Content of Chlorophylls and Carotenes in Rapeseed Oil

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Abstract

The content of the green components in crude rapeseed oil, namely chlorophylls A and B, as well as the products of their decomposition pheophytins A and B, were determined by means of the spectrophotometric method of analysis.

No chlorophyll A was found in any of the analyzed samples but the content of pheophytin A was quite high and amounted to from 17.99 to 25.65 ppm. Because of the fact that the oils were investigated 5 to 6 months after their extraction, the results obtained are not unexpected, bearing in mind that chlorophyll contained in oil easily changes into pheophytin.

No chlorophyll B was found in some of the samples, investigated and in the remaining samples from 0.14 to 1.79 ppm was found. This can be explained by the hypothesis of slower change of chlorophyll B into pheophytin B.

The content of pheophytin B in the investigated samples was between 0.52 and 6.15 ppm. This confirms the results obtained by Mingot (3). With old oils, the results of determining such components, whose content approaches nil in the sample, are less precise because of the influence of the oil which was used as a blank.

The content of carotenes in crude rapeseed oils was also determined by the spectrophotometric method after isolating them by means of the column chromatography on Al_2O_3.

In spite of the small amount of carotenes determined in rapeseed oils, a correlation can be observed between the quantity of chlorophylls and carotenes. It has been found that the higher the chlorophyll content, the higher also the carotene content, even if very slight in some cases, in all the investigated oils either extracted or pressed.

Introduction

MOST VEGETABLE OILS and fats have a specific colour due to the presence of natural vegetable pigments, products of their decomposition and their accompanying substances. Most of the colour is given by chlorophylls, carotenes, xanthophyll and other colour substances related to them. An exception to this is cottonseed oil which has the gossypol pigment.

Investigations on the *green* pigments which appear in oils have established that there are two chlorophyll components—A and B—and the products of their decomposition—pheophytins A and B—present in oils as well as in other products from vegetable sources.

Spectrophotometric curves with characteristic maxima for green pigments are shown in most studies devoted to the analysis of oils. Many authors ascribe these maxima to the presence of chlorophyll. They disregard, however, the difference between chlorophyll A and B and are not interested in the products of its decomposition.

First Kaufmann and Vogelmann (1) determined the wavelengths at which chlorophyll A and pheophytin A give absorption maxima in linseed oil, the values being 600 mμ and 665–670 mμ, respectively. Further, these authors stated that a maximum absorption characteristic for pheophytin A appeared in the linseed oil which was subjected to tests.

In his report, Kurita (2) indicated that the maxima, which soybean oil gives in the visible range at a wavelength of 670 mμ, are to be related to the presence of pheophytin A. An analysis of the spectrophotometric curves of various oils in the range at which chlorophyll A and B and pheophytin A and B give characteristic maxima confirms the fact that the quantity of chlorophyll A or the product of its decomposition pheophytin A prevails in oils. Chlorophyll B and pheophytin B do not give any visible maxima that can be explained by the smaller quantity of those pigments in products from vegetable sources.

The quantity of chlorophylls A and B as well as of the products of their decomposition and accompanying substances depends on the method of producing the oil and on other technological and biological factors.

According to Mingot (3) the presence of pheophytin in olive oil should be explained by oxidation and fermentation which take place while the olives are being processed. The final products of these processes are organic acids which influence the chlorophyll and produce pheophytin. The author has observed an interesting fact, namely, that chlorophyll A changes into pheophytin A more quickly than chlorophyll B changes into its relevant pheophytin. The same fact has been confirmed by Mackinney and Joslyn (4) and Aronoff (5) who investigated chlorophyll in aqueous acetone.

Papers on the quantity of chlorophyll A and B and pheophytin A and B in various kinds of oil are scarce and are concerned almost exclusively with olive oil. Therefore, Mingot (3) has worked out a spectrophotometric method for determining the four above-mentioned components, which appear side by side, and he gave convenient equations for calculations.

In the present work Mingot's method was used for determining the concentration of chlorophyll A and B and pheophytin A and B in 20 crude rapeseed oils, and this was the first purpose of our work. To do this, standards of chlorophyll A and B and pheophytin A and B had to be obtained, analytic wavelengths found, chlorophyll A and B and pheophytin A and B absorption coefficients determined for rapeseed oil, and four equations with four unknowns set up for expressing the components. Further, the equations had to be presented in a convenient form for calculations.

