

Determination of the Unsaponifiable Matter in Fatty Acids by a Rapid Column Method

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ABSTRACT

A rapid, quantitative method for determining the unsaponifiable matter (USM) of commercial fatty acids is described. The fatty acid samples are saponified with a mixture of potassium hydroxide and Celite 545 ground together then heated for a short period. The resulting granular powder is transferred to a glass chromatography column containing a short section of CaCl_2 /Celite mixture, and the USM is eluted with dichloromethane. Samples were analyzed in 2 separate laboratories by the new method with slight variations. Good agreement between duplicates and between laboratories was obtained for all of the acids examined. Samples were also analyzed by Official Methods, and the results were uniformly somewhat higher by the proposed method.

INTRODUCTION

Commercial fatty acids contain varying amounts of dissolved unsaponifiable matter. The analytical methods used to measure the unsaponifiable matter (USM) in these substances are derived from procedures originally developed for fats and oils. The USM content of fatty acids is normally determined by AOCS Method TK 1a-64 (1) and that of tall oils by ASTM Method D-803 (2). Both methods involve lengthy, multiple-step extraction of the saponified solution with ethyl ether or petroleum ether. Many problems may be encountered during an analysis, including: incomplete extraction of the USM, troublesome emulsions, carry-over of soaps into the USM, and potential fire hazards due to the flammability of the solvents.

In a recent report in *JAACS*, a new method for the determination of the USM in fats and oils that differed significantly in principle from the traditional wet methods now commonly employed was described (3). Unlike the wet chemical methods, this "dry column" method required no solvents during the saponification step, and the extraction of the USM was accomplished with a nonflammable solvent. Higher average values for percentage USM were obtained by the proposed method than by the Official AOCS method.

Because a simple, rapid method is also needed to assay the unsaponifiable matter of commercial fatty acids, in continuation of this work we initiated a subsequent study to apply the dry column method to such substances. The method, as proposed for fats and oils, was found to be ineffective for fatty acids. We subsequently developed a modified method, applicable to fatty acids, which we present here. Determinations by the modified method were done in 2 laboratories at separate locations, and the results were compared with those obtained by Official Methods.

EXPERIMENTAL

Materials

We used potassium hydroxide pellets, ACS grade, J.T. Baker Chemical Co., Phillipsburg, NJ; anhydrous calcium

chloride pellets, J.T. Baker Chemical Co.; Celite 545 (grade: not acid-washed C-212), Fisher Scientific Co., King of Prussia, PA; and dichloromethane, distilled in glass, Burdick and Jackson Laboratories, Muskegon, MI. The dichloromethane was residue-free.

Apparatus

We used a porcelain mortar of 400 mL capacity (Coors no. 14); glass pestle, 15 cm length, with smooth sides and no lip; glass jars, screw-neck, 4 oz, having plastic lids with liner removed; tamping rod; teaspoon, stainless steel; and glass chromatography column, 30 mm id and 30 cm overall length having a drip tip 5 cm \times 8 mm od; or a vacuum-assisted chromatography column, prepared by Van Gillum Scientific Glass, Memphis, TN, depicted in Figure 1.

Preparation of Calcium Chloride/Celite Mixture

The mixture may be prepared in advance in a mortar of suitable size. To 1 part anhydrous calcium chloride, add 1 part distilled water and blend with pestle, then add 3 parts of Celite 545 in batches and grind to uniformity. The mixture is stored covered in an amber jar and should be used within a month of its preparation.

Preparation of Potassium Hydroxide/Celite Mixture

If multiple analyses are to be performed, this mixture is prepared in lots of 75 g or more. In a large mortar, 2 parts potassium hydroxide pellets are ground with one part distilled water. Four parts Celite 545 are then added to the contents of the mortar and the mixture is ground until uniform. The mixture is stored in an amber jar and should be used within 10 days of its preparation.

Saponification Procedure

The potassium hydroxide/Celite mixture (10 g) is placed in the 400-mL mortar. The fatty acid sample is weighed accurately (to 0.1 mg) and transferred quantitatively to the bed of KOH/Celite in the mortar. A 5-g sample of fatty acid is used if the expected USM content is $<1\%$ or a 2-4-g sample if the expected value is $>1\%$. The mixture is ground until the sample is distributed throughout the Celite, then a second batch (10 g) of the KOH/Celite is added to the contents of the mortar; the mixture is reground until uniform and then is transferred with the teaspoon from the mortar to the 4-oz jar. Residual sample is recovered with 5 g of Celite 545 placed in the mortar and swept along the sides with the pestle. This mixture is also placed in the jar. The jar is capped and shaken briefly until the mixture is uniform. The cover is then loosened and the jar is heated for 20-30 min in an oven set at 130 C.

