

Calculation of Iodine Value from Measurements of Fatty Acid Methyl Esters of Some Oils: Comparison with the Relevant American Oil Chemists Society Method

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ABSTRACT: A new calculation method for the determination of iodine value (IV) from measurements of fatty acid methyl esters is proposed. The method is based on the quantitative determination of fatty acid methyl esters of vegetable oils by capillary gas chromatography. IV is a measure of the number of double bonds in the unsaturated fatty acids in one gram of oil. The analytical methodology of its evaluation includes the use of rather health dangerous reagents, and for that reason is mostly avoided by laboratory analysts. A calculation procedure to determine the IV of oils from their fatty acid methyl ester composition is in use based on the American Oil Chemists' Society (AOCS) method Cd 1c-85. A new calculation procedure for IV, based also on the evaluation of the fatty acid methyl esters of oils, was developed. The application of the proposed calculation methodology was checked with olive oil, corn oil, soybean oil, cottonseed oil, and sunflower seed oil. The proposed calculation gave results in better agreement with the Wijs method than with the relevant AOCS method.

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KEY WORDS: Fatty acids, iodine value, vegetable oils.

One important parameter of different vegetable oils is the amount of unsaturation of the constituent fatty acids. This has been measured by the iodine value (IV), which is currently determined by the Wijs method (1). Various methods are available to determine the IV of fats and oils, such as that of Hanus and Hubl (2), Hofmann and Green (3), and Rosenmund and Kuhnenn (4). The IV of vegetable oils can provide very useful information in other scientific fields. For example, IV is used for the determination of oil quality of different plant species (5–8), for the study of the effects of insecticides on plants (9), and for the determination of the quality of diesel fuel derived from vegetable oils (10). Although many methods have been developed, the Wijs method is the most widely used as a standard method. Major drawbacks of that method include the use of dangerous iodine

trichloride (Wijs reagent) and the time-consuming procedures for reagent preparation and chemical analysis. For these reasons, IV determination, despite its high diagnostic utility, is not often used in food analysis laboratories. Numerous efforts have been made to reduce the use of dangerous chemicals by substituting instrumental methods for the IV calculation procedure. Near-infrared, Fourier transform nuclear magnetic resonance (NMR), and Fourier transform infrared methods have been used to determine IV (11,12). Techniques were also developed to determine IV by differential scanning calorimetry (13) and based on triglyceride composition (14). A procedure to determine the IV from the fatty acid composition has been proposed by an American Oil Chemists' Society (AOCS) method (15). This method is general and can be applied to any vegetable oil. In a correlation study between the Wijs and AOCS methods, it was found that, depending on the vegetable oil used, the Wijs and AOCS results were not consistently in agreement.

During an oil authenticity check, one of the first analyses performed is the determination of the fatty acid composition. This is effected by transesterification of oil triglycerides and analysis of the produced fatty acid methyl esters by gas chromatography using capillary columns. Capillary columns and the integration facilities of modern gas chromatographs can provide accurate information on the percentages of different fatty acid methyl esters. By using these known percentages of fatty acid methyl esters and a general equation with coefficients specific for every type of vegetable oil, a new calculation procedure for the determination of IV is proposed. The application of the proposed method was checked with olive oil, corn oil, soybean oil, cottonseed oil, and sunflower seed oil. The proposed calculation gave results in better agreement with the Wijs method than with the relevant AOCS method. The development and statistical evaluation of this method is presented in this article.

MATERIAL AND METHODS

Samples and reagents. Authentic samples of virgin olive oil, sunflower oil, soybean oil, cottonseed oil, and corn oil were kindly supplied by the Greek State Chemical Laboratories (Athens, Greece). Ten to 12 samples of each oil variety were examined in order to permit statistical calculation of the results.

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Analytical grade reagents and deionized water were used throughout the study.

Fatty acid methyl ester and IV determination. Analysis of IV and fatty acid methyl esters was done according to Association of Official Analytical Chemists (AOAC) methods (1,16). Both types of analysis were run in triplicate. A gas chromatograph (model 5842, Hewlett-Packard, Palo Alto, CA) equipped with a 30 m, CP-Sil 88 column and a flame-ionization detector was used for fatty acid methyl ester analysis. Helium was used as a carrier gas with a flow rate of 2 mL/min. Separation was done isothermally at 175°C. The injector and detector temperatures were 230°C.