The second purpose of this study was to determine the *yellow* pigments in rapeseed oil. It is worthy of mention that except for one reference by Kaufmann (6) there is no information available on this subject.

In order to determine the carotenes it was necessary to isolate them first from the given substance. After studying the methods in this line it was decided to use the chromatographic method with aluminium oxide as given by Bickoff and Williams (7) who worked it out for determining carotenes in oils.

TABLE I

Determination of Chlorophyll A and B, Pheophytin A and B Absorption Coefficients at Analytic Wavelengths

ppm	$a_{645.5}$	$a_{655.8}$	$a_{663.8}$	a_{668}
1	2	3	4	5
Chlorophyll A				
0.98	0.020	0.061 [a]	0.087	0.076
1.14	0.018	0.053	0.088	0.073
2.10	0.019	0.054	0.083	0.074
2.12	0.018	0.051	0.083	0.076
3.14	0.019	0.053	0.081	0.071
3.38	0.018	0.050	0.082	0.075
3.93	0.018	0.050	0.081	0.071
3.96	0.018	0.051	0.080	0.069
4.76	0.018	0.049	0.082	0.069
4.82	0.020	0.054	0.087	0.076
5.96	0.018	0.049	0.085	0.072
6.73	0.019	0.050	0.081	0.073
8.36	0.020	0.055	0.083	0.073
8.93	0.020	0.054	0.084	0.073
9.90	0.019	0.053	0.081	0.071
Average absorption coefficient	0.019	0.052	0.083	0.073
Chlorophyll B				
0.53	0.055	0.040	0.030	0.023
0.54	0.050	0.037	0.026	0.021
1.07	0.052	0.039	0.028	0.022
1.20	0.051	0.040	0.029	0.022
1.56	0.052	0.039	0.028	0.022
1.84	0.052	0.039	0.029	0.023
1.85	0.052	0.039	0.029	0.023
2.56	0.051	0.038	0.028	0.022
2.83	0.052	0.040	0.030	0.023
2.96	0.052	0.038	0.028	0.022
3.35	0.051	0.037	0.027	0.022
3.62	0.051	0.039	0.029	0.023
3.83	0.053	0.039	0.029	0.022
3.99	0.051	0.037	0.028	0.021
4.36	0.054	0.040	0.029	0.023
Average absorption coefficient	0.052	0.039	0.028	0.022
Pheophytin A				
0.98	0.007 [a]	0.024	0.052	0.060
1.14	0.010	0.026	0.053	0.062
2.10	0.011	0.026	0.053	0.061
2.12	0.011	0.027	0.054	0.064
3.14	0.010	0.025	0.051	0.060
3.38	0.010	0.026	0.052	0.062
3.93	0.009	0.022	0.051	0.057
3.96	0.010	0.025	0.051	0.060
4.76	0.010	0.026	0.052	0.060
4.82	0.011	0.026	0.051	0.062
5.96	0.009	0.023	0.051	0.058
6.73	0.010	0.025	0.052	0.060
8.36	0.011	0.029	0.054	0.062
8.93	0.010	0.026	0.052	0.060
9.90	0.009	0.023	0.051	0.059
Average absorption coefficient	0.010	0.025	0.052	0.060
Pheophytin B				
0.53	0.024	0.040	0.033	0.028
0.55	0.023	0.032	0.027	0.024
1.11	0.022	0.037	0.030	0.025
1.20	0.019	0.038	0.031	0.025
1.64	0.021	0.035	0.029	0.025
1.84	0.022	0.041	0.034	0.028
1.98	0.021	0.034	0.029	0.025
2.79	0.021	0.033	0.029	0.025
2.83	0.020	0.040	0.034	0.027
3.26	0.021	0.032	0.028	0.024
3.74	0.020	0.034	0.029	0.025
3.62	0.020	0.041	0.033	0.028
4.35	0.022	0.037	0.030	0.026
4.57	0.019	0.033	0.028	0.023
5.46	0.019	0.036	0.030	0.025
Average absorption coefficient	0.021	0.036	0.030	0.026

[a] Not in average.

