A Method for The Quantitative Determination of Individual Oils in a Blend

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A matrix inversion method, based on the data obtained by GLC analysis of fatty acids, sterols, 4-methylsterols, triterpene alcohols, tocopherols and squalene, was applied to quantitatively determine ingredients of vegetable oil blends. Identification of individual oils in mixtures was achieved by comparison of a set of data derived on the basis of the level of certain characteristic components in each pure oil, and the concentration of the same components in the blends.

A computer program was prepared in BASIC to facilitate automatic calculations and printout of results. Sets of formulae also were derived to enable manual estimations without the need for computer facilities.

The program was evaluated by analysis of 19 different laboratory prepared mixtures containing two, three and six components of the oils of corn, soybean, sunflower, palm, cottonseed, palm kernel, sesame, coconut and olive. The system was fully successful in the qualitative identification of the ingredients of all blends tested. For the quantitative determination of these ingredients, the system was better than 80% accurate for the two-, three-, and six-component mixtures.

Intentional adulteration of pure oils and fats by admixing with cheaper ones is widely practiced in many parts of the world for economic reasons (1-3). Detection and determination of individual oils in such mixtures present an analytical challenge to the routine quality control laboratory.

An adequate system for the identification of ingredients of oil admixtures has been of wide interest in many laboratories. Earlier simple systems were based on the use of some physical and chemical indices (e.g., refractive index, iodine value, etc.) collected from the routine analyses of these oils. The overlapping ranges of these indices for some important oils made it difficult to identify these oils or determine their proportions in blends. The introduction of gas liquid chromatography (GLC) made a great contribution to the analysis of fats and oils. Identification systems were much improved by the use of fatty acid patterns quantitatively determined by GLC. But, again, some oil mixtures are still difficult to identify due to the wide overlapping ranges of fatty acids. Further improvement of the identification systems necessitated the use of more parameters. Components of the unsaponifiable matter and triglyceride analysis have been used recently and, in conjunction with fatty acids and the physical and chemical indices, resulted in more satisfactory criteria for the identification and quantitative determination of most blends of economic interest (1,3).

Manual handling of the data on great numbers of parameters is difficult and time consuming. Computers made an important contribution to the solution of this problem, and a number of computer programs were developed for the identification of oil samples by comparison of the ratios of fatty acids in those oils to the ratios in pure oils (4,5).

In 1985, a computerized data processing method for estimation of the composition of edible oil mixtures was reported (6). The program used fatty acid composition, sterol content and tocopherol level of edible oils to evaluate the applicability of a generalized least squares estimator and weighed least squares estimator with backwards elimination.

In the present work, a processing method for the data obtained by GLC analysis of fatty acids, sterols, 4-methylsterols, triterpene alcohols, hydrocarbons and tocopherols is proposed for identification and quantitative determination of ingredients of oil blends. The method may be applied using a set of equations for manual calculation or, more easily, using a computer program.

EXPERIMENTAL PROCEDURE

Apparatus and materials. A Packard Model 430 gas liquid chromatograph equipped with a flame ionization detector (FID) and connected to a data processor Chrompac C-R.3A" was used. The column used for fatty acid analysis was 2-m × 2-mm (i.d.) glass packed with 10% DEGS on chromosorb W (100-120 mesh) operated isothermally at 180°C. For the separation of the unsaponifiable matter, a 2-m × 2-mm (i.d.) glass column packed with 3% SE30 on chromosorb W (100-120 mesh) was used with a temperature program retained at 200°C for three min and then raised to 280°C at 7°C/min. The injector and detector were kept at 320°C under both for fatty acid and for unsaponifiable matter analyses. A personal computer model HP-85 with a memory capacity of 32 Kbytes was employed for data processing.

