INTERNATIONAL STANDARD

ISO 10519

Second edition
1997-11-01

Rapeseed — Determination of chlorophyll content — Spectrometric method

Graines de colza — Détermination de la teneur en chlorophylle — Méthode spectrométrique

Foreword

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Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 10519 was prepared by Technical Committee ISO/TC 34, *Agricultural food products,* Subcommittee SC 2, *Oleaginous seeds and fruits.*

This second edition cancels and replaces the first edition (ISO 10519:1992), which has been technically revised.

Annexes A and B of this International Standard are for information only.

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Printed in Switzerland

Rapeseed — Determination of chlorophyll content — Spectrometric method

1 Scope

This International Standard specifies a spectrometric method for the determination of the chlorophyll content of rapeseed. It is not applicable to the determination of chlorophyll in oils.

2 Normative references

The following standards contain provisions which, through reference in this test, constitute provisions of this International Standard. At the time of the publication, the editions indicated were valid. All standards are subject to revision, and the parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 648:1977, *Laboratory glassware - One-mark pipettes.*
ISO 664:1990, *Oilseeds - Reduction of laboratory sample to test sample.*
ISO 665:1977, *Oilseeds - Determination of moisture and volatile matter content.*

3 Definition

For the purposes of this International Standard, the following definition applies.

3.1
chlorophyll content
mass fraction of substances in the sample contributing to the absorption band at a wavelength near 665 nm, as determined under the operating conditions specified in this International Standard and measured as chlorophyll A

NOTE — The chlorophyll content is expressed in milligrams per kilogram.

4 Principle

Extraction of a test portion in a suitable apparatus with a specified extraction solvent. Spectrometric determination of the chlorophyll content of the extracted solution

5 Reagent

Use only reagents of recognized analytical grade unless otherwise stated.

5.1 Extraction solvent

Transfer to a 500 ml beaker 100 ml of anhydrous ethanol. Add to the contents of the beaker 300 ml of anhydrous iso-octane (2,2,5-trimethylpentane) or anhydrous technical *n*-heptane or anhydrous petroleum ether (essentially composed of C_7 hydrocarbons, with a boiling range between 90 °C and 100 °C).

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Analytical balance, capable of weighing to the nearest 0,001 g.

6.2 Mechanical grinder, blade type, or coffee mill or equivalent.

6.3 Mechanical microgrinder (see figure 1), comprising stainless-steel tubes of approximately 50 ml volume which can be securely stoppered, stainless-steel ball-bearings (∅ 16 mm), and an apparatus to shake the securely stoppered tubes horizontally at a frequency of 240 min^{-1}, with a horizontal displacement of 3,5 cm, or a **Dangoumau ball mill**[1].

Dimensions in millimetres

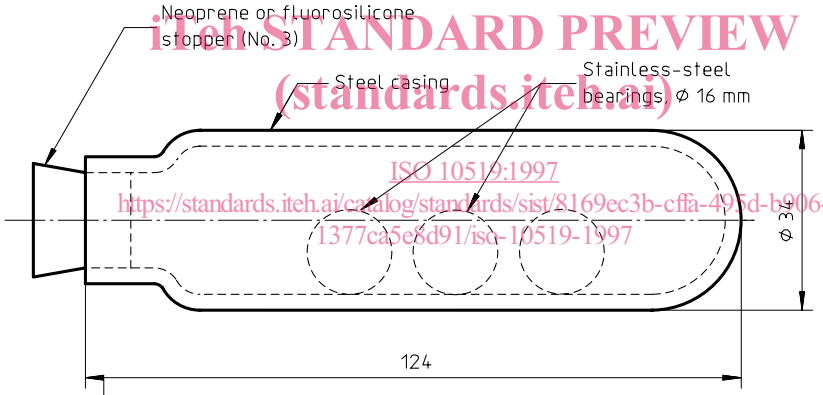

Figure 1 — Mechanical microgrinder

6.4 Filter paper, medium speed, V-folded.

6.5 Spectrometer (preferably with wavelength scanning), suitable for carrying out absorbance measurements at wavelengths between 600 nm and 700 nm, with a spectral bandwidth of 2 nm.

6.6 Optical cells, having a path length of at least 1 cm.

6.7 Pipettes, of 30 ml capacity, complying with the requirements of ISO 648, class A, or a repetitive dispenser capable of dispensing 30 ml with an error of less than 1 %.

6.8 Culture tubes, of 20 ml capacity, provided with stoppers.

7 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 542.

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport and storage.

8 Preparation of test sample

Prepare a test sample in accordance with ISO 664 from the laboratory sample as received, after separation of impurities.

Dry seeds with a moisture content of greater than 10 % (*m*/*m*) for 12 h at 45 °C to reduce the moisture level to 10 % (*m*/*m*) or less in order to reduce the risk of destroying chlorophyll pigments.

Transfer 50 g of the test sample to the mechanical grinder (6.2) and grind to produce a uniformly ground seed. If a small grinder such as a coffee mill is used, grind several portions of 10 g and then combine them and mix thoroughly the ground portions.