Compared with other methods, which are based on the isolation of carotenes by means of unsaponifiables, this method is quicker, simpler, and provides better conditions for preserving the components, which is important for the final results. In order to obtain the unsaponifiables it is first necessary to saponify the sample and subject it to subsequent extractions with a solvent. Then, after distilling the solvent and drying the residue, the separation of carotenes from other substances can be begun. The time necessary for carrying out all those operations is about 2.5 times that required by the direct chromatographic method. Further, carotenes are subjected for a longer time to the influence of temperature while the sample is being prepared, and temperature, as well as light, gives favourable conditions for the formation of isomeric forms, which are generally known as neocarotenes. Those changes take place at an extraordinary speed in the solutions of dissolvents. The above-mentioned reasons were decisive in choosing the direct chromatographic method.

Experimental

Chlorophyll

Obtaining of the Standards of Chlorophyll A and B. Chlorophyll A and B standards were obtained by means of column chromatography according to the modified Stoll and Widemann (8) method. The modification consisted in the alteration of the quantity of chromatographic columns and in the application of a cooling jacket on the columns. This permitted the separation of leaf pigments at the temperature of 4–5C. Identification of the standards obtained in ether solution were carried out by the spectrophotometric method aided by the adsorption maxima in the visual range which are characteristic for chlorophyll A and B. The spectrophotometric curves were compared with the data given by Harris and Zscheile (9) and Zscheile and Comar (10).

Further, absorption coefficients of the investigated compounds were determined at the wavelength of 659.8 mμ for chlorophyll A and 642.4 mμ for chlorophyll B. The values were found to be in compliance with those given in the literature.

In order to determine the concentration of the pure substance in standard solution used while determining the absorption coefficients the complexometric method of determining magnesium was applied as described by Falk (11).

In addition to the spectral characteristic used for checking the obtained pigments for cleanliness, a phase test and a test for ascertaining the presence of the phytol group were applied because of the fact that the phytol group may split off due to the hydrolysis of the chlorophyll.

The Determination of the Analytic Wavelengths. Those wavelengths in which characteristic maxima appear for chlorophyll A and B, and for pheophytin A and B in the investigated rapeseed oil were assumed to be the analytic wavelengths. In order to determine the position of those maxima, solutions were prepared of the above-mentioned substances in rapeseed oil decolourized with activated charcoal. Ranges from 630–680 mμ have been investigated. The maxima which have been found lie at the following wavelengths: 663.8 for chlorophyll A, and 645.5 for chlorophyll B. The measurements were carried out with a Zeiss VSU-1 type spectrophotometer.

Pheophytin A and B standards were obtained from their relevant chlorophylls by the method described by Mingot (3). Solutions of those standards in oil were also investigated in the range of 630–680 mμ. The determined maxima appear at the following wavelengths: 668 mμ for pheophytin A, and 655.8 mμ for pheophytin B.

Following Mingot it was assumed that Beer's law is correct in analytic wavelengths for the investigated range of concentrations.

The Determination of Absorption Coefficients for the Standard Substances in Rapeseed Oil. Decolourized rapeseed oil has been weighed in flasks and various amounts of the ether solution of chlorophyll

TABLE II

Determined Contents of Chlorophyll A and B, Pheophytin A and B in Crude Rapeseed Oils

Samples in oils	Characteristics of crude rapeseed oils: Acid value	Iodine value	Saponification value	Unsaponifiable matter, %	Refractive index n_D^{25}	Content in ppm: Chlorophyll A	Chlorophyll B	Pheophytin A	Pheophytin B
Extracted									
1	2.1	101.2	174.6	0.83	1.4720	.....	.....	25.62	5.36
2	2.8	100.3	172.7	0.82	1.4720	.....	1.06	20.91	2.69
3	3.4	102.9	170.9	0.79	1.4715	.....	.....	25.20	4.86
4	1.8	97.9	173.6	0.84	1.4720	.....	1.00	17.99	1.73
5	5.1	98.0	170.3	0.52	1.4710	.....	.....	24.84	6.15
Pressed									
1	1.7	101.6	172.3	0.74	1.4717	.....	0.70	21.04	4.23
2	1.3	102.2	170.9	0.59	1.4720	.....	.....	22.56	3.72
3	2.7	101.8	176.2	0.63	1.4719	.....	0.62	22.73	3.18
4	1.7	100.0	175.4	0.36	1.4718	.....	.....	25.13	0.52
5	2.0	99.8	170.0	0.42	1.4715	.....	1.23	20.60	1.54
6	2.2	94.6	172.8	0.48	1.4710	.....	0.33	21.89	3.00
7	2.4	97.5	178.9	0.41	1.4710	.....	0.55	23.84	3.41
8	2.2	100.4	176.4	0.44	1.4710	.....	1.19	22.88	2.31
9	2.7	100.3	179.3	0.37	1.4710	.....	.....	21.99	3.96
10	1.9	98.2	176.1	0.22	1.4708	.....	1.79	23.92	2.49
11	2.3	100.8	171.4	0.49	1.4710	.....	0.21	22.58	3.87
12	2.2	93.4	170.5	0.28	1.4709	.....	.....	22.88	4.52
13	2.6	102.1	173.2	0.49	1.4710	.....	0.99	23.43	3.03
14	2.5	93.2	172.5	0.56	1.4707	.....	.....	23.03	3.71
15	2.1	103.3	174.0	0.60	1.4710	.....	0.14	21.79	2.68