The Wijs method was used for the determination of the IV. Iodine chloride was used for double-bond saturation analysis, and the consumed iodine was measured by titration with 0.1 M standard sodium thiosulfate solution.

RESULTS AND DISCUSSION

Measured fatty acid methyl esters and the corresponding IV are depicted in Tables 1–5. The rows C_1 , C_2 , and C_3 correspond to the sum of mono-, di-, and tri-unsaturated fatty acid methyl esters.

In order to develop a suitable correlation procedure between the IV and the fatty acid methyl ester percentages, several factors had to be considered. In the vegetable oil studied, the total percentage of triglycerides amounted to 97–98% of the lipids. Fatty acids contributed to the IV with their relative amounts, the number of their double bonds, and their molecular masses. Unsaturated fatty acids of vegetable oils studied belong mainly in the C_{18} family, and included oleic acid ($C_{18:1}$), linoleic acid ($C_{18:2}$), and linolenic acid ($C_{18:3}$). Palmitoleic acid ($C_{16:1}$), eicosenoic acid ($C_{20:1}$), and erucic acid ($C_{22:1}$) do not belong to this family, and they are present in

TABLE 1
Iodine Value (IV) and Percentages of Mono (C_1)-, Di (C_2)-, and Tri (C_3)-unsaturated Fatty Acid Methyl Esters of Olive Oil Samples

	IV (Wijs)	C_1	C_2	C_3	IV (Eqn 1) ^a	IV (AOCS)
1	86.3	79.19	6.82	0.76	84.8	81.9
2	87.1	74.52	10.84	0.58	85.4	84.4
3	85.6	77.63	8.64	0.78	85.9	83.8
4	83.8	74.93	10.99	0.81	86.6	85.6
5	86.1	78.21	9.19	0.53	86.5	84.4
6	85.3	76.30	9.47	0.66	85.4	83.8
7	86.6	75.97	9.64	0.57	85.1	83.5
8	84.4	76.46	9.85	0.56	85.8	84.3
9	82.5	77.76	7.82	0.55	84.3	81.8
10	85.2	79.14	7.12	0.56	84.7	82.7
11	84.3	77.93	7.95	0.56	84.7	82.3
12	83.4	77.78	7.97	0.59	84.6	82.0
					Mean of differences:	–0.2994 ^b
					SD ^d of differences:	1.7818 ^c
						1.4020
						1.7034

^aIV (Eqn. 1) = $x C_1 + y C_2 + z C_3$; $x = 0.93$, $y = 1.35$, and $z = 2.62$.

^bIV(Wijs) – IV(Eqn. 1).

^cIV(Wijs) – IV(AOCS).

^dSD, standard deviation.

TABLE 2
IV and Percentages of Mono (C_1)-, Di (C_2)-, and Tri (C_3)-unsaturated Fatty Acid Methyl Esters of Corn Oil Samples

	IV (Wijs)	C_1	C_2	C_3	IV (Eqn. 1)	IV (AOCS)
1	118.4	26.43	61.11	0.56	119.6	130.0
2	116.1	26.92	60.44	0.45	118.8	129.0
3	118.6	27.29	58.57	1.07	117.9	127.7
4	117.8	27.16	59.35	1.70	120.6	130.6
5	120.3	27.65	59.95	0.85	119.8	129.8
6	117.1	25.41	60.68	0.72	118.3	132.1
7	123.0	26.21	59.14	0.99	117.5	127.5
8	120.5	29.30	57.54	0.76	117.6	126.8
9	122.9	27.96	58.43	0.73	117.5	127.1
10	118.6	28.09	57.76	0.56	116.1	126.0
11	122.5	25.97	60.46	0.66	118.3	128.7
12	118.3	29.38	57.13	0.82	117.2	126.4
13	120.1	29.26	57.99	0.70	118.1	127.4
					Mean of differences:	–0.2804 ^b
					SD of differences:	2.7793
						3.3789

^aIV (Eqn. 1) = $x C_1 + y C_2 + z C_3$; $x = 1$, $y = 1.5$, and $z = 2.62$.

