Determination of physicochemical constants of fats and oils from the composition of their fatty acids using gas—liquid chromatography and constructed alignment chart

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A method of determination of the most often used physicochemical constants of fats and oils by determining their fatty acids by gas—liquid chromatography and using the combined alignment chart constructed for this aim is presented. From this alignment chart we can read the saponification value, refractive index, neutralization value, specific gravity, combustion heat, iodine-saponification factor, and the hardness number.

If we use for the determination of the iodine value the method described in our previous work then all these constants can be determined practically on the basis of only one chromatographic record of the corresponding mixture of fatty acids.

The time relation of the results obtained in this way is convenient and the values are in good agreement with those given in literature. Conditions for the construction of the alignment chart are also described.

Owing to its high separative efficiency, relatively short time of analysis and because only samples in the order of micrograms are needed, the method of gas—liquid chromatography is one of the most often used and it is also the most effective method in such a region of natural materials as are derivatives of fatty acids and/or triglyceridic fats and oils. A survey of applications of gas—liquid chromatography since its introduction [1] into the assay of this kind of natural materials would already be very difficult to make.

On the other hand, charts — which can be very convenient especially when we have to deal with repeated determination or calculation of some forms of results where a certain degree of inaccuracy can be tolerated — are very rarely used in assay calculations of triglyceridic fats and oils. Physicochemical constants such as the iodine value, the saponification value, the refractive index, the combustion heat, the specific gravity, the neutralization value, the iodine-saponification factor, and the hardness number are up to now the most important and the most often used constants for the determination of the species and for the control of the technological processes in the industry of edible and technical fats.

From nomograms designed for triglyceridic fats and oils the following simple charts can be mentioned: nomogram for the determination of the corrected iodine

value [2], for the determination of the iodine value from the hydrogen value [3], for the determination of the corresponding iodine value from the refractive index [4], triangular chart for the determination of fatty acid composition based on known iodine and thiocyanogen values [5], and chart for determination of fatty acid composition when the iodine value and the specific absorbance coefficient at 233 nm are known [6], further the chart for the determination of the relative ratio of low molecular fatty acids using distillation with water vapour [7]. Charts which can be applied in saponification processes and in the hydrolytic cleavage of triglycerides are summarized in [8]. The chart [9] is an indicator of the selectivity of the process of partial catalytic hydrogenation. The advantages of gas—liquid chromatography analyses stimulated the construction of a combined chart for determination of the iodine value [10].

All above-mentioned charts could be used only for the determination of single constants and mostly only for the determination of the iodine value.

The aim of the present paper was to work out such a procedure and chart which, based on a single gas—liquid chromatogram of a fatty acid mixture, would allow us to determine easily and in one procedure the above-mentioned 7 most important and, in connection with triglycerides, the most often required physicochemical constants.

Experimental

Conditions for construction of the combined alignment chart

The combined alignment chart for the derivation of basic physicochemical constants of fats and oils contains the mapping of the following relations

$$\mathrm{ISF} = \mathrm{SV} - \mathrm{IV}\ [11], \tag{1}$$

$$\mathrm{SG} = 0.8475 + 0.0003\,\mathrm{SV} + 0.00014\,\mathrm{IV}\ [12], \tag{2}$$

