extraction has been developed. Quantitative determination of unsponifiables together with that of the total content of fatty acids and esters have been made.

The unsaponifiables, non-fatty impurities, are always present in certain percentages in fats. These create problems both in fat and food chemistry. The various types of substances in the unsaponifiables, such as sterols, phenols, hydrocarbons, fatty alcohols, waxes, vitamins, antioxidants, coloring matters and indicidental impurities, if not properly removed or treated to eliminate harmful effects, may cause complication in the human system through continuous ingestion along with foods. The fats and fatty impurities are also known to be at the root of faulty fat metabolism, [1-3] leading to serious consequences of atherosclerosis and perhaps heart failure. [4-5] The best procedure adopted in this investigation for tackling the problems of unsaponifiables was to determine their exact quantities. A very simple apparatus for countercurrent extraction has been developed and used in place of the official manual procedures of A.O.C.S.[6] and Agricultural Chemists[7] (A.O.A.C.). As usual with the unsaponifiables, the contents of total fatty acids (TFA) and total fatty ester (TFE) have also been determined.

The prerequisites taken as guide in developing the apparatus were as follows: (*a*) the extractor is simple to construct and easy to maintain; (*b*) it is low in cost; (*c*) the extractor may lend itself to a wide variety of applications; and it eliminates emulsion problems often encountered in manual procedures.

Extractor

The apparatus illustrated in Fig. 1 is self-explanatory. Despite its apparent simplicity, there may be difficulties in the sequence of setting up the apparatus. It is to be noted that the procedures described in the text are to be followed in the right order. The optimum conditions for the most efficient extraction require a balance between minimum agitation and maximum solvent dispersion. Vigorous agitation may give emulsions and certain amounts of carry-over of undesirable portions. The inner-tube tip must be over the magnetic bar for efficient extraction. A magnetic bar (1″ in length) will disperse the solvent without setting up a vigorous agitation at the solvent interface. The height of the tube above the 1-litre (wide-mouth) bottle and below the outlet arm, is critical in the sense that the column of solvent in this portion helps better separation of emulsion. The onset of emulsification can be controlled through experience by addition of small amounts of alcohol (2-3 ml. at a time) over a short period of time. Each fat may need special treatment according to its composition. Rubber stoppers are used for convenience. They are heated with 20% alkali for four hours and washed free from alkali before use. Our experience indicates that the rubber stoppers should be replaced every year. Ground-joints may be used instead, if possible.

Quantitative Determination of the Unsaponifiables

The procedures through the extractor developed are applicable to any type of fat. For a fat with high

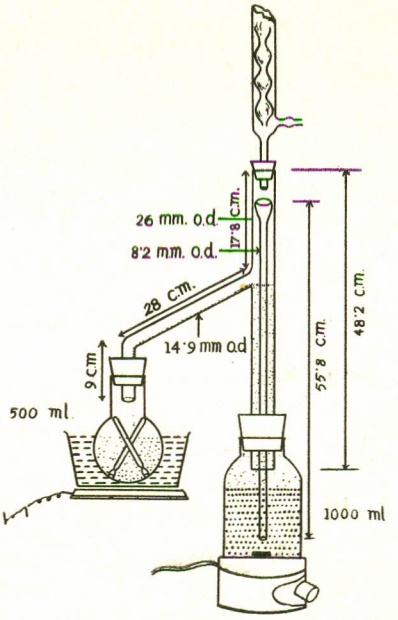

Fig. 1.—Extractor for determination of unsaponifiables.

amounts of unsaponifiables (over 7.0%) a slight increase in the alcohol percentage of the extractable solution, as noted in the foregoing section, may solve the emulsion problems. Three types of solvents, petroleum ether, ethyl ether and mixed solvent of petroleum ether+ethyl ether (1: 1) are chosen for trial in extraction. The mixed solvent being most efficient was employed in the procedures described below. The alcohol contents in soap stock solution for having no emulsion with the solvents are: petroleum ether, 33.0-37.5%, ethyl ether, 14.0-20.0%, and mixed solvent, 24.0-26.0%.

Apparatus and Reagents

The main apparatus as shown in Fig. 1; Erlenmeyer flasks, 250 ml., 2 separatory funnels, each 1 liter in capacity; 1-liter suction flask; and 50 ml. Erlenmeyer flask. Ethyl alcohol (absolute) distilled over KOH pellets and powdered zinc; aqueous potassium hydroxide solution, 100% KOH by weight (117.5 g. 85% KOH pellets in 100 ml. H_2O); ethyl ether (absolute) distilled over KOH pellets; petroleum ether (35-75°C.) distilled over KOH pellets; sodium hydroxide solution, 0.02N accurately standardized; and phenolphthalein indicator solution, 1.0% in 95% alcohol.

