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DETERMINATION OF CHLOROPHYLL PIGMENTS IN CRUDE VEGETABLE OILS

Results of a collaborative study and the standardized method

(Technical Report)

Prepared for publication by

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Determination of total chlorophyll pigments in crude vegetable oils: Results of a collaborative study and the standardized method (Technical Report)

Synopsis

The development, by collaborative study, of a standardized method for the determination of chlorophyll pigments (mainly pheophytins, which are produced by loss of magnesium from the molecules of respective chlorophylls) in crude vegetable oils is described. The chlorophyll pigments are determined by spectrophotometric measurement at 670 nm.

INTRODUCTION

Chlorophyll pigments (mainly chlorophyll a) are indicators of the quality of oilseeds, and the method for their spectrophotometric determination by measurement of the extract [1] or by reflectance measurement [2] were proposed for the standardization [3].

During industrial seed processing, chlorophyll pigments (mainly pheophytins, which are better soluble in oil and solvents) are extracted into crude oils, and become indicators of their quality, particularly in rapeseed, soybean and olive oils. The spectrophotometric determination of chlorophyll pigments in soybean oil has been standardized [4], but the development of spectrophotometric techniques made a revision of the method necessary [5].

In addition to the direct spectrophotometry of total chlorophyll pigments, the fluorometric determination [6, 7] and the HPLC of individual pigments [8-12] are possible but for most purposes, the determination of total chlorophyll pigments is sufficient for the quality assessment of crude oils.

COLLABORATIVE STUDIES AND RESULTS

The procedure given below was used, measuring the absorbance at 670 nm, where the readings are in the best correlation with the content of total chlorophyll pigments.

The statistical analysis of results was calculated in agreement with the standard procedure [13]. The following symbols were used: n = number of laboratories; m = mean value; Sr = repeatability standard deviation; r = repeatability; kr = coefficient of variation of repeatability; SR = reproducibility standard deviation; R = reproducibility; kR = coefficient of variation of reproducibility.

The preliminary test was carried out in 1990. Six samples were analyzed in 12 laboratories:
A = crude rapeseed oil obtained by expeller pressing;
B = oil A diluted by refined rapeseed oil (1:2 m/m);
C = oil B diluted by refined rapeseed oil (1:2 m/m);
D = crude rapeseed oil obtained by solvent extraction of expeller cakes;
E = oil D diluted by refined rapeseed oil (1:1 m/m);
F = oil E diluted by refined rapeseed oil (1:1 m/m).
All samples were analyzed in the duplicate. The samples were presented coded in random order. The two duplicate determinations are given in Tab. I.

The statistical evaluation is summarized in Tab. II. As the results were considered not entirely satisfactory, it was decided to repeat the test in 1991 with the presentation of a reference sample with the known content of chlorophyll pigments, and to synchronize the analyses.

In 1991, ten laboratories participated, and the following six samples were analyzed:
A = crude expeller pressed rapeseed oil;
B = crude rapeseed oil obtained by solvent extraction of expeller cake;
C = bleached rapeseed oil;
D = fully refined rapeseed oil;
E = oil A diluted with refined rapeseed oil D (2:3 m/m);
F = oil B diluted with refined rapeseed oil D (2:3 m/m).
Oils were presented coded in duplicates in the random order. The results are given in Tab. III.
In some laboratories, the operators were not able to determine the content of chlorophyll pigments in bleached and fully refined oils. Therefore, the method should be applied only for the analysis of crude oils.

Table I RESULTS OF TOTAL CHLOROPHYLL PIGMENTS:
(content of chlorophyll pigments in samples A - F given in mg per kg):
Collaborative study 1990

Lab code	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F
01	27.04	4.97	2.23	30.06	10.31	2.35
	29.70	11.10	1.90	29.59	11.40	3.11
02	37.34	14.85	5.21	37.22	15.50	6.15
	37.38	15.09	5.25	36.55	14.71	6.85
03	33.00	13.50	3.50	33.40	12.50	4.80
	39.10	9.60	3.80	33.90	11.50	6.10
04	37.48	9.74	2.75	37.33	16.33	3.18
	32.15	10.43	1.59	37.48	12.70	5.63
05	29.37	10.01	2.02	24.47	12.98	5.25
	28.12	7.03	3.33	28.30	13.50	4.09
06	34.53	16.75	6.73	36.26	18.82	8.89
	35.91	17.44	8.29	36.95	17.78	8.89
07	33.67	18.78	3.80	33.67	16.40	3.80
	33.67	12.78	5.01	34.01	16.75	7.25
08	36.30	16.10	4.60	35.40	17.00	6.00
	37.00	15.10	5.00	36.00	17.30	6.40
09	34.66	14.61	5.66	28.89	15.82	6.76
	37.98	6.83	4.93	33.36	16.84	6.71
10	31.67	3.93	4.58	34.76	14.84	6.68
	37.24	4.79	4.67	31.48	16.58	6.27
11	35.07	15.02	1.45	33.53	18.07	6.72
	36.38	13.47	2.50	35.01	17.85	6.77
12	37.15	11.31	2.47	36.19	18.27	6.03
	36.96	13.95	5.56	38.00	15.17	6.44