$$\mathrm{CH} = 11\,380 - \mathrm{IV} - 9.15\,\mathrm{SV}\ [13], \tag{3}$$

$$\mathrm{HN} = \mathrm{ISF} + 3.7\,\mathrm{T}\ [11], \tag{4}$$

$$\mathrm{RI} = 1.4515 + 0.0001171\,\mathrm{IV}\ [14], \tag{5}$$

$$\mathrm{NV} = 0.000236\,\mathrm{SV}^2 + \mathrm{SV}\ [15], \tag{6}$$

$$\mathrm{SV} = 3 \cdot 56\,108/(3 \cdot M + 38.01)\ [15]. \tag{7}$$

Relation (*1*) was changed in the following way

$$\mathrm{IV} - 50 + \mathrm{ISF} + 120 = \mathrm{SV} + 70$$

and was represented by alignment chart *I* with equations

$$x_1 = 0; \qquad y_1 = m_1 \cdot (\mathrm{IV} - 50),$$

$$x_2 = d_1; \qquad y_2 = m_2 \cdot (\mathrm{ISF} + 120),$$

$$x_3 = d_2; \qquad y_3 = m_3 \cdot (\mathrm{SV} + 70),$$

where we used the units

$$m_1 = 1\text{ mm};\quad m_2 = 1\text{ mm};\quad m_3 = m_1 \cdot m_2/(m_1 + m_2) = 0.5\text{ mm},$$
$$d_1 = 270\text{ mm};\quad d_2 = d_1 \cdot m_1/(m_1 + m_2) = 135\text{ mm}.$$

Relation (*2*) was changed in the following way

$$0.00014\,(\mathrm{IV} - 50) + 0.0003\,(\mathrm{SV} + 70) = \mathrm{SG} - 0.8475 + 0.014$$

and was mapped by alignment chart *II* with equations

$$x_4 = 0; \qquad y_4 = m_4 \cdot 0.00014\,(\mathrm{IV} - 50),$$
$$x_5 = d_3; \qquad y_5 = m_5 \cdot 0.0003\,(\mathrm{SV} + 70),$$
$$x_6 = d_4; \qquad y_6 = m_6 \cdot (\mathrm{SG} - 0.8475 + 0.014),$$

where the following units were used

$$m_4 = 7143\text{ mm};\quad m_5 = 1666.7\text{ mm};\quad m_6 = m_4 \cdot m_5/(m_4 + m_5) = 1351.3\text{ mm},$$
$$d_3 = 135\text{ mm};\quad d_4 = d_3 \cdot m_4/(m_4 + m_5) = 109.5\text{ mm}.$$

Relation (*3*) was transformed into

$$\mathrm{IV} - 50 + 9.15\,(\mathrm{SV} + 70) = 11380 - \mathrm{CH} + 590.5$$

and was mapped by alignment chart *III* with equations

$$x_7 = 0; \qquad y_7 = m_7 \cdot (\mathrm{IV} - 50),$$
$$x_8 = d_5; \qquad y_8 = m_8 \cdot 9.15\,(\mathrm{IV} + 70),$$
$$x_9 = d_6; \qquad y_9 = m_9 \cdot (11\,380 - \mathrm{CH} + 590.5),$$

where the following units were used

$$m_7 = 1\text{ mm};\quad m_8 = 0.0546\text{ mm};\quad m_9 = m_7 \cdot m_8/(m_7 + m_8) = 0.05177\text{ mm},$$
$$d_5 = 135\text{ mm};\quad d_6 = d_5 \cdot m_7/(m_7 + m_8) = 128\text{ mm}.$$

Relation (*4*) was transformed into

$$3.7\,\mathrm{T} + 77.8 + \mathrm{ISF} + 120 = \mathrm{HN} + 197.8$$

and was mapped by alignment chart *IV* with equations

$$x_{10} = 0; \qquad y_{10} = m_{10} \cdot (3.7\,\mathrm{T} + 77.8),$$
$$x_{11} = d_7; \qquad y_{11} = m_{11} \cdot (\mathrm{ISF} + 120),$$
$$x_{12} = d_8; \qquad y_{12} = m_{12} \cdot (\mathrm{HN} + 197.8),$$

where the following units were used

$$m_{10} = 1.35\text{ mm};\quad m_{11} = 1\text{ mm};\quad m_{12} = m_{10} \cdot m_{11}/(m_{10} + m_{11}) = 0.5745\text{ mm},$$
$$d_7 = 120\text{ mm};\quad d_8 = m_{10} \cdot d_7/(m_{10} + m_{11}) + 69\text{ mm}.$$

The scales "IV" and "SV" were represented by the same equation and have the same unit in alignment charts *I*, *II*, *III*. Thus we were able to associate them using the scales "IV" and "SV" and so a new alignment chart — the combined one

was formed. Into the combined alignment chart the chart *IV* was added in such a way that the "ISF" scales in both alignment charts were made identical. This scale in chart *IV* and in the combined alignment chart has the same unit and the same sense.

