condenser are minimal. By continuous addition of a certain quantity of make-up water to the system, a corresponding amount of water is removed. This water, however, meets the most demanding standards and may consequently be drained directly to the river.

The standards imposed by most European countries for cooling water that may be drained into the rivers are given below. You will note that these standards are fairly similar to those prevailing in North America: (a) pH between 6.5 and 8.5; (b) dissolved oxygen content of at least 4 mg per litre; (c) temperature may not exceed 30 C (86 F); (d) SS (suspended solids) 30 ppm higher than the SS of influent; (e) BOD_5 (biological oxygen demand over 5 days) not exceeding 30 mg/L and COD (chemical oxygen demand) not exceeding 60 mg/L.

The first four conditions are easily met; the fifth depends directly on the fatty content of the water. The wastewater drained from a plant equipped with the double condensation system described above contains a maximum of 8 ppm of fatty matter which corresponds to less than the COD standard of 60 mg/L allowed for wastewater.

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Use of Unsaponifiable Matter for Detection of Ghee Adulteration with Other Fats

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ABSTRACT

Gas liquid chromatography (GLC) was used for the detection of lard and margarine added to buffalo and cow ghee. The chromatograms of the unsaponifiable matter could be divided into two parts representing hydrocarbons and sterols. Hydrocarbons were fractionated by GLC into 3-6 different compounds depending on the lipid origin. The sterols were cholesterol and β-sitosterol. The content of cholesterol in lipid samples was in the following decreasing order: cow > buffalo > lard > margarine. With β-sitosterol, the concentration order was: margarine > buffalo > cow > lard. The ratios of total hydrocarbons to total sterols in the unsaponifiable matter for margarine and lard were the most different for the various lipids. Adulteration of cow and buffalo ghee with various levels of lard or margarine caused significant changes in the unsaponifiable compounds. It is possible to determine the extent of admixture of lard or margarine to either cow or buffalo ghee by applying a simple regression equation for each unsaponifiable component. Hence, an examination of unsaponifiable matter appears to provide a rapid and simple laboratory method for the detection of ghee adulteration.

INTRODUCTION

There is a growing need for thorough and reliable information on the nutrient composition of all human foods. Sources of dietary fat have been changing continuously during the past two decades. Consumption of vegetable oils has risen and there has been a shift from butter to margarine and an increase in the use of solid fat for cooking. Owing to the high price of butter, unethical suppliers adulterate butter with other lipids which are similar in structure and less expensive. Various methods have been proposed for detecting the presence of foreign fats in dairy products. The differences in the melting diagrams and crystallization patterns of various lipids, as determined by differential thermal analysis, provide a basis for the determination of adulteration in cow or buffalo ghee (1). Such adulteration causes a significant change in the concentrations of certain fatty acids (2,3,4). Adulteration can be detected by the changed ratios of some fatty acids in the lipid extracts (4,5). The detection of adulteration of oils and fats by sterol analysis have been reported for: vegetable fats in milk fat (6), margarine in butter (7), other vegetable oils in olive oil (8) and animal fats in vegetable fats and oils (9).

Procedures for isolating and separating sterols from butter and margarine by Florisil column or thin layer chromatography (TLC) followed by gas chromatography (GLC) of the sterol fraction has been used to characterize lipid adulteration with plant fats (7,10). The present paper describes the examination of unsaponifiable components directly by gas liquid chromatography (GLC) without use of other preliminary chromatographic methods. This approach permits examination of both hydrocarbons and sterols for detecting the presence of lard or margarine in cow or buffalo ghee.

MATERIALS AND METHODS

Sources of Samples

Pure cow and buffalo ghee were obtained from the Food Science and Technology Department, Dairy Science Division, Faculty of Agriculture, Cairo University. Margarine and crude lard were purchased from the Abu Zaabal Company and the local market, respectively. The dissected lard was heated at 70 C and the melted fat was filtered while warm through Whatman no. 1 filter paper to obtain tissue-free and water-free lard lipids. These lipid samples can be considered as an authentic materials. Mixtures were prepared containing 5, 10, 15, 20, 25 and 30% (w/w) of margarine or lard in cow or buffalo ghee.