TABLE II STATISTICAL EVALUATION OF THE RESULTS: Collaborative study 1990

Symbol	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F
n	12	12	12	12	12	12
m	34.54	11.30	4.02	33.95	15.37	5.88
Sr	2.24	2.99	0.90	1.27	1.14	0.97
kr	6.49	26.45	22.29	3.75	7.43	16.46
r	6.34	8.46	2.54	3.60	3.23	2.74
SR	3.39	4.02	1.78	3.09	2.47	1.66
kR	9.81	35.57	44.20	9.40	16.06	28.33
R	9.58	11.38	5.03	9.03	6.99	4.71

In the interlaboratory test 1992, thirteen laboratories participated, and the following 6 samples were analyzed:
P = crude rapeseed oil obtained by expeller pressing,
R = crude rapeseed oil obtained by solvent extraction of expeller cakes,
S = oil P diluted with refined rapeseed oil (1 : 1 v/v),
T = oil R diluted with refined rapeseed oil (1 : 1 v/v),
V = oil P diluted with refined rapeseed oil (1 : 7 v/v),
Z = oil R diluted with refined rapeseed oil (1 : 7 v/v).
All samples were presented coded in random number in the duplicate. The analytical results are summarized in Tab. V. Results marked with *x* have been rejected as out-liers. Some results were lacking, others had to be rejected because of out-lying values. The statistical evaluation is summarized in Tab. VI, showing good repeatability and good reproducibility of the method.

TABLE III DETERMINATION OF CHLOROPHYLL PIGMENTS IN RAPESEED OIL:
(content of chlorophyll pigments in the samples A-F is given in mg per kg):
Collaborative study 1991

Lab code	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F
01	48.00	52.60	0.20	0.40	18.30	21.10
	47.80	52.30	0.20	0.40	18.20	21.20
02	49.00	53.20	-	-	18.30	21.20
	48.60	52.70	-	-	18.30	21.00
03	48.80	53.20	0.28	0.43	18.60	21.10
	48.90	53.20	0.28	0.43	18.60	21.10
04	49.00	53.38	0.28	0.43	19.04	21.20
	48.60	53.37	0.28	0.41	19.08	21.33
05	47.07	53.16	-	0.14	13.71	20.72
	47.51	52.94	-	0.17	14.51	20.13
06	35.63	40.38	-	-	13.14	-
	36.90	41.18	-	-	13.47	-
07	49.60	53.70	0.25	0.37	*19.10*	*21.60*
	49.40	53.70	0.23	0.40	18.90	21.50
08	49.06	53.98	0	-	16.95	21.30
	48.23	53.49	0	-	18.43	21.24
09	43.96	50.45	0.07	0.14	13.74	19.30
	43.96	50.45	0.10	0.10	14.61	19.72
10	45.50	48.70	0.30	0.30	18.90	21.20
	45.70	48.60	0.30	0.40	16.30	20.80

TABLE IV. STATISTICAL EVALUATION OF RESULTS
Collaborative study 1991

Symbol	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F
n	10	10	7	7	10	9
m	46.56	51.23	0.20	0.32	17.01	20.93
Sr	0.38	0.25	0.01	0.03	0.72	0.21
kr	0.82	0.49	5.04	9.72	4.26	0.98
r	1.09	0.72	0.03	0.09	2.05	0.58
SR	4.01	4.02	0.11	0.13	2.29	0.63
kR	8.62	7.84	57.69	40.73	13.47	3.01
R	11.36	11.37	0.32	0.13	6.48	1.78

The Tab. VI shows comparable or better results of the 1992 study. For the quality assessment of crude oils, the repeatabilities and reproducibilities obtained in the interlaboratory tests in 1991 and, particularly, in 1992 are fully satisfactory.

The fluorometric method was found satisfactory in our laboratory, but the equipment is not available in most laboratories. The HPLC method gives good results in our laboratory, and obviously, in other laboratories as well, but it is not yet sufficiently developed for the standardization. For the quality evaluation of crude oils, the determination of total chlorophyll pigments is quite satisfactory, and it is not necessary to separate them.

TABLE V. DETERMINATION OF CHLOROPHYLL PIGMENTS IN VEGETABLE OILS
Collaborative test 1992
(The concentration of chlorophyll pigments is given in mg/kg.)