The scale "IV" in the combined alignment chart was then changed to the doublescale: on the right side of the scale it was plotted

$$y_{13} = m_{13} \cdot (\mathrm{RI} - 1.4515)/0.0001171 - 50,$$

where $m_{13} = 1$ mm.

By this doublescale relation (*5*) was mapped. The shift by -50 in the scale "RI" corresponds to the shift of the scale "IV".

Similarly the scale "SV" was changed to the doublescale in such a way that on the right side of the scale it was plotted

$$y_{14} = m_{14} \cdot 46.0287\ (2118.644 + 2\,\mathrm{NV} - 46.0287) + 35,$$

where $m_{14} = 0.5$ mm.

By this doublescale relation (*6*) was mapped. The shift by $+35$ in the scale "NV" corresponds to the shift of the "SV" scale.

Finally, the doublescale representing relation (*7*)

$$M = \frac{3 \cdot 56\,108 - 38.01\,\mathrm{SV}}{3\,\mathrm{SV}}$$

is placed on the horizontal lines in the lower part of the figure. On the lower side of the doublescale it was plotted

$$x_{15} = m \cdot M.$$

For different intervals the unit m was chosen according to Table 1.

On the upper side of the doublescale it was plotted

$$x_{16} = \frac{3 \cdot 56\,108 - 38.01 \cdot \mathrm{SV}}{3\,\mathrm{SV}} \cdot m.$$

The same unit m was chosen in the corresponding intervals of the variable SV as for x_{15} scale.

Because the doublescale on the lower part of the figure would be too long, we broke it into five parts in such a way that the most often occurring values of SV or M could be read from the doublescale with the highest accuracy.

The samples used

Plant oil samples extracted from the following industrially stored oil seeds were used: coconut, groundnut, sunflower, linseed, and rape seed. Their acid values were: 4.79, 10.1, 3.81, and 10.77 mg KOH/g, respectively. From animal fats we used a sample of commercial lard having an acid value of 0.33 mg KOH/g.

Conditions for the experimental determination of constants

Determination of iodine value was done according to Hanuš using a solution of iodomonobromine and by back titration with 0.1 N solution of sodium thiosulfide [16].

Simultaneously, the iodine values were derived by determination of unsaturated fatty acids using gas—liquid chromatography and by reading from chart as shown in [10].

Determination of the azometric constant — the saponification value — was done by current method of the saponification of the sample with 0.5 N alcohol solution of KOH followed by titration with 0.5 N alcohol solution of HCl [16]. The theoretical values of these constants were compared with those from *Tables* [8].

The freezing point of free fatty acids (titre — T) was expressed after saponification of samples and after hydrolysis of soaps in Zhukov's device [16].

Conditions for gas—liquid chromatography

Methyl esters of fatty acids of coconut oil, groundnut oil, sunflower oil, linseed oil, rape seed oil, and lard prepared by direct reesterification in a micropressure device according to *Peisker* [17] were analyzed using Research Gas Chromatograph Hewlett—Packard, mod. 7620 A connected with HP 3370 A electronic digital integrator. The conditions of analysis were the same as in [10].

Procedure of determination of individual physicochemical constants by using the constructed alignment chart

The chromatogram of separated and qualitatively identified and quantified fatty acids of sample of triglyceridic oil or fat is completed by corresponding values of molecular weights. Using these values we then calculate the weight average of molecular weights of fatty acids of sample. Then we look for the above-mentioned average values of molecular weights (M) on the horizontal double scale on the lower part of the figure; the given value of molecular weight gives us directly the corresponding *saponification value* on the upper part of the same scale. Such a determined constant of saponification value is then sought and marked on the left hand side of the vertical scale SV. Since this carrying line is common for both the saponification and neutralization values as a double-scale, the plotted saponification value gives us the corresponding neutralization value on the right hand side of the same line.