Sources of Authentic Hydrocarbons and Sterols

A set of hydrocarbons (*n*-eicosane, *n*-docosane, squalene, *n*-triacontane and *n*-dotriacontane) and sterols (cholesterol, campesterol, brassicasterol, stigmasterol, β-sitosterol, stigmasterol and fucosterol) was purchased from Sigma Chemical Company. The purity of these compounds was checked by GLC; each compound gave one peak. The relationship between log retention times of hydrocarbons and their number of carbon atoms was used to characterize unknown hydrocarbons (*n*-hencosane, *n*-tricosane, *n*-tetracosane,

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TABLE I

Composition (%) of the Unsaponifiable Matter of Cow Ghee, Buffalo Ghee, and Lard Adulterated Samples

Extent of admixture		C_{28}	C_{29}	C_{31}	Cholesterol	β-Sitosterol	TH/TS[a]
Cow ghee	Lard						
100	0	1.0	10.2	0.4	87.4	1.0	0.13:1
95	5	1.0	13.0	0.6	84.5	0.9	0.17:1
90	10	0.9	15.8	0.8	81.6	0.9	0.21:1
85	15	0.9	19.3	1.0	78.0	0.8	0.28:1
80	20	0.8	21.8[b]	1.2[b]	75.4	0.8	0.31:1
75	25	0.8	25.0[b]	1.4[b]	72.1	0.7	0.37:1
70	30	0.7	27.1[c]	1.4[c]	70.1[b]	0.7	0.41:1
0	100	0.0	68.2	4.0	27.7	0.1	2.59:1
Buffalo ghee	Lard						
100	0	3.3	16.2	0.6	78.1	1.8	0.25:1
95	5	3.2	18.7	0.8	75.6	1.7	0.29:1
90	10	3.0	21.5[b]	0.9	73.0	1.6	0.34:1
85	15	2.8	24.1[c]	1.2	70.4[b]	1.5	0.39:1
80	20	2.6	26.8[c]	1.3	67.9[c]	1.4	0.44:1
75	25	2.4	29.5[c]	1.5	65.3[c]	1.3	0.50:1
70	30	2.3	29.5[c]	1.7	65.2[c]	1.3	0.50:1
0	100	0.0	68.2	4.0	27.7	0.1	2.60:1

[a]Ratio of total hydrocarbons to total sterols.
[b]Mixtures are significantly different from the pure ghee at 5% level.
[c]Mixtures are significantly different from the pure ghee at 1% level.

n-pentacosane, *n*-hexacosane, *n*-heptacosane, *n*-octacosane, *n*-nonacosane and hentriacontane).

Other Reagents

All solvents used were Analytical Reagent Grade and redistilled before use.

Extraction of Unsaponifiable Matter

Lipid samples were saponified with methanolic KOH (20%, w/v) for 24 hr at room temperature in the dark under atmospheric nitrogen. The unsaponifiable matter was extracted three times with petroleum ether (bp, 40/60 C). The combined extract was washed several times with distilled water until the washings were neutral to phenolphthalein indicator and then dried over anhydrous sodium sulfate.

Separation of Unsaponifiable Matter by GLC

The unsaponifiable substances in the lipid samples and the authentic compounds were analyzed with a GCV Pye Unicam gas chromatograph equipped with dual flame ionization detectors. A coiled glass column (2.8 m × 4 mm) was used, packed with acid-alkali and silanized Diatomite C (100-120 mesh) and coated with 1% OV-17. For separating the unsaponifiable materials injector, column and detector temperatures of 280, 270 and 300 C, respectively, were used. The gas flow rates were 30, 33 and 330 mL/min for nitrogen, hydrogen and air, respectively. Recorder chart speed and range were 1 min/1 cm and 32×10^2, respectively. Under these conditions each sample was completely resolved in about 10 min.

Peaks were identified by comparing the relative retention times with those of standard materials. The values of relative retention times of C_{20}, C_{21}, C_{22}, C_{23}, C_{24}, C_{25}, C_{26}, C_{27}, C_{28}, C_{29}, squalene, C_{30}, C_{32}, cholesterol, brassicasterol, campesterol, stigmasterol, β-sitosterol, stigmasterol and fucosterol were 0.03, 0.06, 0.08, 0.12, 0.15, 0.18, 0.20, 0.23, 0.26, 0.29, 0.33, 0.37, 0.64, 1.00, 1.13, 1.26, 1.35, 1.53, 1.56 and 2.18, respectively. Peak areas were measured by triangulation and the relative proportions of the individual compounds were estimated as the ratio of the partial areas to the total area.

Statistical Analysis

Analysis of variance and the least significant differences (LSD) values were calculated in order to show the influence of adulteration on the unsaponifiable matter composition of pure lipid samples. Simple correlations and regressions were used to demonstrate the relation between the different adulteration levels and various unsaponifiable components. Percentages were transformed using arcsine transformation for correlation analysis (11).

