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ON-LINE GAS CHROMATOGRAPHIC EVALUATION OF IODINE VALUES IN EDIBLE FATS AND OILS

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SUMMARY

A method is reported where an on-line evaluation of gas-chromatographic data allows direct calculation of iodine values of fats and oils. Iodine values are printed out in the final analysis report of fatty acid methyl ester gas chromatographic analysis. For this purpose a simple equation was established which included double-bond increments of fatty acids, mean molecular weights and molecular weight contributions of each single component of the mixture. In a further step, a computer program was developed (Spectra-Physics 4100 Integrator) that allowed the final on-line print-out of the results.

To evaluate the reliability of the method, comparative analyses by titration were carried out. No significant differences in the results could be observed.

INTRODUCTION

The addition of halogen for analytical purposes was probably first suggested by Von Hübl[1] a hundred years ago, and has led to the development and application of numerous iodometric methods for the characterization of fats and oils by their degree of unsaturation. However, these methods have always had several specific limitations[2]. The principal procedures for the determination of iodine values (IVs), known by the names of their authors, produce identical results only in two cases, *e.g.* the methods of Wijs and Kaufman[3]. This tendency is caused principally by incomplete addition of iodine in the case of α,β-unsaturated fatty acids. Moreover, fats and oils that also contain conjugated fatty acids undergo stoichiometric addition of iodine only with sodium hypochlorite in acetic acid in the presence of mercuric chloride after a 1-h reaction time[4]. Other improvements in the field of IV methodology are still restricted by the possibility of errors caused by the unsaponifiable constituents present in fats and oils[3].

A gas chromatographic (GC) determination of IVs from the fatty acid composition of fats and oils represents an attractive alternative, because the limitations related to iodometry would be circumvented. Theoretically, the IV is computed for a single fatty acid as follows[5]:

$$IV = \frac{nb \cdot 25,384}{MW} \tag{1}$$

where nb is the number of double bonds, 25,384 is the molecular weight of iodine × 100, and MW is the molecular weight of the fatty acid.

For GC results showing the complete fatty acid composition of a given fat or oil, the following equation can be used:

$$IV = \frac{\Sigma \% FA_i \cdot DBC_i}{\overline{MW}} \tag{2}$$

where $i = 1, 2, 3, \ldots n$, FA = fatty acid, DBC = double bond contribution (*i.e.* nb × 253.84), $\overline{MW}$ = average MW (*i.e.* $\overline{MW} = \Sigma \% FA_i \cdot MWF_i$), and MWF = MW factor (*i.e.* $MWF_i = 0.01 \cdot MW_i$).

GC and iodometric results showed significant correlations mostly in the case of unhydrogenated vegetable fats and oils[6-8]. The restricted validity of the GC methods was attributed to incomplete resolution of certain fatty acid methyl esters (FAMEs), as well as their isomers, by GC columns using the stationary phases coated on a powdered support. However, rapid progress in the technology of GC columns offers the possibility of a significant improvement in IV methodology. Both fused-silica and glass capillary columns of high separation efficiency can be prepared by a simple laboratory procedure and thus become available for routine analyses of FAMEs[9]. Moreover, a combination of capillary GC with automatic processing of the detector output by a programmable integrator means that iodine and other values can be computed instantly from the fatty acid composition via a user's written program[10,11].

This paper describes both the analysis of FAMEs by capillary GC and the essential instructions for programming a Spectra-Physics 4100 (SP-4100) computing integrator for an on-line determination of the IV from the fatty acid composition. Included for this purpose are two routines written in BASIC, which allow an automatic print-out of IV as well as a rapid entering of molecular weight factors (MWF) and double bond contributions (DBC) of the fatty acids analysed. Mean ($\bar{x}$) and standard deviation (SD) values are given for both IV and average MW ($\overline{MW}$) results obtained by repeated GC analyses of a standard mixture containing nineteen FAMEs. Also a recent procedure[12] to estimate the regression line for bivariate data sets was used to correlate GC and iodometric results obtained by analysing a series of cocoa butter samples as well as palm oil fractions. Consequently, an equation, correlation coefficient, confidence intervals and other data are included for the best-fit regression line.

EXPERIMENTAL

Apparatus

A Carlo Erba Series 4160 gas chromatograph, equipped with a flame ionization detector and an on-column injection (OCI) controller, was used for the quantitative analyses of FAMEs. GC conditions, 25 m × 0.32 mm I.D. fused-silica column coated with a 0.15-μm film of immobilized phase (IP) Carbowax 20M, as described pre-

viously[9], injection, cold OCI; detector temperature, 320°C; oven temperature, 80°C, 2 min isothermal, 8°C/min to 135°C, 1 min isothermal, 4°C/min to 195°C, 1 min isothermal, 3°C/min to 210°C; carrier gas, hydrogen 0.35 kg/cm². Chromatograms were recorded using a SP-4100 computing integrator (San José, CA, U.S.A.) programmable in BASIC.