9 Procedure

9.1 Test portions

Weigh, to the nearest 0,001 g, 2 g of the test sample (clause 8) into a stainless-steel tube or the extraction vessel of the Dangoumau ball mill (6.3).

9.2 Extraction

9.2.1 Add, using a pipette (6.7), 30 ml of the extraction solvent (5.1) to the tube or vessel. If using a tube, add three stainless-steel balls to the tube and shake for 1 h. For Dangoumau ball mills, add at least four medium-sized steel balls to the vessel and extract for 20 min.

9.2.2 Allow the extract to settle for 10 min and then decant a sufficient volume of the extract through the filter paper (6.4) into a culture tube (6.8) to fill the optical cell (6.6). Stopper the tube as soon as possible to minimize evaporation.

NOTE — The presence of more than one phase in the extraction solvent indicates the presence of excessive moisture, either in the sample [which should contain less than 10 % (*m*/*m*) moisture] or in the solvents (which should be anhydrous).

9.3 Determination

Transfer the filtered extract to a cell (6.6) and determine by means of the spectrometer (6.5) the absorbance at wavelengths of 665 nm, 705 nm and 625 nm. (The readings at 705 nm and 625 nm are used to calculate a baseline correction.)

10 Expression of results

The chlorophyll content, w, in milligrams per kilogram of the product as received, is given by the formula

$$w = \frac{k \times A_{corr} \times V}{m \times l}$$

where

A_{corr} (the corrected absorbance) is equal to $A_{665} - (A_{705} + A_{625})/2$;

A_{665} is the absorbance at 665 nm;

A_{705} is the absorbance at 705 nm;

A_{625} is the absorbance at 625 nm;

k is a constant which is equal to 13;

l is the path length, in centimetres, of the optical cell;

m is the mass, in grams, of the test portion;

V is the volume, in millilitres, of solvent added to the tube (9.2.1).

If it is desired to express the chlorophyll content relative to the dry product, take into account in the calculation the moisture content of the sample, determined in accordance with ISO 665.

11 Precision

Details of an interlaboratory test on the precision of the method are summarized in annex A. The values derived from this interlaboratory test may not be applicable to concentration ranges and matrices other than those given.

11.1 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, should not be greater than 10 % of the arithmetic mean of the two results.

11.2 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment should not be greater than 20 % of the arithmetic mean of the two results.

12 Test report

The test report shall specify

— the method in accordance with which sampling was carried out, if known,
— the method used,
— the test result(s) obtained, and
— if the repeatability has been checked, the final quoted result obtained.

It shall also mention all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the result(s).

The test report shall include all information necessary for the complete identification of the sample.

Annex A
(informative)

Results of interlaboratory test

An interlaboratory test was carried out by 16 laboratories in accordance with ISO 5725[2]. The repeatability and reproducibility values shown in table A.1 were obtained.

Table A.1 - Statistical results of interlaboratory test

Parameter	Sample [1]									
	A	B	C	D	E	F	H	I	J	Mean
Number of labs. participating	16	16	16	16	16	16	16	16	16	
Number of outliers [2]	1	1	1	1	1	1	1	1	4	
Mean value (mg/kg)	25,8	31,0	40,4	49,8	75,7	82,9	10,5	20,4	31,2	
Repeatability standard deviation s_r, mg/kg	1,13	1,47	1,45	1,75	2,74	2,99	0,49	0,68	0,99	
Reproducibility standard deviation s_R, mg/kg	1,93	2,15	3,02	3,44	4,9	5,68	1,02	1,63	2,19	
Repeatability coefficient of variation, %	4,39	4,76	3,59	3,52	3,62	3,61	4,66	3,35	3,16	3,85
Reproducibility coefficient of variation, %	7,47	6,94	7,49	6,91	6,47	6,85	9,79	8,00	7,04	7,44
Repeatability limit (r), mg/kg	3,17	4,13	4,06	4,90	7,68	8,38	1,38	1,93	2,79	
Reproducibility limit (R), mg/kg	5,39	6,03	8,47	9,63	13,71	15,90	2,89	4,61	6,21	
Repeatability (r), %	12,3	13,3	10,1	9,9	10,1	10,1	13,1	9,5	8,9	10,81
Reproducibility (R),%	20,9	19,4	21	19,3	18,1	19,2	27,5	22,5	19,9	20,88

[1] Samples A through E were from the 1992 study; samples F through H were from the 1990 study.

A: 25 mg/kg chlorophyll
B: 30 mg/kg chlorophyll
C: 40 mg/kg chlorophyll
D: 50 mg/kg chlorophyll
E: 75 mg/kg chlorophyll
F: 80 mg/kg chlorophyll
H: 10 mg/kg chlorophyll
I: 20 mg/kg chlorophyll
J: 30 mg/kg chlorophyll

[2] One laboratory in each of the studies was eliminated due to failure to carry out the method correctly. With sample J, three additional laboratories were eliminated because of a difference greater than 3 mg/kg between two values.

2) ISO 5725:1986, *Precision of test methods — Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests* (now withdrawn), was used to evaluate the precision data.

Annex B
(informative)

Bibliography

[1] ISO 542:1990, *Oilseeds — Sampling.*

[2] ISO 5725-1:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*.