^bIV(Wijs) – IV(Eqn. 1).

^cIV(Wijs) – IV(AOCS). For abbreviations see Table 1.

minor amounts (between 0.5 and 2% for all of them). Thus, the fatty acid molecular weights do not seem to significantly affect the determination of the IV. The two other factors, namely, the relative amounts of the different acids and their number of double bonds, are crucial for the determination of IV, and they were taken into consideration for the development of the proposed Equation 1.

$$\text{Calculated IV: } IV = x C_1 + y C_2 + z C_3 \quad [1]$$

where: C_1 , C_2 , and C_3 correspond to the sum of relative percentage concentrations of the unsaturated fatty acids with one, two, and three double bonds, respectively, and x , y , and z , are coefficients that must be determined for each type of oil. These coefficients must incorporate the effects of the number of the double bonds which is 1 for x , 2 for y , and 3 for z . They should also incorporate the existing differences in molecular weights, the existence of small and variable amounts of unsaponifiable materi-

TABLE 3
IV and Percentages of Mono (C_1)-, Di (C_2)-, and Tri (C_3)-unsaturated Fatty Acid Methyl Esters of Soybean Oil Samples

	IV (Wijs)	C_1	C_2	C_3	IV (Eqn. 1)	IV (AOCS)
1	123.8	19.15	57.80	8.30	121.8	138.3
2	126.6	19.26	59.61	7.32	121.9	138.9
3	121.9	24.02	54.33	6.95	118.3	132.9
4	121.6	27.13	49.22	8.56	118.5	125.4
5	126.2	20.20	58.20	8.60	124.2	138.1
6	125.8	19.80	59.11	7.64	122.6	137.2
7	124.0	20.30	58.60	7.30	121.5	137.7
8	125.1	19.70	59.11	7.75	122.8	138.0
9	124.9	20.65	58.50	7.06	121.0	136.5
10	126.4	23.90	57.30	7.33	123.3	137.8
					Mean of differences:	3.0456 ^b
					SD of differences:	2.7793
						–11.450 ^c
						2.7594

^aIV (Eqn. 1) = $x C_1 + y C_2 + z C_3$; $x = 1$, $y = 1.4$, and $z = 2.62$.

^bIV(Wijs) – IV(Eqn. 1).

^cIV(Wijs) – IV(AOCS). For abbreviations see Table 1.

TABLE 4
IV and Percentages of Mono (C₁)-, Di (C₂)-, and Tri (C₃)-unsaturated Fatty Acid Methyl Esters of Sunflower Oil Samples

	IV (Wijs)	C ₁	C ₂	C ₃	IV (Eqn. 1) ^a	IV (AOCS)
1	125.8	23.92	63.56	1.35	128.0	134.2
2	128.0	24.08	62.81	1.76	128.0	134.0
3	128.7	25.44	65.25	0.21	129.1	135.4
4	124.8	25.55	63.69	0.29	126.9	132.9
5	124.3	34.26	55.47	0.18	121.8	120.7
6	125.6	27.80	60.40	1.24	126.3	131.7
7	126.1	27.32	61.25	1.31	127.4	132.4
8	124.9	26.32	60.59	0.33	122.8	130.7
9	127.8	24.82	63.05	1.03	127.2	131.5
10	126.5	24.91	60.87	1.65	125.4	133.8
11	125.3	26.58	61.02	1.34	126.4	130.6
	Mean of differences:				-0.1267 ^b	-5.4636 ^c
	SD of differences:				1.5804	3.2751

^aIV (Eqn. 1) = $x C_1 + y C_2 + z C_3$; $x = 0.95$, $y = 1.6$, and $z = 2.62$.

^bIV(Wijs) - IV(Eqn. 1).

^cIV(Wijs) - IV(AOCS). For abbreviations see Table 1.

als (some of them highly unsaturated, such as carotene and squalene), and perhaps some other unforeseen and noncalculated effects on the IV. For these reasons, empirical coefficients should be used for x , y , z , instead of $x = 1$, $y = 2$, and $z = 3$.