The *iodine value* as a second constant which determines the use of the constructed chart is determined from the same chromatogram record of the mixture of fatty acids by using the combined chart as described in previous work [10].

The determined iodine value is plotted on the doublescale IV—RI and on its right hand side we read the refractive index corresponding to the iodine value.

Table 1

Intervals and corresponding units of molecular weights

Intervals M	Unit m [mm]
80— 90	10
90—100	8
100—110	6
110—130	5
130—150	4
150—170	3
170—550	2

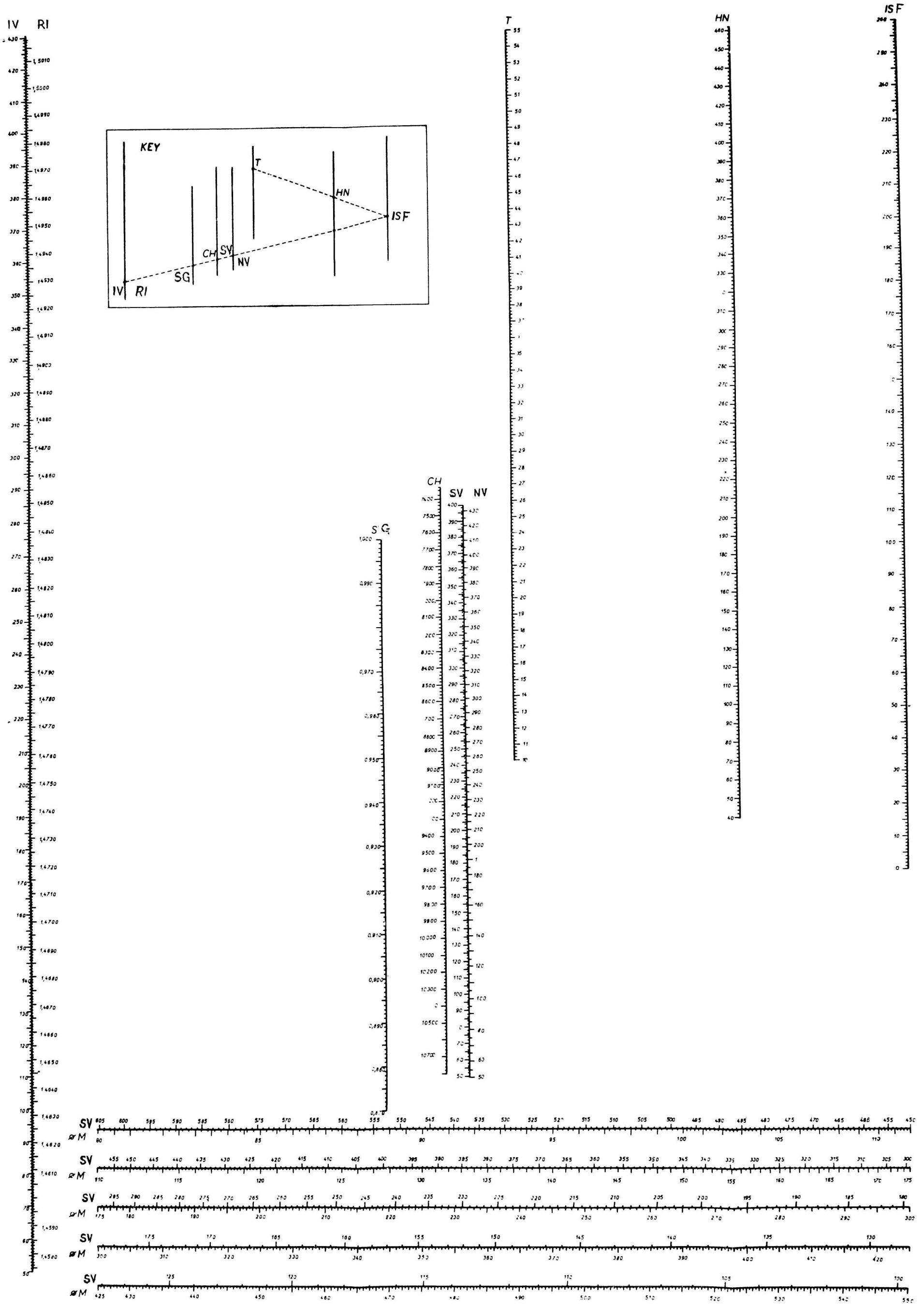

Fig. 1. Combined alignment chart for the determination of the most important physicochemical constants of fats and oils based on known fatty acid composition. (Koman, V. and Danielová, E.), *Chem. zvesti* **29,** 256 (1975).

Connecting the points IV and SV which were determined by the above-mentioned way we get the straight line which intersects on the scales SG and CH the corresponding values of specific gravity and combustion heat of the given sample of fat or oil.

Elongating the connected line of IV—SV till on the scale ISF situated on right hand of the chart and then further in the direction as marked by the key, we get the difference of saponification value and iodine value. If the titre of fatty acids is known we get then also on the scale HN the hardness number of the triglyceridic fat or oil.

Results and discussion

The combined alignment chart constructed under the conditions as described in Experimental, allowing the reading of seven the most important and most frequently used physicochemical constants of fats and oils is presented in Fig. 1.

Table 2

Fatty acids of some fats and oils as determined by gas—liquid chromatography

Fatty acid		*M*	Per cent of fatty acids in oil					
			Coconut	Ground-nut	Sunflo-wer	Linseed	Rape seed	Lard
Caprylic	($C_{8:0}$)*	144.21	7.48	—	—	—	—	—
Capric	($C_{10:0}$)	172.26	6.23	—	—	—	—	0.18