Thin layer glass plates (20 × 20 cm) precoated with 0.5 mm silica gel were used to separate the main components of unsaponifiable matter (E. Merck, Darmstadt, Federal Republic Germany). Six mixtures containing esters of lauric, myristic, palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, arachidic and eicosenoic acids in different proportions were purchased from Applied Science Laboratories, State College, Pennsylvania. Standard materials of cholesterol, brassicasterol, campesterol, stigmasterol, β-sitosterol, lanosterol, α-tocopherol, squalene and normal chain aliphatic hydrocarbons were obtained from the same source. A mixture of β-amyrin, cycloartenol and 24-methylenecycloartanol and also a mixture of normal chain aliphatic alcohols (from C20 to C36) were donated by the Scientific and Applied Research Center, Qatar University.

Pure oils of corn, soybean, sunflower, rapeseed, palm, cottonseed, palm kernel, coconut, sesame seed and olive were obtained from different commercial sources and also by extraction from seeds (1).

Gas liquid chromatography was used for the separation and determination of fatty acids as methyl esters, but for the unsaponifiable matter components both TLC and GLC were used (1).

Data processing. The analytical data obtained by GLC analyses of (m) components of composition in (n)

vegetable oils were used (1). The values of (m), from 1 to 26, represented the components of unsaponifiable matter and fatty acids as listed in Table 1, and (n), from 1 to 10, represented corn, soybean, sunflower, rapeseed, plam, cottonseed, palm kernel, coconut, sesame seed and olive oils, respectively. The concentration of each component of fatty acids and unsaponifiable matter in the different oils a(m,n) were compared with each other, and those showing characteristically high concentrations in certain oils a_c (m,n) were chosen for identification of these oils. A list of these characteristic components is shown in Table 2. The maximum concentrations of the same components in the other oils were denoted by $a_{max}(m)$.

For identification of oils in admixtures, a threshold value, $V_t(m,n)$, was derived for each characteristic component in the different oils so that

$$V_t(m,n) = a_c(m,n) \times P(n) > a_{max}(m)$$

where P(n) is a fraction directly proportional to $a_{max}(m)/a_c(m,n)$. An assigned P(n) value for each oil, depending on the fraction $a_{max}(m)/a_c(m,n)$ was taken as the minimum detection limit of that oil (MDL). For palm kernel, coconut, sesame and rapeseed oils the assigned P(n) value was 5%, and for the other oils it was 10%.

Assuming that a blend, made up from a number of oils (j) at different proportions, X(n), was analyzed for a number of components (i) of fatty acids, sterols, etc., the concentration of each of these components in the blend, A(m), is related to the concentration of the same component in the pure oils, a(m,n), by the following relationship:

$$A(m) = \Sigma\, a(m,n) \times X(n) \quad [1]$$

where n = 1 to j and m = 1 to i. Equation [1] can be written in matrix form:

$$[A(m)]_{i,1} = [a(m,n)]_{i,j} \times [X(n)]_{j,1} \quad [2]$$

$$\text{Or } A = B \times X \quad [3]$$

Where A = [A(m)] a column matrix of dimension i × 1, and B = [a(m,n)] a rectangular matrix of dimension i × j and X = [X(n)] a column matrix of dimension j × 1.

The elements of column matrix [X] are the proportions of oils in the blend, to be determined.

Multiplying the transpose of matrix B (i.e., B^t) by both sides of equation (3) the following relationship is derived:

TABLE 1

The Mean Value of Concentration of Fatty Acids (g/10 g oil) and Components of Unsaponifiable Matter (mg/g oil) Used for Quantitative Determination of Vegetable Oil Blends