Procedures

An amount of fat between 9.0-10.0 g. was weighed accurately into the 250 ml. Erlenmeyer flask. To this was added by means of a syringe 80 ml. absolute alcohol, and 5 ml. of aqueous KOH. This was boiled steadily under a reflux condenser for one hour. Complete saponification was thus ensured.

The mixed solvent, ethyl ether+petroleum ether (1:1, 1000 ml.) was agitated with 24% alcohol (1250 ml.) and two phases were saved ($ether_2$ and $alcohol_2$ as stock solvents). The magnetic bar and 50 ml. $ether_2$ were introduced into the extraction bottle (Fig. 1) which was clamped to the rack of ring stands used for the extraction apparatus. The soap was carefully washed into the bottle with required portion of $alcohol_2$ (soap layer occupying 3/4th of the volume in the bottle) so that two layers resulted on stirring by magnetic system. $Ether_2$ was then added to bring the level to the neck of the bottle, without touching the stopper. The glass bumper and 500 ml. $ether_2$ were put into the stoppered 1/2-liter flask (Fig. 1) while setting up the extraction apparatus (without the condenser). Enough $ether_2$ was added to bring the level of solvent over the side arm. Now the condenser (with cold water flowing) was inserted in place, followed by necessary heating and stirring. Time for extraction with a heavy reflux (short of flooding) was limited to five hours.

The extract solution was carefully transferred to a 1-liter separatory funnel and washed three times with 100 ml. portions of 10% alcohol in distilled water. The first one was only swirled and the latter two were shaken. The combined wash liquid was extracted in the second separatory funnel, two times with 100 ml. portions of $ether_2$. This second extract was washed twice with distilled water.

The combined extracts were again twice washed with distilled water and dried over anhydrous sodium sulfate. The solvent was evaporated in 1-liter filtering flask under suction with a glass bumper (Fig.1) to a few ml. remaining. This was carefully transferred with ethyl ether to a 50-ml. Erlenmeyer flask and evaporated to dryness on steam of a water-bath under a gentle stream of clear and dry air. The drying was completed to constant weight. The flask with the unsaponifiables was warmed to 110°C. for five minutes in an oven and put while hot into a vacuum desiccator that was later subjected to the suction of a water pump for two hours and weighed. The vacuum oven drying method may also be used, if available.

The analysis and calculation were completed according to A.O.C.S. official method[8] which runs as follows :

After weighing, the residue was taken up in 50 ml. of warmed (ca. 50°C.) 95% alcohol containing indicator and previously neutralised to a faint pink colour. It was then titrated with 0.02N NaOH to the same colour.

Weight of fatty acids in the extract, in g.
=ml. of 0.02N NaOH $\times$0.0056

Unsaponificable matter, %

$$= \frac{\text{(Weight of residue - weight of fatty acids) } 100}{\text{Weight of sample}}$$

Results and Discussion

Quantitative estimation of the unsaponifiables is very important, before their nature is studied. The procedure developed in the present investigation is helpful for determination of the unsaponifiable contents in several fats of economic value in East Pakistan. Table 1 shows the comparative results from the extractions by three solvents. The mixed solvent consistently gave better results. Consequently, this solvent was used in the present investigations for the extrac-

TABLE 1.—EFFICIENCY OF SOLVENT IN EXTRACTION OF UNSAPONIFIABLES.

Samples		Petroleum ether (% by wt.)	Ethyl ether (% by wt.)	Petroleum ether + ethyl ether =1:1 (% by wt.)
Hydrogenated cottonseed oil	*a.*	0.53	0.90	1.23
	b.	0.57	0.94	1.19
	c.	0.55	0.97	1.17
Sesame oil		1.83	3.18	3 .56

TABLE 2.—UNSAPONIFIABLES IN DIFFERENT FAT SAMPLES.

Fat samples	Unsaponifiables	
	Percentage in fat	Presence of steroids
1. Market puti fish oil, (plain heating)	1.58	++
2. Solvent-extracted puti fish oil	2.35	++++
3. Oil from hilsa fish heads (solvent)	1.25	++++
4. Safflower oil ..	1.7	+++
5. Coconut oil ..	1.25	++
6. Coconut cake oil ..	2.20	+++
7. Sesame oil ..	3.56	++
8. Sesame cake oil ..	4.23	+++
9. Tamarind seed oil ..	3.80	++
10. Titgila oil ..	4.08	++
11. Groundnut oil ..	1.45	++
12. Mustard cake oil ..	4.85	+++

tion procedures. Table 2 summarizes the data on unsaponifiable contents which gave tests for steroids.

The main feature of the extractor is that it can be set up under any conditions of the laboratory; one-litre wide-mouth reagent or other bottle, rubber stoppers, glass tubes, half-a-litre flask, one small plate heater and one condenser are easily available. The magnetic stirrer may be arranged with one-inch magnetic bar and another rotating magnet (horse-shoe or otherwise) over a controlled motor underneath the bottle.

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