Materials

A standard mixture of nineteen FAMEs (see Table I) was purchased from Nu Chek (Elysian, MN, U.S.A.; Cat. No. A-68). After dilution to 0.05% (w/v) in heptane, a 0.5-μl aliquot of this solution was used for GC analyses. A series of cocoa butter samples was purchased from Coprodal (Itabuna, Brazil). Palm oil fractions were produced in our laboratory.

TABLE I

COMPONENT TABLE SHOWING THE PRINCIPAL ENTRIES USED FOR ON-LINE DETERMINATION OF IVs BY CAPILLARY GC

No.	FAMEs	RT	MWF	DBC	AF*	RW	CW
1	$C_{14:0}$	11.99	2.284	0			
2	$C_{14:1}$	12.54	2.264	253.84			
3	$C_{16:0}$	16.06	2.564	0	RP, IS**	0.015	0.005
4	$C_{16:1}$	16.41	2.544	253.84			
5	$C_{18:0}$	20.28	2.845	0			
6	$C_{18:1}$	20.50	2.825	253.84			
7	$C_{18:2}$	21.31	2.804	507.68	RP (1)	0.010	0.003
8	$C_{18:3}$	22.50	2.784	761.52			
9	$C_{20:0}$	24.42	3.125	0			
10	$C_{20:1}$	24.63	3.105	253.84			
11	$C_{20:2}$	25.46	3.085	507.68			
12	DHGLA***	25.90	3.065	761.52			
13	$C_{20:4}$	26.28	3.045	1015.36			
14	$C_{20:3}$	26.75	3.065	761.52			
15	$C_{22:0}$	28.83	3.406	0			
16	$C_{22:1}$	29.08	3.386	253.84			
17	$C_{22:6}$	33.08	3.285	1523.04	RP (2)	0.005	0.003
18	$C_{24:0}$	33.76	3.827	0			
19	$C_{24:1}$	34.15	3.807	253.84			
	HELP						
	PEAK	70.00	–	–	RP (3)	0.005	0.003

  * Attributed function during integration.
 ** Response factor reference peak or "internal standard".
*** Methyl dihomo-γ-linolenate.

Methods

Sodium methoxide was used as the catalyst for transesterification of cocoa butter samples. The resulting FAMEs were diluted to 0.05% (w/v) in heptane. A 1-μl aliquot of this solution was used for GC injections. IVs were determined according to the method of Wijs[13]. Because IV results determined by either GC or iodometry are prone to non-specific errors (discussed later), the regression lines for bivariate data sets were estimated according to a recently published method[12], which suggests

three alternatives for λ ($\lambda = \sigma^2 y / \sigma^2 x$, where σ^2 is the error variance): $\lambda = 0$ (x upon y regression), $\lambda \to \infty$ (y upon x regression) and λ unknown (the best-fit regression line).

Programming the SP-4100 integrator

The integrator was first programmed via "Dialog", which is a built-in routine[17] to request automatically most information necessary. The entries specified by the operator were as follows:

— Method 1
— The end of run time in min (ER)
— Retention times (RT) of both the component FAMEs and "Help Peak" (an imaginary peak with $RT = 2 \times ER$)
— Response factors (RF) for all FAMEs
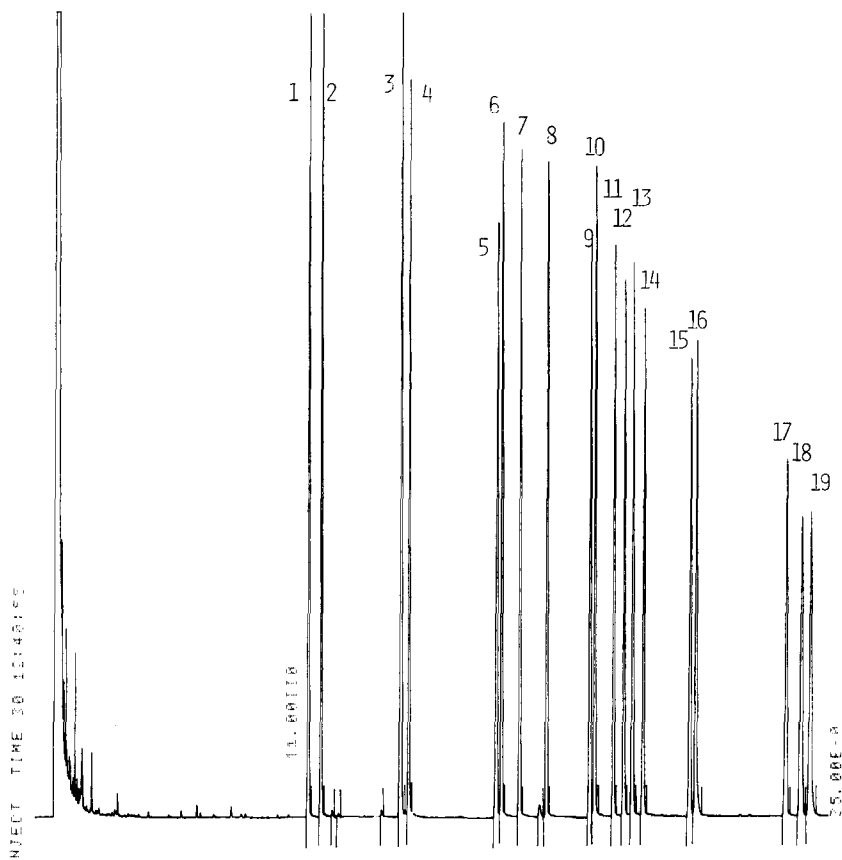— Name of each compoonent FAME

Fig. 1. Gas chromatogram of FAMEs standard mixture. Peaks: 1 = $C_{14:0}$; 2 = $C_{14:1}$; 3 = $C_{16:0}$; 4 = $C_{16:1}$; 5 = $C_{18:0}$; 6 = $C_{18:1,cis}$; 7 = $C_{18:2}$; 8 = $C_{18:3}$ $n - 3$; 9 = $C_{20:0}$; 10 = $C_{20:1}$; 11 = $C_{20:2}$; 12 = $C_{20:3,n-6}$; 13 = $C_{20:4,n-6}$; 14 = $C_{20:3,n-9}$; 15 = $C_{22:0}$; 16 = $C_{22:1}$; 17 = $C_{22:6}$; 18 = $C_{24:0}$; 19 = $C_{24:1}$. For chromatographic conditions see Experimental.

— *RT* of reference peaks (RP) including "Help Peak"
— *RT* of a response factor reference factor reference peak (referred to as "INT STD" in the "Dialog").

The role of both the reference and the response factor reference peaks should be attributed to the best resolved component FAMEs present in most fasts and oils (see Table I and Fig. 1).

After the end of "Dialog", additional parameter values for all reference peaks (including "Help Peak") are entered via the keyboard in order to define the reference as well as the component peak window values (RW and CW). As shown in Table I, RW(1) = 0.01 in the case of $C_{18:2}$ (*RT* = 21.31 min). Consequently, any peak found in the range of 21.31 ± (21.31 × 0.01) min is identified as RP(1). Alternatively, any peak detected in the range of 21.31 ± (21.31 × 0.003)min [CW(1) = 0.003] is identified as $C_{18:2}$ component. The choice of RP, RW or CW is entirely optional. Complex FAMEs mixtures (*e.g.* animal fats and oils) with minimum *RT* differences require generally lower RW and CW parameter values than suggested in the present case (Table I). Parameter values for both the last "real" reference peak and "Help Peak" are always identical, as shown in Table I.

A routine to enter MWF and DBC

As already defined (eqn. 2), both MWF and DBC are indispensable for computing the IV of fats and oils from their fatty acid composition. These values (Table I) were entered rapidly via a user's written program, which is as follows:

```
 90  REM ROUTINE TO ENTER MWF & DBC
100  ! "ENTER NUMBER OF FAMES";
110  INPUT A
120  !
130  FOR I = 1 TO A
140  ! I, $5.03CN(I) CM(I) "MWF =", INPUT MW(I)
150  ! TAB26"DBC=", INPUT DB (I)
160  NEXT I
170  END
```

After initiation (RUN 100) and entry of the number of FAMEs analysed, the program prints successively the names of all FAMEs specified previously via "Dialog" to request their corresponding MWF and DBC, respectively.