	Oil									
Component	I	II	III	IV	V	VI	VII	VIII	IX	X
Squalene	0.127	0.090	0.050	0.000	0.040	0.030	0.120	0.050	0.010	1.410
δ-Tocopherol	0.008	0.200	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
γ-Tocopherol	0.245	0.950	0.310	0.180	0.040	0.364	0.180	0.000	0.100	0.000
Sesamolene	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.420	0.000
Brassicasterol	0.002	0.000	0.400	0.620	0.000	0.000	0.000	0.120	0.130	0.000
Campesterol	1.613	0.620	0.470	1.780	0.140	0.260	0.130	0.050	0.810	0.050
Stigmasterol	0.607	0.570	0.210	0.000	0.070	0.020	0.140	0.120	0.430	0.020
Obtusifoliol	0.045	0.040	0.160	0.060	0.000	0.010	0.030	0.010	0.060	0.020
β-Sitosterol	5.339	0.710	1.950	3.290	0.280	2.790	0.640	0.370	2.420	1.160
β-Amyrin	0.002	0.090	0.000	0.020	0.000	0.000	0.010	0.000	0.100	0.090
Δ^7-Stigmasterol	0.029	0.050	0.320	0.050	0.000	0.000	0.000	0.000	0.000	0.000
Cycloartenol	0.185	0.090	0.310	0.040	0.030	0.040	0.200	0.170	0.100	0.270
24-Methylene cycloartanol	0.212	0.060	0.130	0.020	0.000	0.060	0.040	0.060	0.080	0.710
Citrostadienol	0.085	0.070	0.300	0.040	0.000	0.000	0.010	0.010	0.090	0.020
Caprylic acid	0.000	0.000	0.000	0.000	0.000	0.000	0.291	0.817	0.000	0.000
Capric acid	0.000	0.000	0.000	0.000	0.000	0.000	0.310	0.647	0.000	0.000
Lauric acid	0.039	0.005	0.001	0.050	0.023	0.024	4.600	4.818	0.005	0.000
Myristic acid	0.015	0.010	0.000	0.016	0.015	0.094	1.580	1.731	0.025	0.000
Palmitic acid	0.050	0.009	0.644	0.509	3.860	2.246	0.872	0.856	0.950	1.102
Palmitoleic acid	0.018	0.010	0.004	0.034	0.000	0.097	0.000	0.000	0.025	0.079
Stearic acid	0.196	0.350	0.441	0.164	0.467	0.263	0.213	0.297	0.470	0.275
Oleic acid	2.854	2.234	2.235	5.510	4.261	1.815	1.800	0.654	4.250	7.620
Linoleic acid	5.537	5.376	6.450	2.420	1.174	5.345	0.279	0.174	4.250	0.800
Linolenic acid	0.114	0.814	0.032	0.896	0.061	0.006	0.007	0.000	0.050	0.050
Arachidic acid	0.041	0.053	0.033	0.050	0.010	0.024	0.010	0.000	0.050	0.050
Eicosenoic acid	0.027	0.031	0.038	0.173	0.006	0.003	0.000	0.000	0.025	0.045

I, Corn oil; II, soybean oil; III, sunflower oil; IV, rapeseed oil; V, palm oil; VI, cottonseed oil; VII, palm kernel oil; VIII, coconut oil; IX, sesame oil; X, olive oil.

$$[B^t] \times [A] = [B^t \times B] \times [X] \quad [4]$$

Where B^t is matrix of dimension j × i, $B^t \times B$ is a square matrix of dimension j × j. Therefore, the value of X is given by:

$$X = [B^t \times B]^{-1} \times [B^t] \times [A] \quad [5]$$

$[B^t \times B]^{-1}$ is the inverse of the matrix $[B^t \times B]$ and of the same order (j × j). The result of multiplying $[B^t \times B]^{-1} \times [B^t]$ is a rectangular matrix of dimension j × i, denoted as (C).
Therefore,

$$X = C \times A \quad [6]$$

The latter equation (No. 6) can be written in the following matrix form:

$$[X(n)]_{j,1} = [C(n,m)]_{j,i} \times [A(m)]_{i,1} \quad [7]$$

Or:

$$\begin{bmatrix} X(1) \\ X(2) \\ \\ X(j) \end{bmatrix} = \begin{bmatrix} C(1,1) & C(1,2) & C(1,i) \\ C(2,1) & C(2,2) & C(2,i) \\ \\ C(j,1) & C(j,2) & C(j,i) \end{bmatrix} \times \begin{bmatrix} A(1) \\ A(2) \\ \\ A(i) \end{bmatrix}$$

Or:

$$\begin{aligned} X(1)\% &= C(1,1) \times A(1) + C(1,2) \times A(2) \\ &\quad + \ldots + C(1,i) \times A(i) \\ X(2)\% &= C(2,1) \times A(1) + C(2,2) \times A(2) \\ &\quad + \ldots + C(2,i) \times A(i) \\ X(j)\% &= C(j,1) \times A(1) + C(j,2) \times A(2) \\ &\quad + \ldots + C(j,i) \times A(i) \end{aligned} \quad [8]$$

Equations [8], therefore, predict the proportions of the oils constituting a blend X(j) from the concentrations of the components of fatty acid, ..., etc., obtained by GLC analyses of the mixture and constants C(n,m) derived from Equation [5].