A added. Further, the ether was evaporated in an atmosphere of inert gas and the absorption of the standard prepared in this way measured at four analytic wavelengths. The same was done with the standard solution of chlorophyll B and pheophytin A and B.

The standard solution of pheophytin A were prepared from the chlorophyll A solutions in rapeseed oil. To each of those solutions, 0.5 ml of glacial acetic acid was added. Then, the solution was heated till its colour changed from yellow to brown. When the solution was still hot, an inert gas was blown through it in order to evaporate the acetic acid. The pheophytin concentration has been determined in ppm of chlorophyll A.

Pheophytin B standard solutions were prepared in the same way.

The absorption coefficients were then calculated and results are compiled in Table I.

Using the additivity of absorption as a base, it is possible to represent the absorption of a multicomponent mixture by the total sum of the absorptions of all its components.

The following system of equations with four unknown was obtained for the analyzed four-component mixture, where x_1, x_2, x_3, x_4 represent the concentrations of chlorophyll A, chlorophyll B, pheophytin A and pheophytin B, respectively.

$$A_{645.5} = 0.019x_1 + 0.052x_2 + 0.010x_3 + 0.021x_4$$
$$A_{655.8} = 0.052x_1 + 0.039x_2 + 0.025x_3 + 0.036x_4$$
$$A_{663.8} = 0.083x_1 + 0.028x_2 + 0.052x_3 + 0.030x_4$$
$$A_{668} = 0.073x_1 + 0.022x_2 + 0.060x_3 + 0.026x_4$$

Those equations were solved and given in such a form as to make it convenient to calculate the concentrations of chlorophyll A and B, and pheophytin A and B after putting in the absorption values of the samples measured at four analytic wavelengths.

$$x_1 = 0.38\ A_{645.5} - 23.76\ A_{655.8} + 72.50\ A_{663.8} - 52.34\ A_{668}$$
$$x_2 = 34.75A_{645.5} - 28.83\ A_{655.8} + 18.20\ A_{663.8} - 9.33\ A_{668}$$
$$x_3 = 3.96\ A_{645.5} - 0.12\ A_{655.8} - 59.46\ A_{663.8} + 67.00\ A_{668}$$
$$x_4 = -40.52\ A_{645.5} + 92.71\ A_{655.8} - 81.78\ A_{663.8} + 38.10\ A_{668}$$

The Determining of the Quantity of Chlorophyll A and B, and Pheophytin A and B in Oils. Samples of crude rapeseed oils were subjected to investigation. The characteristics of the oils and the determined quantities of chlorophyll and pheophytin have been compiled in Table II.

Carotenes

First of all, the available beta-carotene sample was tested for cleanliness by determining its absorption curve, according to the wavelength. Further, a standard straight line was drawn for the further use for taking the readings of carotene concentrations in the investigated samples.

Then, rapeseed oil solutions in dried petroleum ether was prepared. A weighed portion of 10 g oil was dissolved in ether and taken over quantitatively to a 100 ml capacity volumetric flask. Then, the volume of the flask was replenished with the same solvent. The solution obtained in this way contained 0.1 g /ml of oil.

