

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/239233631>

Modified method for the determination of unsaponifiable matter in oils and fats

ARTICLE *in* THE ANALYST · AUGUST 1994

Impact Factor: 4.11 · DOI: 10.1039/an9941901793

READS

212

3 AUTHORS, INCLUDING:



[Suely Pereira Freitas](#)

Federal University of Rio de Janeiro

57 PUBLICATIONS 218 CITATIONS

SEE PROFILE

Modified Method for the Determination of Unsaponifiable Matter in Oils and Fats

Leopold Hartman, Hudson Soares Viana and Suely Freitas

National Centre of Food Technology Research, CTAA-EMBRAPA, Avenida das Américas 29.501, Guaratiba, Rio de Janeiro RJ, 23020-470, Brazil

An inversion of the existing methods for the determination of unsaponifiable matter in oils and fats is proposed. The modified procedure consists in saponifying a 2–2.5 g sample of oil or fat with 2 mol l⁻¹ ethanolic potassium hydroxide solution, dissolving the resultant mixture in 50 ml of pure cyclohexane, removing the soap by addition of 25 ml of water containing 0.5 g of sodium hydrogencarbonate and extracting the separated soap layer with 25 ml of cyclohexane. The combined cyclohexane layers are washed twice with 25 ml portions of 50% aqueous ethanol, cyclohexane is evaporated and the residue is dried *in vacuo* at 80 °C. Results obtained with the above procedure in the determination of the unsaponifiable matter in four different vegetable oils were in agreement with those given by the IUPAC standard hexane method II.D.5 with economy of solvents and operational time.

Keywords: Unsaponifiable matter; oil and fat; saponification; cyclohexane

Introduction

The unsaponifiable matter in oils and fats, which consists mainly of hydrocarbons, sterols and aliphatic alcohols of high molecular mass, serves for the identification of oils and fats and for the detection of incidental and intentional impurities (e.g., falsification with mineral oils). All current methods of its determination include the saponification of the sample with ethanolic potassium hydroxide dilution with water and extraction with solvents such as diethyl ether, light petroleum or hexane.^{1–4} Because of the solubility of the unsaponifiable matter in ethanolic alkaline soap solution, multiple extractions are necessary and this, together with the removal of the entrained soap and the drying of the final residue, requires a long time and considerable amounts of reagents. Pelloquin *et al.*,⁵ after examining 13 international methods, recommended two procedures, one requiring nine extractions with hexane and the other three extractions with diethyl ether, with numerous washings in both instances.

A casual observation that a methanolic soap solution is soluble in diethyl ether and an ethanolic soap solution is soluble in hexane has led us to an attempt to invert the current methods for the determination of unsaponifiable matter. The inversion consisted in removing the soaps from a cyclohexane solution of the unsaponifiable matter, instead of extracting this matter from an ethanolic aqueous soap solution (cyclohexane having been adopted as the solvent for reasons to be stated subsequently).

Several procedures were investigated. A fat sample was saponified with methanolic potassium hydroxide and the product obtained was dissolved in 50 ml of diethyl ether, followed by an attempt to extract the soaps with water. The attempt was unsuccessful, because the dilution with water produced three phases instead of the expected two, the upper ethereal phase being small. Satisfactory results were achieved when an ethanolic soap solution was dissolved in hexane

followed by the extraction of the soaps with water. Similar results were obtained with the less volatile cyclohexane, which was adopted as the solvent on safety grounds (reduced fire risk) and for environmental reasons.

Experimental

Materials

Samples of crude soya bean, maize, babassu and palm oils were used.

Apparatus

The apparatus used consisted of 250 ml flat-bottomed and round-bottomed flasks with ground joints, separating funnels, a rotary evaporator, a water pump (aspirator), a 2 ml pipette, measuring cylinders of 10, 25 and 50 ml, a water-bath and a hot-plate. Grease should not be used for lubricating the ground-glass joints and stopcocks.

Reagents

A 50% m/m aqueous solution of pure potassium hydroxide, cyclohexane (analytical-reagent grade), 95% ethanol, a 50% v/v mixture of ethanol and water and sodium hydrogencarbonate were used.

Procedure

A 2–2.5 g amount of a well mixed oil sample is weighed accurately in a dry, 250 ml flat-bottomed flask with a ground joint, 10 ml of 95% ethanol are added with a measuring cylinder, 2 ml of 50% aqueous potassium hydroxide are added with pipette, followed by two pieces of pumice, and the mixture is boiled gently under reflux on a hot-plate for 30 min. To the hot mixture 50 ml of cyclohexane are added with a measuring cylinder and shaken. The mixture becomes completely clear. To the mixture 25 ml of distilled water containing 0.5 g of sodium hydrogencarbonate are added with a measuring cylinder and the mixture is shaken well. If an emulsion occurs, the flask is heated under reflux to incipient boiling of its contents, until a separation of the phases takes place. The hot mixture is transferred quantitatively into a separating funnel and after settling, which requires a few minutes, the soap layer is returned to the flask followed by the addition of 25 ml of cyclohexane with a measuring cylinder and vigorous shaking. Any emulsion is removed by heating the flask again under reflux. The hot mixture is transferred quantitatively into a second separating funnel, the bottom soap layer is discarded and the upper layer is added to the first separating funnel. The combined cyclohexane layers are washed twice with 25 ml of 50% v/v ethanol–water each time, adding 5 ml of the washing solution without shaking and, after its removal, the remaining 20 ml with shaking for 1 min.