The calculation of x , y , z empirical coefficients was based on regression analysis of the relative percentages of already determined fatty acid methyl esters. Chemical and mathematical restrictions were used during x , y , z evaluation. Chemical restrictions used for x , y , z determination were: (i) x , y , $z > 0$, (ii) $z > y > x$, and (iii) IV of x , y , z used in calculations were $x = 1$, $y = 2$, and $z = 3$. The mathematical restriction was to select suitable x , y , z coefficients in order to minimize difference of IV calculated by the Wijs and Equation 1 methods. Results of these calculations are tabulated in Tables 1–5 for olive oil, corn oil, sunflower oil, soybean oil, and cottonseed oil, respectively. Coefficients x , y , and z for the different oils studied were found to be between 0.95 and 1.1 (mean 1) for x , between 1.35 and 1.6 (mean 1.5) for y , and 2.62 for z (Table

TABLE 5
IV and Percentages of Mono (C₁)-, Di (C₂)-, and Tri (C₃)-unsaturated Fatty Acid Methyl Esters of Cottonseed Oil Samples

	IV (Wijs)	C ₁	C ₂	C ₃	IV (Eqn. 1)	IV (AOCS)
1	111.5	19.15	57.30	0.10	113.0	112.5
2	107.6	19.17	54.39	0.22	108.7	111.3
3	108.6	17.83	55.33	0.25	108.8	111.9
4	110.1	16.31	57.43	0.18	110.3	112.2
5	108.9	17.35	56.04	0.18	109.2	110.7
6	109.6	18.31	54.50	0.16	107.8	111.4
7	110.8	16.05	56.32	0.20	108.3	111.7
8	111.1	15.97	59.42	0.17	113.0	112.6
9	108.2	16.60	56.53	0.24	109.3	110.9
10	107.9	19.30	53.96	0.28	108.3	111.0
	Mean of differences:				-0.2457 ^b	-2.0889 ^c
	SD of differences:				1.4141	0.9116

^aIV (Eqn. 1) = $x C_1 + y C_2 + z C_3$; $x = 1$, $y = 1.5$, and $z = 2.62$.

^bIV(Wijs) - IV(Eqn. 1).

^cIV(Wijs) - IV(AOCS). For abbreviations see Table 1.

TABLE 6
Coefficients for the Evaluation of Iodine Value from the Percentages of Unsaturated Fatty Acid Methyl Esters

Oil	x	y	z
Olive oil	0.93	1.35	2.62
Corn oil	1	1.5	2.62
Soybean oil	1	1.4	2.62
Sunflower oil	0.95	1.6	2.62
Cottonseed oil	1.1	1.6	2.62
Mean values:	1	1.5	2.62

6). Mean values of the calculated coefficients could be applied with less accuracy for all types of vegetable oils studied.

Application of paired t -tests for probability $P = 0.01$ between results of the Wijs method and from calculations according to Equation 1 for the oils tested gave: for olive oil, $t = 1.21$ (3.11); for corn oil, $t = 2.12$ (3.05); for soybean oil, $t = 0.40$ (3.25); for sunflower oil, $t = 2.78$ (3.17); and for cottonseed oil, $t = 0.12$ (3.25). Values in parentheses are the corresponding highest t values (for the same degrees of freedom) from the relevant statistical tables (17) suggesting that the null hypothesis is fulfilled and that measured and calculated iodine numbers do not differ significantly at $P = 0.01$.

Calculation of the IV according to the AOCS method for the oils studied are tabulated in Tables 1–5. A comparison of the results from Equation 1 and the AOCS procedure for every type of vegetable oil shows that results from the AOCS method exhibited a general bias toward consistently higher or lower values. On the contrary, results from Equation 1 were both higher and lower than the Wijs method (2).