A routine to compute IVs

After printing a complete report on the fatty acid composition (see Fig. 2), the SP-4100 automatically starts the IV routine, which is as follows:

```
190  REM IV ROUTINE
200  D=0:M=0
210  FOR I=1 TO 50
220  M(I)=LC(I)*MW(I)
230  M=M+M(I)
240  D(I)=LC(I)*DB(I)
250  D=D+D(I)
```

```
260   NEXT I
270   IV = D/M
280   !
290   ! "IODINE VALUE = " IV, "AVERAGE MW = " M
300   FOR I = 1 TO 72:! " = "; :NEXT
310   GOTO 4850
4840  !! PLOT 0
4845  RUN200
```

The IVs and MWs of the fatty acids are the final results printed by this routine (Fig. 2).

```
IODINE VALUE METHOD               30 12:40:55

FILE 1        METHOD  1.      RUN  18        INDEX   5


NAME                    CONC        RT        AREA BC      RF        RRT

C14:0 =                5.134     12.04     169598 01      1.       0.747
C14:1 =                5.058     12.6      167116 01      1.       0.782
C16:0 =               10.567     16.12     349092 01      1.       1.
C16:1 =                5.164     16.47     170628 01      1.       1.022
C18:0 =                5.018     20.35     165751 02      1.       0.952
C18:1 =                5.168     20.56     170742 03      1.       0.962
C18:2 =                5.062     21.37     167233 01      1.       1.
C18:3 =                5.087     22.56     168051 01      1.       1.056
C20:0 =                5.003     24.48     165286 02      1.       1.146
C20:1 =                5.027     24.68     166079 03      1.       1.155
C20:2 =                5.083     25.52     167937 01      1.       1.194
DHGLA                  4.915     25.97     162387 01      1.       1.215
C20:4 =                5.105     26.34     168632 01      1.       1.237
 20:5 -                4.999     26.82     165151 01      1.       1.255
C22:0 =                4.861     28.89     160590 02      1.       1.352
C22:1 =                4.948     29.16     163481 03      1.       1.365
C22:6 =                4.611     33.16     152323 01      1.       1.552
C24:0 =                4.585     33.84     151476 01      1.       1.584
C24:1 =                4.605     34.24     152145 01      1.       1.602

TOTALS                100.                3303698


IODINE VALUE=  122.48496      AVERAGE MW=  297.02879
=============================================================================
```

Fig. 2. Typical print-out from gas chromatographic analysis. CONC = Concentration in %; RT = retention time in min; RRT = relative retention time.

RESULTS AND DISCUSSION

A typical print-out of results obtained by the GC analysis of FAMEs (Fig. 1) is shown in Fig. 2. The fatty acid composition was computed by a built-in routine (Method 1) which, modified by a ROM overlay (see lines 4840 and 4845), branched automatically to the user's written program to print both IVs and MWs at the end of the principal report.

Obviously, a routine analysis of IVs by capillary GC combined with automatic

computing requires, apart from a column of sufficient separation efficiency, a non-discriminatory injection technique (*e.g.* cold OCI) as well as high reproducibility of *RT* over extended periods of time. Such requirements are best reflected by the use of IP capillary columns in which the phase-stripping effect mediated via cold OCI is reduced to a negligible level[9,14]. In this context, the reliability of the IP Carbowax 20M was tested over a period of two weeks by measuring ΔRT for the first ($C_{14:0}$) and the last ($C_{24:1}$) compound eluted after injection of the nineteen standard FAMEs. IV as well as MW were determined simultaneously. As shown in Table II, the values of ΔRT were subjected to minor fluctuations (S.D. = 0.03 for n = 10) without any time-dependent relationship.

TABLE II

VARIATIONS OF RETENTION TIMES, MOLECULAR WEIGHT AND IODINE VALUES DURING TEN INJECTIONS

Injection No.	IV	MW	$\Delta RT^{\star}$
1	122.015	297.024	22.16
2	121.697	297.292	22.15
3	122.256	297.095	22.20
4	122.241	297.131	22.17
5	121.717	297.063	22.17
6	122.485	297.029	22.14
7	122.113	297.101	22.12
8	122.637	297.099	22.20
9	122.420	296.979	22.19
10	122.116	297.218	22.17
$\bar{x}$	122.170	297.103	22.17
SD	0.308	0.093	0.03

$\star$ For $C_{14:0}$ and $C_{24:1}$ FAMEs.

The analysis of cocoa butter samples by both capillary GC and iodometry (Wijs's method) is summarized in Table III. As expected, the values obtained by Wijs's method (variable y) are systematically lower (ΔIV) than the authentic GC results (variable x_1) because the latter were computed for pure fatty acids whereas the former were for total fat. Optionally, a correction of the GC values was carried out (variable) by taking into account the percentage of glycerolrest[7], computed on the basis of MW, which is a known variable. In this case, the IV differences ($\Delta IV'$) were significantly lower, as shown in Table III.