Gravity Extraction Procedure

The heated jar is removed from the oven and its contents are returned to the mortar as soon as the jar can be handled comfortably. The mixture is reground (about 0.5 min) until it is of uniform granular consistency. The glass chromatog-

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raphy column is loosely fitted with a plug of glass wool in its drip tip and then is charged with 5 g of the $\text{CaCl}_2/\text{Celite}$ mixture, which is compressed tightly with the tamping rod. A tared, 150-mL tall form beaker is then placed under the column. The contents of the mortar are transferred to the column with the teaspoon, and the column packing is compressed with the tamping rod to a depth of 50-60 mm to permit uniform solvent flow. Residual amounts of sample mixture are washed from the spoon, tamping rod, pestle, jar and lid over the mortar with about 25 mL of dichloromethane. The washings are swirled in the mortar and then quickly pipetted into the column along the walls. After the rinsings have percolated into the column bed, additional dichloromethane is added along the column walls until the entire bed is wet and a few drops of eluate have been collected in the beaker. Then, the column is charged with 150 mL of dichloromethane, and the entire volume is collected (in about 25 min) in the beaker. The column may be checked for completeness of extraction with an additional 20 mL of dichloromethane. This second fraction is collected separately, evaporated to dryness, and examined for residue. If any residue is observed, the procedure is repeated with an additional 20 mL of dichloromethane.

Solvent from the main fraction may be removed under a stream of nitrogen with gentle heating while the eluate is being collected. The contents of the beaker are taken to constant weight under vacuum, and the weight of the residue in the beaker is used to calculate the percentage USM in the sample.

Vacuum-Assisted Extraction Procedure

This procedure differs from the above in column packing, solvent elution and drying steps. Because a fine glass frit is part of the modified chromatography column, no glass wool is used in the column tip. The $\text{CaCl}_2/\text{Celite}$ mixture is packed (55 mm deep) to fill the 18 mm id portion of

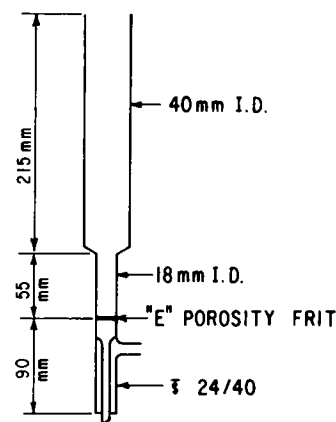


FIG. 1. Vacuum-assisted chromatography column.

the column (see Fig. 1). After the contents of the mortar are transferred to the column, they are compressed to a depth of about 50 mm. Dichloromethane washings and fresh solvent are placed on the column as before; however, the rate of solvent flow is adjusted via aspirator vacuum so that a consistent elution time of about 10 min is attained for each sample. Solvent is allowed to collect directly into a tared, 250-mL round-bottomed flask (24/40 joint). The solvent is removed and the sample is taken to constant weight on a rotary evaporator at about 60 C under 30 mm vacuum (normally 15-20 min).

Examination of the USM for Soap Residue

The method used to determine the presence of soap has been described elsewhere (3). With this procedure, the amount of soap found in any of the USM residues from the fatty acids listed in Table I was less than 60 μg .

TABLE I

Determination of the Unsaponifiable Content of Commercial Fatty Acids by Two Methods

Fatty acid principal component(s)	Origin	Dry column		AOCS ^b (% USM)
		Laboratory A (% USM) ^a	Laboratory B (% USM) ^a	
90% 8:0 ^c	Coconut	0.20	0.22	0.08
95% 12:0	Coconut	0.21		0.11
90% 14:0	Coconut	0.55	0.63	0.48
80% 16:0	Palm kernel	0.45	0.37	0.31
57% 18:0	Tallow	0.37	0.41	0.32
50% 16:0; 45% 18:0	Tallow	1.12	1.09	0.74
27% 16:0; 70% 18:0	Tallow	0.26	0.26	0.12
14:0, 16:0, 18:0	Mixed	1.16	1.14	0.79
80% 18:1; 10% 18:2	Tallow	0.93	0.61	0.44
57% 18:2	Soy	0.60	0.61	0.54
25% 18:1; 60% 18:2	Corn ^d	0.31	0.34	0.26
28% 16:0; 23% 18:1; 41% 18:2	Cottonseed	3.10	3.24	2.38
92% 22:1	Rapeseed	1.48	1.28	1.17
16:0, 16:1, 18:1, 18:2, 20:0, 20:1	Menhaden	2.34	2.18	1.82
30% 16:0; 30% 18:0; 30% 22:0	Herring	0.90	0.87	0.44
20% 16:0; 34% 18:1; 10% 20:1; 10% 22:0	Dogfish liver ^d	17.74	18.25	9.25
50% 18:1; 45% 18:2	Tall oil ^d	0.95	0.87	0.49
50% 18:1; 45% 18:2	Tall oil ^d	1.49	2.46	1.14
30% 18:1; 25% 18:2 ^d	Tall oil distillate	32.90	32.80	24.80
80% 36:1 (Dimer acid) ^d	Tall oil	0.20	0.19	0.15