Application of paired t -tests for probability $P = 0.01$ between results of the Wijs method and from calculations according to the AOCS method Cd 1-85 for the oils tested gave: for olive oil, $t = 4.52$ (3.11); for corn oil, $t = 17.34$ (3.05); for soybean oil, $t = 21.2$ (3.25); for sunflower oil, $t = 10.1$ (3.17); and for cottonseed oil $t = 7.03$ (3.25). Values in parentheses are the corresponding highest t values (for the same degrees of freedom) from relevant statistical tables (17). In this case, the corresponding t values show that IV measured by the Wijs method compared to IV calculated according to AOCS method do differ significantly at $P = 0.01$.

Accordingly, the proposed procedure for the calculation of the IV from the percentages of fatty acid methyl esters by using Equation 1 and coefficients specific for every vegetable oil (Table 6) can be used successfully for the determination of IV. The proposed procedure also seems to have a better accuracy than the relevant AOCS method Cd 1-85.

REFERENCES

1. *Official Methods of Analysis of AOAC International*, AOAC International, Arlington, 1984 AOAC Official Method 28.023, Iodine Absorption Number Wijs Method.
2. Paquot, C., *Standard Methods for the Analysis of Oils, Fats and Derivatives*, 6th edn., Pergamon Press, Oxford, 1979, pp. 66–70.
3. Cocks, L.V., and C. Van Rede, *Laboratory Handbook for Oil and Fat Analysis*, Academic Press, London, 1966, pp. 109–113.

4. Rossel, G.B., Classical Analysis of Oils and Fats, in *Analysis of Oils and Fats*, edited by R.G. Hamilton, and J.B. Rossel, Elsevier, London, 1987, pp. 10–12.
5. Misra, N., Sangita-Batra, A. Rathore, and S. Batra, Effects of Storage Moulds on the Nutritional Components of *Foeniculum vulgare* Mill, *Int. J. Trp. Plant Dis.* 6:67–72 (1988).
6. Rowland, G.G., A. McHughens, Rh.S. Bhatti, and Andro Flux, *Can. J. Plant Sci.* 69:911–913 (1989).
7. Tosheva-Tsvetkova, T.S., Tsvetkova-TS-Tosheva, The Role of Food in the Fat Metabolism of Overwintering Larvae of the Plum Fruit Moth, *Gratinarska - I - Losarska - Nauka* 20:43–48 (1983).
8. Bergman, J.W., G. Carlson, G. Kushnak, N.R. Riveland, G. Stalknecht, L.E. Welty, and D. Wichman, Registration of Finch Safflower, *Crop Sci.* 29:829–832 (1989).
9. Schynowski, F., and W. Schwack, Photochemistry of Parathion on Plant Surfaces. Relationship Between Photodecomposition and Iodine Value of the Plant Cuticles, *Chemosphere* 33:2255–2262 (1996).
10. Mittelbach, M., Diesel Fuel Derived from Vegetable Oils. VI Specifications and Quality Control of Biodiesel, *Bioresour. Technol.* 56:7–11 (1996).
11. Gee, P.T., Iodine Value Determination by FTIR Spectroscopy, *Malays. Oil Sci. Technol.* 4:182–185 (1995).
12. Van De Voort, F.R., J.E. Sedman, and A.A. Ismail, Rapid and Direct Iodine Value and Saponification Number Determination of Fats and Oils by Attenuated Total Reflectance/Fourier Transform Infrared Spectroscopy, *J. Am. Oil Chem. Soc.* 69:1118–1123 (1992).
13. Haryati, T., Y.B. Che Man, H.M. Ghazali, B.A. Asbi, and L. Buana, Determination of Iodine Value of Palm Oil by Differential Scanning Calorimetry, *Ibid.* 74:939–942 (1997).
14. Haryati, T., Y.B. Che Man, H.M. Ghazali, B.A. Asbi, and L. Buana, Determination of Iodine Value of Palm Oil Based on Triglyceride Composition, *J. Am. Oil Chem. Soc.* 75:789–792 (1998).
15. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, edited by David Firestone, 4th edition, American Oil Chemists' Society, Champaign, 1989, Cd 1c-85.
16. *Official Methods of Analysis*, AOAC International, Arlington, 1984, AOAC Official Method 28.060.
17. Miller, J.C., and J.N. Miller, *Statistics for Analytical Chemistry*, 3rd edn., Ellis Harwood, Prentice Hall, New York, 1993, p. 58.

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