Lauric	($C_{12:0}$)	200.31	50.31	0.15	—	—	—	0.11
Myristic	($C_{14:0}$)	228.37	20.07	—	—	—	—	1.52
Palmitic	($C_{16:0}$)	256.42	8.51	17.40	7.06	8.77	3.84	23.73
Palmitoleic	($C_{16:1}$)	254.40	—	0.60	—	—	0.32	3.35
Stearic	($C_{18:0}$)	284.47	2.76	2.30	9.41	5.67	1.49	15.16
Oleic	($C_{18:1}$)	282.45	4.64	39.00	23.33	21.98	14.77	45.42
Linoleic	($C_{18:2}$)	280.43	—	37.00	56.66	12.26	15.12	8.81
Linolenic	($C_{18:3}$)	278.41	—	1.38	2.10	51.32	6.29	0.51
Eicosenoic	($C_{20:1}$)	310.50	—	2.62	—	—	9.44	0.74
Erucic	($C_{22:1}$)	338.55	—	0.55	—	—	47.93	—

* The digit before the colon indicates number of "C" atoms in the chain of fatty acid and the digit after the colon marks the number of double bonds.

Table 3

Iodine values of samples of fats determined by different methods and titres

Oil	By Hanuš [16]	From *Tables* [8]	From chart [10]	Titre
Coconut	4.20	7.5— 10.5	4.9	23.40
Groundnut	99.00	84—100	106.7	29.70
Sunflower	129.55	125—136	129.8	18.90
Linseed	181.55	170—204	183.5	17.70
Rape seed	103.55	97—108	104.0	11.20
Lard	59.73	53—77	61.3	37.00

It can be seen that the scales for various constants are associated around the basic scales for the iodine and saponification values, *i.e.* that for the reading of individual constants from the chart the known iodine and saponification values are pertinent. The procedure for the determination of these two basic constants as well as of remaining other constants were described in detail in Experimental.

The basic prerequisite for the determination of physicochemical constants using the presented chart is the possibly most exact knowledge of qualitative and quantitative composition of fatty acids in a given triglyceride sample. As we have mentioned before, at the present time gas—liquid chromatography of fatty acids is the most effective method for this purpose.

The application and verification of the described method for constant determination was done using lard and coconut, groundnut, sunflower, linseed, and rape seed oil.