Laboratory code	Sample P	Sample R	Sample S	Sample T	Sample V	Sample Z
01	39.78x	38.45x	21.86x	19.99x	5.99	5.39x
	40.12x	38.28x	22.10x	20.01	6.22	5.28x
02	55.2	53.8	28.9	26.3	7.4	6.9
	55.9	53.5	28.8	26.2	7.7	7.1
03	55.33	52.69	28.33	26.17	7.47	6.80
	55.20	52.88	28.26	26.02	7.44	6.78
04	53.7	51.6	27.7	25.6	7.3	6.5
	53.8	51.7	27.7	25.5	7.3	6.6
05	54.3	52.2	28.1	25.8x	7.3x	6.7
	54.3	52.2	28.2	-	-	6.7
06	55.09	52.98	28.41	26.17x	7.47	6.84
	55.07	52.93	28.59	-	7.61	6.91
07	55.5	52.6	28.8	25.9	7.2	6.6
	54.9	52.4	28.4	25.9	7.3	6.8
08	53.6	52.4	27.2	25.9x	7.0	6.8
	54.1	52.3	27.1	27.6x	6.9	6.8
09	55.2	53.2	28.7	25.5	7.5	6.5
	55.2	53.1	28.7	26.3	7.6	6.5
10	54.2	52.2	27.8	25.9	6.6	6.3
	54.3	52.2	27.7	26.0	6.7	6.4
11	53.65	51.61	27.98	25.95	7.43	6.79
	53.65	51.42	28.14	25.94	7.38	6.84
12	53.1	51.1x	27.8	25.8x	7.3x	6.7
	52.7	-	27.7	-	-	6.7
13	54.94	52.66	28.40	25.90	7.39	6.75
	54.90	52.61	28.28	26.00	7.46	6.75

TABLE VI. STATISTICAL EVALUATION OF THE RESULTS Collaborative study 1992

Symbol	Sample P	Sample R	Sample S	Sample T	Sample V	Sample Z
n	12	11	12	8	11	12
Sr	0.233	0.104	0.109	0.080	0.098	0.067
r	0.658	0.294	0.310	0.227	0.277	0.190
kr	0.427	0.158	0.389	0.308	1.360	0.999
SR	0.845	0.632	0.502	0.260	0.463	0.182
R	2.390	1.790	1.420	0.737	1.310	0.514
kR	1.550	1.204	1.783	1.001	3.431	2.706

DETERMINATION OF CHLOROPHYLL PIGMENTS IN CRUDE VEGETABLE OILS

1. SCOPE AND FIELD OF APPLICATION

This standard describes a method for the determination of total chlorophyll pigments, expressed as pheophytin a, in crude vegetable oils. The method is suitable for the determination of quantities of chlorophyll pigments higher than 1 mg/kg.

2. DEFINITION

The content of chlorophyll pigments in vegetable oils is expressed as mg of pheophytin a in 1 kg of oil.

3. PRINCIPLE

Chlorophyll pigments are determined by measuring the absorbance at 670 nm, correcting the result for the background absorption, and calculating the content with use of the absorptivity of pheophytin a, which is the main chlorophyll pigment in crude vegetable oils.

4. APPARATUS

4.1. Spectrophotometer allowing the measurement in the range of 630 to 710 nm.

4.2. Glass cells, thickness 5 mm or 10 mm.

4.3. Glass funnel (diameter 40 - 60 mm).

5. PROCEDURE

5.1. Preparation of the test sample

The sample is heated to the temperature higher than 5 °C than the melting point, if the sample is solid at room temperature. The liquid sample is homogenized, and if turbid, filtered immediately before the analysis, using a medium-pore size filter paper.

5.2. Measurement

The sample is measured at 630 nm, 670 nm and 710 nm in a 5 mm or a 10 mm spectrophotometer cell against air instead of a reference cell.

6. EXPRESSION OF RESULTS

The content of chlorophyll pigments is expressed in mg of pheophytin a, which is equal to:

$$C = 345.3 \times (A_{670} - 0.5 \times A_{630} - 0.5 \times A_{710}) : L$$

where: C = content of chlorophyll pigments in mg of pheophytin a in 1 kg of oil,
A = absorbance at the respective wavelength (nm),
L = thickness of the spectrophotometer cell (mm).

7. REPEATABILITY OF THE METHOD

The repeatability, expressed by the coefficient of variation, is equal to:
0.4 at high contents of chlorophyll pigments,
1.0 at medium contents of chlorophyll pigments,
1.4 at low contents of chlorophyll pigments.

8. REPRODUCIBILITY OF THE METHOD

The reproducibility, expressed by the coefficient of variation, is equal to:
1.3 at high contents of chlorophyll pigments,
3.5 at medium contents of chlorophyll pigments,
6.0 at low contents of chlorophyll pigments.

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