RESULTS AND DISCUSSION

The composition of the unsaponifiable portion of various lipid samples is shown in Tables I and II. The hydrocarbons of the unsaponifiable fraction were separated by GLC into 3-6 compounds depending upon the lipid origin. Only two sterols, i.e., cholesterol and β-sitosterol, were present in detectable concentrations in any of the lipid samples. The amount of a particular compound refers to its percentage distribution in the total unsaponifiable matter.

n-Octacosane (C_{28}) occurred in all the samples except lard. The levels of *n*-nonacosane (C_{29}) were relatively high, the lowest and highest values being 4.1% and 68.2% in margarine and lard, respectively. In contrast, the C_{29} contents of pure cow and buffalo ghee were 10.2% and 16.2%, respectively. High concentration of this compound therefore seems to be characteristic of lard. Margarine contained *n*-triacontane (C_{30}), UC_{32} and dotriacontane (C_{32}) as minor (> 1% − < 10%) and major (> 60%) constituents, respectively. UC refers to a hydrocarbon having a definite number of carbon atoms with unknown molecular formula. There was no C_{32} in ghee (cow or buffalo) and as much as 3% in 5% margarine in ghee. Hence, *n*-dotriacontane (C_{32}) could be used to indicate the presence of a margarine adulterant in other lipids. Three major hydrocarbons from butter fat were identified as the C_{20} compounds (phyt-1-ene, phyt-2-ene and neo-phytadiene). The following hydrocarbons were also present: 2,6,10-trimethyl tridecane, *n*-pentadecane, *n*-hexadecane, 5-methyl hexadecane, *n*-heptadecane, *n*-octadecane and phytane (12).

The spectrum of sterol compounds in the lipid samples investigated here was simpler than that of the hydrocar-

TABLE II

Composition (%) of the Unsaponifiable Matter of Cow Ghee, Buffalo Ghee, Margarine and Margarine Adulterated Samples

Extent of admixture		C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	UC_{32}	Cholesterol	β-Sitosterol	TH/TS[a]
Cow ghee	Margarine									
100	0	1.0	10.2	0.0	0.4	0.0	0.0	87.4	1.0	0.13:1
95	5	1.0	9.8	0.1	0.8	3.3	0.2	83.0	1.8	0.18:1
90	10	1.0	9.4	0.2	1.2	6.2	0.3	79.2	2.5	0.22:1
85	15	1.0	9.1	0.3	1.5[b]	10.5[c]	0.5[b]	73.6[b]	3.5	0.30:1
80	20	1.1	8.8	0.5	1.9[c]	14.6[c]	0.6[c]	68.5[c]	4.0	0.38:1
75	25	1.1	8.5	0.6	2.2[c]	15.6[c]	0.8[c]	66.1[c]	5.1[b]	0.40:1
70	30	1.1	8.2	0.8[b]	2.5[c]	18.8[c]	0.9[c]	61.8[c]	5.9[c]	0.48:1
0	100	1.2	4.1	1.7	7.2	64.3	2.9	1.2	17.4	4.38:1
Buffalo ghee	Margarine									
100	0	3.3	16.2	0.0	0.6	0.0	0.0	78.1	1.8	0.25:1
95	5	3.2	15.5	0.1	0.6	3.3	0.2	75.4	1.7	0.30:1
90	10	3.1	15.8	0.2	0.7	6.7[c]	0.3	71.4	1.8	0.37:1
85	15	2.8	14.5	0.3	1.4	10.2[c]	0.6	67.4	2.8	0.42:1
80	20	2.8	13.9	0.4	1.6	14.9[c]	0.6	62.2[b]	3.6	0.52:1
75	25	2.4	13.8	0.5	2.2	16.4[c]	0.8[b]	59.2[c]	4.7	0.56:1
70	30	2.7	13.6	0.6[b]	2.5	19.7[c]	0.9[b]	54.8[c]	5.2[b]	0.67:1
0	100	1.2	4.1	1.7	7.2	64.3	2.9	1.2	17.4	4.38:1

[a]Ratio of total hydrocarbons to total sterols.
[b]Mixtures are significantly different from the pure ghee at 5% level.
[c]Mixtures are significantly different from the pure ghee at 1% level.

TABLE III

Correlation Coefficient (r) Matrix between Various Adulteration Ratios and Individual Unsaponifiables

Adulterant	Unsaponifiable components								
	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	UC_{32}	Cholesterol	β-Sitosterol	TH/TS[a]
					Cow				
Lard	-0.987	+0.991	0.00[b]	+0.995	0.00[b]	0.00[b]	-0.991	-0.991	+0.955
Margarine	+0.984	-0.994	+0.982	+0.993	+0.993	+0.994	-0.991	+0.991	+0.945
					Buffalo				
Lard	-0.991	+0.984	0.00[b]	+0.992	0.00[b]	0.00[b]	-0.985	-0.992	+0.955
Margarine	-0.981	-0.981	+0.993	+0.979	+0.993	+0.994	-0.990	+0.974	+0.950

[a]Ratio for total hydrocarbons to total sterols.
[b]Indicates the absence of the component in the adulterant.
All correlations were significant at 0.1% level.