A very close linear relationship was found in the case of GC and iodometric results (correlation coefficient significant at 5% level with Student's *t*-test value t = 44.7 for 7 degrees of freedom), as shown in Table IV. Consequently, the envelope for the best-fit regression line, defined by the cases when $\lambda = 0$ and $\lambda \to \infty$, confirms the reliability of the calculated slope (1.020). This statistical approach is indispensable when an increased tendency towards non-specific errors is present. Such a tendency is primarily associated with the presence of new chemical species in hydrogenated as

TABLE III

COMPARISON OF IODOMETRICALLY AND GAS CHROMATOGRAPHICALLY OBTAINED IODINE VALUES

Sample No.	MW	By GC* (x)	Wijs** (y)	ΔIV	By GC*** (x₁)	ΔIV'
1	276.20	35.68	34.22	1.46	34.12	−0.10
2	275.80	36.24	33.97	2.27	34.65	0.68
3	276.44	39.78	38.02	1.76	38.03	0.01
4	275.93	40.82	38.58	2.24	39.02	0.44
5	276.04	41.93	40.33	1.60	40.09	−0.24
6	276.01	43.78	41.72	2.06	41.86	0.14
7	275.94	50.64	47.14	3.50	48.41	1.27
8	276.82	60.64	59.66	0.98	57.98	−1.68
9	275.82	60.83	59.11	1.72	58.16	−0.95
10	276.47	61.81	60.49	1.32	59.10	−1.39

  * Authentic IV for 100 g fatty acids.
 ** Authentic IV for 100 g fat.
*** Corrected IV for 100 g fat.

TABLE IV

STATISTICAL DATA SHOWING THE CORRELATION OF BOTH IODOMETRIC AND UNCORRECTED GC RESULTS

	λ = 0	λ unknown	λ → ∞
Slope	1.021	1.020	1.017
95% Confidence inerval	0.971–1.077	0.976–1.088	0.965–1.071
x- and y-axis intercepts	2.836	2.803	2.690
	−2.900	−2.860	−2.738
Mean x, y		47.215, 45.324	
Correlation coefficient*		0.9980	
Best-fit equation		y = 1.020x − 2.860	

  * Significant at 5% level with t = 44.7 for 7 degrees of freedom.

well as heat-abused fats and oils[15]. The interest in obtaining the best possible correlation of GC and titrimetric IVs lies in the possibility of automatically computing a whole range of physicochemical constants (saponification value, refractive index, neutralization value, specific gravity, combustion heat, iodine-saponification factor and hardness number) derived from the fatty acid composition, as already suggested[16].

REFERENCES

1 A. von Hübl, *Dinglers Polytech. J.*, 253 (1884) 281; *J. Soc. Chem. Ind., London*, 3 (1884) 641.
2 J. Pokorny, *Chem. Listy*, 66 (1972) 22.
3 H. A. Boekenoogen (Editor), *Analysis and Characterization of Oils, Fats and Fat Products*, Vol. 1, Interscience, London, 1964, pp. 32–58.
4 S. Mukherjee, *J. Amer. Oil Chemists Soc.*, 32 (1955) 351.
5 B. M. Margosches, W. Hinner and L. Friedmann, *Z. Angew. Chem.*, 37 (1924) 334.
6 V. Koman and E. Danielova, *Chem. Zvesti*, 28 (1974) 218.

7 A. Huyghebaert and H. Hendrickx, *Milchwissenschaft*, 25(9) (1970) 506.
8 A. H. G. Chin and W. L. Siew, *MARDI Res. Bull.*, (1981) 1986.
9 H. Traitler, L. Kolarovic and A. Sorio, *J. Chromatogr.*, 279 (1983) 69.
10 Anonymous, *Lab. Pract.*, 33 (2) (1984) 21.
11 A. Braithwaite, *Eur. Spectroscopy News*, 51 (1983) 20.
12 G. N. Wiggans and A. Sahgal, *Lab. Pract.*, 32 (11) (1983) 77.
13 *Bestimmung der Jodzahl nach Wijs, DGF-Einheitsmethoden CV 11d(53)*, Wissenschaftliche Verlags-gesellschaft mbH, Stuttgart, F.R.G.
14 K. Grob, G. Grob and K. Grob, Jr., *J. Chromatogr.*, 211 (1981) 243.
15 A. E. Waltking, W. E. Serry and G. W. Bleffert, *J. Amer. Oil Chemists Soc.*, 52 (1975) 96.
16 V. Koman and E. Danielova, *Chem. Zvesti*, 29 (1975) 256.
17 *SP-4100 Computing Integrator Instruction Manual, No. A0099-085*, Spectra Physics, San Jose, CA, June 1981.