In our case, the matrix B, referred to as Standard Calibration Matrix (SCM), contained the mean concentration values of the 26 components of fatty acids and unsaponifiable matter obtained by GLC analyses of the 10 pure oils studied (1).

The computer program. The program started with the assumption that all the 10 oils were present in the test sample. For the purpose of producing a more informative final report a number of parameters, including peroxide value, iodine value and refractive index, obtained by analyses of the mixture, were introduced. An input loop was included in the program to facilitate the questionnaire procedure for feeding in the values of A(m) of the test mixture. The next part of the program carried out pre-identification of oil ingredients of the sample through logic statements which eliminated oils from the mixture if the concentration of their characteristic components were below V_t. The data of the pure oils, as pre-identified, are read from the original SCM, and respective a(m,n) values are assigned.

The levels of the oils in the mixture were calculated by solution of equation [5], and those estimated at concentrations below their MDL were removed and the concentrations of the remaining oils in the blend recalculated. The process is repeated until all the estimated amounts of oils are over their MDL. A final report is printed including the names and proportions of oils in the test mixture as well as the other parameters introduced from the keyboard during the program run. A theoretical iodine value is also calculated from the fatty acid composition of the blend (1) and printed on the report.

RESULTS AND DISCUSSION

Identification of oil ingredients of a mixture in this work was approached by the selection of certain characteristic components for each oil. Analyses (1) suggested that low carbon number fatty acids (C8 to C14) at any concentration in a mixture indicates the presence of palm kernel and coconut oils. Detection of sesamine and sesamoline in a mixture identify sesame seed oil. The presence of brassicasterol above certain limits characterizes rapeseed oil, while any detected amount of δ-tocopherol indicates the presence of soybean oil. Though palmitic acid is found in all vegetable oils, its presence above a certain limit indicates the presence of palm oil. Other components used in the identification process in this work but considered of lower characterizing power include squalene, which is contained in olive oil at a level higher than its level in the other vegetable oils; obtusifoliol, citrostadienol and Δ 7-stigmasterol, which are found at high concentration in sunflower oil, and β-sitosterol, which is present at higher concentration in corn oil.

The presence of an oil in a mixture is confirmed if its characteristic components were at a concentration above

TABLE 2

Threshold Values[a] of Characteristic Components in Different Vegetable Oils

Oil	Characteristic components	V_t[b]
Corn	β-Sitosterol	2.200
Soybean	δ-Tocopherol	0.010
	γ-Tocopherol	0.050
	Linolenic acid	0.110
	β-Amyrin	0.003
Sunflower	Obtusifoliol	0.050
	Citrostadienol	0.050
	Δ7-Stigmasterol	0.050
Rapeseed	Brassicasterol	0.040
Palm	Palmitic acid	1.200
Cottonseed	Palmitic acid	1.200
Palm kernel	Caprylic acid	0.001
and coconut	Capric acid	0.001
	Lauric acid	0.040
	Myristic acid	0.010
Sesame seed	Sesamine and sesamoline	0.010
Olive	Squalene	0.100
	Palmitoleic acid	0.010

[a]The unit used for fatty acids is g/10 g oil; the unit used for the components of unsaponifiable matter is mg/g oil.
[b]Threshold values of the characteristic components.

their V_t value in that blend. However, as some pairs of oils were characterized by the same components, such as palm kernel and coconut and palm and cottonseed oils, the identification of the characteristic components of such critical pairs of oils indicates the presence of either or both oils. In such cases, additional steps were required to confirm the actual oil composition of the blend. This was achieved by eliminating the oils which were pre-identified in the blend but estimated below the MDL for that oil. Estimation of the oils depended on the comparison of the 26 components of fatty acids and unsaponifiable matter of the pure oils, as identified in the blend, and composition of the same components in that blend.