The second washing solution usually does not produce any colour on addition of phenolphthalein. If it does, a third washing with 25 ml of 50% v/v ethanol–water should be applied. The washed solution is transferred into a weighed 250 ml round-bottomed flask and the solvent is distilled off in a rotary evaporator. The flask is then connected with a water pump (aspirator), immersed in a water-bath and heated under vacuum at 80 °C for 30 min. The flask is cooled under vacuum by immersing it in a beaker filled with cold water, the vacuum is carefully released and the flask wiped dry with a piece of cloth, placed for a few minutes in a desiccator and weighed. Another drying period of 15 min *in vacuo* usually produces a constant mass. A blank determination is carried out in a similar manner but omitting the oil or fat.

The amount of the unsaponifiable matter is calculated according to the equation

$$\text{unsaponifiable matter (\%)} = \frac{m_1 - m_2}{m} \times 100$$

where m_1 is the mass of dry residue (g), m_2 the mass of the residue obtained with the blank (g) and m the mass of the fat sample (g).

Results and Discussion

The four different oils considered were analysed using the IUPAC II.D.5 standard hexane method and the modified procedure described here. The results of triplicate analyses of each oil are given in Table 1.

The ratios of the averages of the results obtained by the IUPAC and the modified procedure demonstrate good agreement between the two methods. The IUPAC method required nine extractions with 50 ml of hexane each time, three washings with 150 ml of 50% aqueous ethanol each time and titration of the residue with 0.01 mol l⁻¹ hydrochloric acid in acetone owing to the presence of soap. In the modified procedure, the soap was removed from the unsaponifiable matter dissolved in 50 ml of cyclohexane by the addition of 25 ml of water containing 0.5 g of sodium hydrogencarbonate. One extraction of the soap layer with 25 ml of cyclohexane was required to recover the small amount of the unsaponifiable matter entrained by the soap layer. Two washings with 25 ml of 50% aqueous ethanol were needed to remove the excess of alkali and there was no need to titrate the residue with dilute hydrochloric acid. Hence the inversion of the existing methods for the determination of unsaponifiable matter resulted in considerable decreases in the working time and the amounts of reagents used.

Another time-saving device is the suggested moderate heating to speed up the separation of phases when emulsions are formed, a procedure that does not produce any deleterious effects. Finally, in order to decrease the number of time-consuming blank determinations, it is advisable to reserve at least 1000 g of cyclohexane for the determination of the unsaponifiable matter, which makes it possible to carry out 15 determinations with only one blank assay. The mean mass of the residues obtained when using 75 ml of cyclohexane in the blank assay was 0.0064 g.

Results of analyses performed in duplicate on five consecutive days using crude soya bean oil compared with those obtained by the IUPAC method are given in Table 2. The standard deviations are low (0.01 and 0.03%) and as the mean values are identical there is no statistical difference between the standard method and the modified procedure.

To examine the accuracy of the modified procedure, the following test was carried out. The soaps resulting from the analyses performed according to the IUPAC method and therefore free of unsaponifiable matter were acidified with hydrochloric acid and the liberated fatty acids were extracted with diethyl ether. The ethereal solution was washed with water, the solvent removed in a rotary evaporator and the residue dried *in vacuo*. To approximately 2 g samples of the recovered fatty acids various amounts of pure tetradecan-1-ol were added and the amounts of unsaponifiable matter were determined according to the modified procedure. The results are given in Table 3.

References

- 1 IUPAC Standard Methods for the Analysis of Oils, Fats and Derivatives, 6th edn., Determination of Unsaponifiable Matter, Hexane Method, II.D.5, Pergamon Press, Oxford, 1979.

Table 1 Determination of the unsaponifiable matter in four vegetable oils according to the standard IUPAC method and the proposed modified procedure

Oil	Unsaponifiable matter (%)		Average of results A Average of results B
	IUPAC method (A)	Modified procedure (B)	
Soya bean	0.66	0.67	1.00
	0.71	0.70	
	0.69	0.68	
Maize	1.79	1.78	1.00
	1.73	1.73	
	1.78	1.77	
Babassu	0.20	0.21	0.99
	0.25	0.23	
	0.24	0.26	
Palm	0.72	0.78	1.00
	0.75	0.71	
	0.76	0.74	

Table 2 Statistical treatment of the results for the determination of unsaponifiable matter in crude soya bean oil

IUPAC method			Modified procedure		
Unsaponifiable matter (%)	Mean (%)	Standard deviation (%)	Unsaponifiable matter (%)	Mean (%)	Standard deviation (%)
0.66	0.69	0.03	0.68	0.69	0.01
0.71			0.67		
0.69			0.67		
			0.69		
			0.70		
			0.69		
			0.68		
			0.71		
			0.67		
			0.70		

Table 3 Recovery of unsaponifiable matter using the modified procedure

Fatty acids/g	Tetradecan-1-ol added/g	Dried residue after subtraction of the blank/g
2.0082	0.0185	0.0181
2.0154	0.0253	0.0255
2.0443	0.0345	0.0344
2.0017	0.0422	0.0417

-
- 2 *Official and Tentative Methods of the American Oil Chemists' Society*, AOCS Champaign, IL, Official Methods Ca 6a-40 and Ca 6b-53, 1980.
 - 3 *BS 684: 1958. Determination of Unsaponifiable Matter*, British Standards Institution, London, 1958.
 - 4 *DGF—Einheitsmethoden*, Determination of the Unsaponifiable Matter C-III 1a(77) and C-III 1b(77) VCH, Stuttgart, 1984.
 - 5 Pelloquin, A., Dimitriadis, C., and Naudet, M., *Rev. Fr. Corps Gras*, 1977, **24**, 551.

Paper 3/07033F

Received November 26, 1993

Accepted February, 1, 1994