Qualitative and quantitative data on the content of various fatty acids in the triglyceride samples used as determined by gas—liquid chromatography are presented in Table 2. The table contains also literary data on molecular weights of the assayed fatty acids [8].

The iodine values of the assayed triglyceride samples as determined by different methods are presented and compared in Table 3.

The average molecular weights of fatty acids as calculated from Table 2 and their corresponding saponification values as read on the horizontal scales of the chart are listed in Table 4. For comparison also the saponification values as determined by the classical titration method and literary data on these values are also given in this table. It can be seen, that the saponification values of triglyceride samples as determined by the combination of chromatography and nomogram are closest to literary data.

Using the saponification values read out from the constructed chart (listed in Table 4) and using the iodine values as determined by procedure [10] (listed in Table 3) it was possible to read the other physicochemical constants of the

Table 4

Comparison of saponification values of fatty oils found by various methods

Method of determination	Coconut	Ground-nut	Sunflo-wer	Linseed	Rape seed	Lard
Middle molecular weights computed from fatty acids; molecular weight averages	210.91	280.56	269.92	277.96	308.11	274.46
SV determined classically by the titrimetric method [16]	265.30	191.00	183.90	186.60	170.60	188.00
SV found by presented chromatogram-nomogram method	251.80	191.80	198.60	193.20	174.90	195.75
Theoretical SV as found in *Tables* [8]	250—264	188—195	188—194	188—196	170—180	190—202

Table 5

Physicochemical constants of fats and oils determined by the presented chromatogram—nomogram method

Oil	Refractive index (40°C)	Specific gravity [15/15°C]	Combustion heat	Neutralization value	Iodine-saponification factor	Hardness number
Sunflower	1.46670[a]	0.9208	9570	192.5	53.5	123.5
	1.466—1.468[b]	0.922—0.926	9486	203.7	?	?
Groundnut	1.46480[a]	0.9210	9510	201.0	76.0	196.5
	?[b]	0.917—0.921	9637	199.8	102.0	196.3
Linseed	1.47279[a]	0.9290	9485	196.0	4.6	70.0
	1.472—1.475[b]	0.930—0.938	9494	201.1	15.0	70.5
Rape seed	1.46365[a]	0.9134	9715	177.0	66.0	107.5
	1.464—1.468[b]	0.930—0.938	9682	180.4	61.0	107.6
Lard	1.45840[a]	0.9145	9520	205.0	76.0	266.0
	1.459—1.461[b]	0.934—0.938	9555	204.2	134.0	292.4

a) As found from the described chart.
b) From *Tables* [8].

assayed triglyceride samples. In Table 5 these values are presented and compared with literary data. In Table 5 no physicochemical constants for coconut oil are presented because its iodine value is lower than 50 and thus the chart could not be used. This points to the application limitations of the chart for physicochemical constant determination of triglyceridic fats and oils. The chart cannot be used when the iodine value is lower than 50 or higher than 430 and/or the saponification value is lower than 50 or higher than 400. There are only about 15 such cases among natural fats and oils.

It should be added in connection with the presented procedure for the determination of physicochemical constants using our alignment chart that not only the assay as mentioned in [10] can be used for iodine value determinations but also any of the methods described in the recently published review [18] may be employed. However, the first mentioned method for the determination of the iodine value makes it possible to determine the whole set of physicochemical constants by a single gas—liquid chromatogram of a fatty acid mixture. It was the aim of our work.

Taking into account the rate of the determination of physicochemical constants of triglyceride samples using the presented procedure, *i.e.* gas—liquid chromatography combined with the use of our chart, the exactness of the obtained results can be considered to be acceptable for the requirements of the fatty oil industry and research.

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Abbreviation used

IV iodine value
RI refractive index at 40°C
SG specific gravity [15/15°C]
CH combustion heat
SV saponification value
T titre of free fatty acids
HN hardness number
ISF iodine-saponification factor (difference of SV and IV)
$\varnothing M$ average molecular weight

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