The amount of 10 ml of the prepared rapeseed oil solution in petroleum ether was given on a column with an internal diameter of 11 mm. The column was filled with 12 g of active aluminum oxide /80 mesh/. The column of the aluminum oxide was covered with a layer formed of 1 g of anhydrous sodium sulfate. After the components were adsorbed on the column, the carotenes were eluted with petroleum ether containing 2% acetone. Small quantities of this solvent were given so that the surface of the solvent was kept at the same level. Preliminary tests proved that a quantity of 20 ml of the solvent is sufficient for the eluting of the coloured carotene band which was distinctly separated and moving along the column at the highest speed. The eluent containing carotenes was collected into a volumetric flask

TABLE III

Content of Carotenes in Crude Rapeseed Oil

Rapeseed oils	Absorption at wavelength 450 mμ	Content as β-carotene ppm
Extracted N°		
1	0.169	7.01
2	0.155	6.43
4	0.151	6.26
Pressed N°		
1	0.121	5.02
2	0.103	4.27

at the outlet of the column. The carotene content was determined by measuring the absorption at the wavelength of 450 mμ and taking readings of its concentration from the standard curve prepared previously. The above-described procedure was applied with all the determinations which were carried out. The carotene contents were expressed in its predominant component, i.e. beta-carotene.

The results obtained for the investigated rapeseed oils are given in Table III.

Further investigations are being continued on the accuracy of quantitative determinations of chlorophylls and pheophytins in rapeseed oil.

Studies on carotenes contents in rapeseed oil are also being continued.

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A Simplified and Precise Flavor Evaluation Procedure for Determining Oxidative Rancidity in Vegetable Oils[1]

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Abstract

High correlations were observed between the flavor evaluation procedure and objective chemical tests for measuring oxidative deterioration in stored corn, cottonseed, safflower, and soybean oils. The high correlations observed are believed to result from the precision of the flavor threshold testing procedure. Therefore, when suitable flavor testing methods are employed, the chemical methods become more valuable in assessing the quality of an oil. Milk is recommended as the testing media because of its desirable properties.

Introduction

IN THE DEVELOPMENT, evaluation and quality control of edible oils, stability of the oils to oxidative deterioration is of primary importance. Sensory evaluation of the oils has long been the most sensitive method of assessing quality but it is well recognized that it generally lacks precision and reproducibility. Hence, many chemical methods have been developed to measure oxidative deterioration with the objective of correlating the data with flavor deterioration. Varied experiences have been reported in this regard and most researchers are still seeking the ideal test. While additional chemical methods certainly merit attention, we have observed that considerable improvement in research results is obtainable by applying a more precise flavor evaluation technique and by recognizing the reciprocal relationship of chemical data to the quantitative sensory evaluation data. This relationship was originally observed by Lillard and Day (3) in studies on milk fat. The purpose of this investigation was to ascertain if a similar relationship existed with vegetable oils.

Experimental Procedures

Cottonseed, soybean, safflower, and corn oil, containing no added antioxidants, were dispensed into 2 oz open vials and exposed to 100 ft-c of light at 30C for 16 weeks. Analyses were conducted at 2-week intervals on 2 oz of each oil.

Peroxide values were determined by the AOCS method (1) and expressed as milligram of peroxide per kilogram of oil. The 2-thiobarbituric acid number (TBA), was measured by the procedure of Sinnhuber and Yu (4), and the free carbonyls were measured as described by Keith and Day (2).

Flavor evaluations were carried out with a panel of 12 trained judges. The panel was selected from a group of 22 people by presenting milk, containing an oil with an oxidized flavor, at the approximate threshhold of detection, in a triangular test. Twelve panel members were selected after completion of ten triangular tests. The selected panel members were trained by using the aforementioned oils, to familiarize them with oxidative rancidity in the oils.

Samples were prepared for the flavor test in the following manner. The oil to be evaluated was emulsified in pasteurized skim milk to a level of 4% by means of a Waring blendor. The skim milk-oil sample was then mixed in different proportions with fresh pasteurized-homogenized milk containing 4% fat. The samples prepared in this manner contained a total of 4% lipid but different concn of the vegetable oil, depending on the amt required to give a perceptible oxidized flavor. A series of dilutions were thus prepared to cover the range of two above and two below

Name_____

Date_____

Can You Detect an Oxidized Flavor?

Sample No.					
Yes					
No					
Other Flavor (Comments)					

FIG. 1.

[1] Technical Paper No. 1833, Oregon Agricultural Experiment Station, Corvallis.