bons. Cow ghee had the highest cholesterol and the lowest β-sitosterol concentrations. It has already been reported that the major sterol in butter is cholesterol (13,14). In contrast, margarine was characterized by the highest β-sitosterol and the lowest cholesterol contents. The content of cholesterol in lipid samples was in the following decreasing order: cow > buffalo > lard > margarine. With β-sitosterol, the concentration order was: margarine > buffalo > cow > lard. The sterol fraction of six samples of butter contained one compound, i.e., cholesterol. The sterols from six samples of margarine apparently comprised three major components: β-sitosterol, γ-sitosterol and stigmasterol (7). A different sterol pattern for margarine has been described (14) in which the major components of margarines were β-sitosterol, stigmasterol, Δ^7-stigmasterol and campesterol. The discrepancies in sterol compounds in different margarines may reflect the original vegetable oils used in their formulation. Our values for the concentration of sterol compounds differ greatly from the reported results (7,14). However, there is a broad agreement with the literature in the concentration order for cholesterol and β-sitosterol.

The ratios of total hydrocarbons to total sterols (TH/TS) in the total unsaponifiable matter for margarine and lard were the most different for the various lipids. Lard and margarine contained 19.9 and 33.7 times as much hydrocarbons as pure cow ghee, and 10.4 and 17.5 times as much hydrocarbons as pure buffalo ghee, respectively.

Adulteration of cow and buffalo ghee with various levels of lard caused the following significant changes in the unsaponifiable compounds: a decrease in the concentrations of C_{28}, cholesterol and β-sitosterol and an increase in the quantities of C_{29} and C_{31}. Mixing ghee with margarine caused the appearance of C_{30}, C_{32} and UC_{32}; and an increase in the quantities of C_{31}, C_{32}, UC_{32}, β-sitosterol and TH/TS ratio. The amount of C_{28} was about 2.8 times as high in buffalo ghee as in margarine and exceeded the levels in cow ghee by a factor of 1.13.

The simple correlation coefficient between percentage adulterant and concentration of various hydrocarbons and sterols was highly significant at the 0.1% level. Hence, such decrease or increase in any of the unsaponifiable substances was mainly due to the presence of the adulterant in the mixture. It is possible to determine the extent of admixture of lard or margarine to either cow or buffalo ghee by applying a simple regression equation for each unsaponifiable component. By analyzing the unsaponifiable matter of lipid

TABLE IV

Linear Regression Equations for the Adulteration Ratios (Y) of Adulterants Mixed with Pure Ghee and the Individual Unsaponifiables (X)

Unsaponifiable component	Mixing with lard	Mixing with margarine
	Cow	
C_{28}	Y = 100.41 - 96.33 X	Y = 732.16 + 718.28 X
C_{29}	Y = -17.52 + 1.72 X	Y = 166.51 - 16.53 X
C_{30}	–	Y = -4.44 + 56.07 X
C_{31}	Y = -12.24 + 27.87 X	Y = -6.59 + 14.66 X
C_{32}	–	Y = -0.72 + 1.56 X
Cholesterol	Y = 145.64 - 1.67 X	Y = 101.28 - 1.16 X
β-Sitosterol	Y = 111.59 - 116.96 X	Y = -6.20 + 6.1 X
TH/TS[a]	Y = 4.66 + 37.52 X	Y = 8.44 + 21.25 X
	Buffalo	
C_{28}	Y = 99.73 - 30 X	Y = 147.29 - 45.08 X
C_{29}	Y = -30.69 + 1.92 X	Y = 133.60 - 9.05 X
C_{30}	Y = -18.93 + 29.61 X	Y = 2.38 + 57.59 X
C_{31}	–	Y = -4.63 + 14.38 X
C_{32}	–	Y = -0.82 + 1.56 X
Cholesterol	Y = 155.82 - 1.87 X	Y = 101.26 - 1.29 X
β-Sitosterol	Y = 105.38 - 16.19 X	Y = -3.75 + 6.02 X
TH/TS[a]	Y = -0.57 + 39.54 X	Y = 4.89 + 22.08 X

[a]Ratio of total hydrocarbons and total sterols.

samples and according to the following equation, the adulteration levels can be easily determined (Tables III and IV).

$$Y = A + BX$$

where: Y = adulteration ratio; A = constant value, the intercept of the regression line; B = regression coefficient; and X = the concentration of individual unsaponifiables.

The present investigation describes a rapid and simple laboratory method for detecting certain kinds of lipid adulteration. For simplicity and speed for determining adulteration levels, the application should only be confined to the compounds in the adulterants and not in the pure lipids, e.g., C_{30}, C_{32} and UC_{32}, or to substances occurring in the adulterants in amounts higher than that in pure lipids such as C_{31} and β-sitosterol, especially in detecting margarine mixed with buffalo ghee. Compounds present in higher quantities in pure lipids than in adulterants should not be used as an indicator of adulterations, e.g., C_{28} and cholesterol. Further study is needed concerning the lipid samples mixed with more than one adulterant.

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