^aAverages of duplicate determinations.

^bAOCS TK 1a-64 (ref. 1).

^cNumber of carbons:number of double bonds.

^dRequired 30-min heating period.

RESULTS AND DISCUSSION

When the dry column method previously proposed (3) was initially applied to most commercial fatty acids, several problems were encountered: the sample/Celite mixture formed a lumpy rather than a free flowing powder; the flow of solvent through this mixture in the column was extremely slow; suspended matter was carried through into the eluate along with the USM. These problems may, in part, be due to the rapid reaction of soap formation from fatty acids in the presence of strong base resulting in nonuniform distribution of soap over the Celite matrix. Since the method, as developed for fats and oils, could not be used directly for most fatty acids, we explored several modifications. The procedure found to be satisfactory for fatty acids differs from the original in the following manner: a fat or oil is first blended with an aqueous KOH solution, heated, then blended with Celite 545, whereas fatty acids must be ground together with a preformed mixture of KOH/Celite 545 prior to being heated. We also found that the modified fatty acid procedure requires less elapsed time for most fatty acids (50-60 min) than the dry column method proposed for fats and oils (3) partly because more rapid and uniform solvent flows through the column bed.

The dry column method can be used for the determination of the USM in liquid or solid fatty acid. It is only necessary to insure uniform distribution of the fatty acid over the KOH/Celite mixture by thorough grinding before they are heated. Soap formation was complete in all cases during the 20-30 min heating period, regardless of sample composition. Heating of this mixture is necessary for uniform distribution of the soap over the Celite matrix. Since good results are dependent on the sample mixture being heated at 130 C for at least 20 but no longer than 30 min, we suggest that, if possible, a mechanical convection rather than a gravity oven be used for these determinations.

Occasionally, solvent elution time with certain higher molecular weight fatty acid samples was quite long (30-40 min) with a gravity feed column. To minimize analysis time with these samples as well as to make all solvent flow rates uniform, we developed the partial vacuum technique. With use of the modified chromatography column (Fig. 1), an aspirator vacuum can be adjusted to give the desired flow rate. The diameter of the bottom portion of the column is restricted somewhat to give a greater CaCl_2 /Celite bed depth and thus allow a longer contact time with

the eluent. The use of a rotary evaporator with a hot water bath is convenient in that solvent removal and sample drying under vacuum are accomplished in one step.

Samples listed under laboratory A were eluted with a partial vacuum while those under laboratory B were eluted from columns by gravity (Table I). A comparison of these results shows that close agreement was obtained, regardless of the elution technique employed. Also, good agreement between laboratories was found over a wide range of fatty acids differing significantly in composition, physical properties and USM content (0.19-33%).

Samples of the USM residues were checked for the presence of soap by a method reported earlier (3). Only negligible amounts of soap (<0.1% of the USM) were detected in any of the residues examined, thus obviating the need for a titration to correct for these contaminants. We previously determined, however, that slight amounts of soap may contaminate the USM if the CaCl_2 /Celite trap is not in place below the column bed (3). Recovery studies were also done to determine whether major constituents of the USM such as sterols are isolated quantitatively by the dry column method. Cholesterol was added to oleic acid (+99.9%), the mixture was saponified, and the USM was recovered by the described procedure. Duplicate experiments gave quantitative recovery of cholesterol.

The fatty acid samples also were analyzed by AOCS Method TK 1a-64 (Table I). For most examples, higher values were obtained by the dry column than by Official Method, possibly for reasons noted in the Introduction. These differences were especially apparent for samples such as dogfish liver fatty acids and tall oil acid distillate. Because contaminants were undetectable, these limited comparative data indicate that the results obtained by the dry column method are more representative of the actual USM content of fatty acids than those determined by the Official Method.

REFERENCES

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