The computer program was written in BASIC language to facilitate data processing and will be made available upon request. The program was evaluated by analyzing 19 laboratory-prepared mixtures of different known compositions for the 26 components A(m). In both pure oils and mixtures, concentrations of fatty acids were expressed as g/10 g oil, while the components of unsaponifiable matter were expressed in units of mg/g oil. The results obtained with the 19 mixtures are summarized in Table 3. Generally there was good agreement between the estimated and actual composition. The accuracy of estimation of a mixture containing (j) oils was evaluated by the following formula:

$$\text{Accuracy of estimation of mixture} = 100 - \sum_{m=1}^{j} \sqrt{[AC(m)-ES(m)]^2}$$

Where ES(m) and AC(m) are the estimated and actual concentration of the oil component number (m) in the mixture, respectively.

The program was 100% accurate for the qualitative identification of oil ingredients of all blends. For quantitative determination of the concentration of oils in the binary mixtures containing soybean and rapeseed oils, the program was from 80 to 88% accurate; for the other binary mixtures the accuracy was from 92 to 98%. The relatively low determination accuracy for the former mixtures was due to the use of a common characteristic component, i.e., linolenic acid contained in both oils at close levels. This problem is now being treated by adding extra weight for the other characteristic components for these two oils. For the three component oil mixtures, the accuracy of quantitative determination was from 80 to 90%. Good agreement was observed between actual and estimated composition of the six component oil mixtures where the accuracy was 80 and 89%.

Manual calculation of the proportions of oil ingredients of a blend may be carried out by substituting the concentration values of fatty acids and unsaponifiable components in the appropriate formulae of Equations [8]. For that, several sets of formulae each corresponding to a mixture of different oils were prepared by computerized processing of Equation [5]. In each case, the elements of matrix (B) were the data from the oils in question and the set of formulae obtained were applicable to the determination of mixtures of the same oils. These equations will also be made available upon request. The use of this manual system, therefore, requires that the ingredients of the mixture initially be identified qualitatively by comparison of the threshold values of the characteristic components of each oil to the same components in the blend.

TABLE 3

The Actual and Estimated Composition of Mixtures of Vegetable Oils Using the Method of Data Processing

Mix No.	Oil composition												Accuracy of estimation
	Palm kernel		Corn		Sunflower		Soybean		Rapeseed		Palm		
	AC*	ES**	AC	ES	AC	ES	AC	ES	AC	ES	AC	ES	
1			45	42	55	58							94
2			75	73	25	27							96
3			65	64	35	36							98
4			61	59					39	40			97
5			20	22					80	78			96
6			55	51					45	49			92
7							50	45	50	60			80
8							71	64	29	36			86
9							60	54	40	46			88
10							70	79			30	21	82
11							40	35	60	65			90
12			70	66							30	34	92
13					26	24	44	41	30	35			90
14			44	51	23	16	33	33					86
15			60	60	20	28	20	12					84
16			30	21			30	36			40	43	82
17			30	32	40	30			30	38			80
18	10	12	11	16	29	24	20	20	19	15	11	13	82
19	11	10	21	22	10	10	17	16	15	13	25	29	89

AC, actual composition; ES, estimated composition.

In the present work, the two steps involved in oil identification improved the efficiency of identification methods previously used (1,6). The use of characteristic components for oil pre-identification in this program improved the detection of sunflower oil, where it was identified with 100% accuracy, while it was not detected in three samples (of a total 34 samples) in the work of Niekerk and Burger (6). The problem of critical pair identification as previously reported (1) was overcome in the present work by using an additional identification step including the use of minimum detection limits and elimination of oils estimated below these limits.

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[Received March 21, 1988; accepted June 6, 1988]