SLOVENSKI STANDARD
SIST EN 17908:2024

01-februar-2024

Alge in izdelki iz alg - Metode vzorčenja in analize - Določevanje skupnih lipidov z metodo Ryckebosch-Foubert

Algae and algae products - Methods of sampling and analysis - Determination of total lipids content using the Ryckebosch-Foubert method

Algen und algenbasierte Produkte oder Zwischenprodukte - Methoden zur Probeentnahme und Analyse - Bestimmung von Gesamtlipiden mit der Ryckebosch-Foubert Methode

Algues et produits à base d'algues - Méthodes d'échantillonnage et d'analyse - Détermination de la teneur en lipides totaux à l'aide de la méthode de Ryckebosch-Foubert

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ICS:

13.020.55	Biološki izdelki	Biobased products

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EUROPEAN STANDARD

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EN 17908

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English Version

Algae and algae products - Methods of sampling and analysis - Determination of total lipids content using the Ryckebosch-Foubert method

Algues et produits d'algues - Méthodes d'échantillonnage et d'analyse - Détermination de la teneur en lipides totaux à l'aide de la méthode de Ryckebosch-Foubert

Algen und algenbasierte Produkte oder Zwischenprodukte - Verfahren zur Probenahme und Analyse - Bestimmung von Gesamtlipiden mit der Ryckebosch-Foubert-Methode

This European Standard was approved by CEN on 6 November 2023.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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European foreword

This document (EN 17908:2023) has been prepared by Technical Committee CEN/TC 454 “Algae and algae products”, the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by June 2024, and conflicting national standards shall be withdrawn at the latest by June 2024.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

This document has been prepared under a standardization request addressed to CEN by the European Commission. The Standing Committee of the EFTA States subsequently approves these requests for its Member States.

Any feedback and questions on this document should be directed to the users’ national standards body. A complete listing of these bodies can be found on the CEN website.

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Introduction

General

The European Committee for Standardization (CEN) was requested by the European Commission (EC) to draft European standards or European standardization deliverables to support the implementation of Article 3 of Directive 2009/28/EC for algae and algae-based products or intermediates.

This request, presented as Mandate M/547[1], also contributes to the Communication on “Innovating for Sustainable Growth: A Bio Economy for Europe”.

The former working group CEN Technical Board Working Group 218 “Algae”, was created in 2016 to develop a work programme as part of this Mandate. The technical committee CEN/TC 454 'Algae and algae products' was established to carry out the work programme that will prepare a series of standards.

The interest in algae and algae-based products or intermediates has increased significantly in Europe as a valuable source including but not limited to, carbohydrates, proteins, lipids, and several pigments. These materials are suitable for use in a wide range of applications from food and feed purposes to other sectors, such as textiles, cosmetics, biopolymers, biofuel and fertilizer/biostimulants. Standardization was identified as having an important role in promoting the use of algae and algae products.

The work of CEN/TC 454 should improve the reliability of the supply chain, thereby improving the confidence of industry and consumers in algae, which include macroalgae, microalgae, cyanobacteria, Labyrinthulomycetes, algae-based products or intermediates and will promote and support commercialization of the European algae industry.

Considerations in relation to the method

There is an objective among the algae community to have an accepted standardized method for the determination of total lipids in algae. There are other methods for the determination of total lipid content currently utilized in specific areas, like food and feed, and non-food and non-feed applications, each one producing consistent results when used in one laboratory, but many times not consistent between different methods or laboratories.

The aim of this document is to define one suitable laboratory method of analysis for the determination of total lipids in algae. This method could also be used as a reference method for the validation of other applied methods. The Ryckebosch-Foubert method determines the total lipid content in micro- and macroalgae. This method has a lower reproducibility when applied to algae with lower lipid content.

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[1] Available at https://ec.europa.eu/growth/tools-databases/mandates/index.cfm?fuseaction=refSearch.search#

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1 Scope

This document specifies a laboratory method for the determination of the total lipid content in micro- and macroalgae by the Ryckebosch-Foubert method.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 17399, *Algae and algae products — Terms and definitions*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN 17399 and the following apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
total lipid content
all lipid substances extracted from the test portion under the operating conditions specified, expressed in mg/gram relative to dry weight

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4 Principle

The lipids in dried and homogenized algal biomass are extracted with chloroform/methanol (see Annex B). Afterwards a counter extraction with water is performed to remove the non-lipids. Two consecutive extractions of two phases each are performed, as has been optimized as described in Annex A. After removal of the solvents, the amount of total lipids is determined gravimetrically.

5 Apparatus

5.1 Rotary evaporator, including a vacuum pump with an adjustable vacuum level

5.2 Centrifuge, at room temperature

5.3 Vortex

5.4 Analytical balance, with a readability of 0,01 mg

5.5 Bead beater (only in case procedure 8.3 is used)

Different bead beaters and/or different cells can lead to different results. Therefore, when using procedure 8.3, the specifications of the bead beater and cell used shall be indicated in the report.

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6 Reagents and materials

6.1 Pyrex®[2] screw capped tubes, 10 ml (one unit per test portion) or comparable glassware

6.2 Pyrex® screw capped tubes, 20 ml (one unit per test portion) or comparable glassware

6.3 Funnels, (one unit per test portion)

6.4 Filter paper of 180 µm, (one unit per test portion), e.g. of Whatman, type nr. 1

6.5 Pasteur pipettes, 2 ml (one unit per test portion), e.g. a Soda-lime glass, non-sterile pipette with a total length of 145 mm

6.6 Round-bottom flasks, useable for analytical balance (not above the maximum mass) for the rotary evaporator system (one unit pertest portion), at the temperature of the balance (to avoid weighing error)

6.7 Micropipettes

6.8 Chloroform (with a purity not less than a volume fraction of 99,0 %

6.9 Methanol (with a purity not less than a volume fraction of 99,0 %

6.10 Chloroform/methanol (1/1 by volume)

6.11 Anhydrous sodium sulphate

6.12 Demineralized water

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7 Sampling and sample handling

Sampling and sample handling is not part of the method specified in this document. A recommended sample handling procedure is given in EN 17605:2022 with the following adaptations/additions:

— fine grinding shall be conducted as defined in 3.13 in EN 17605:2022;

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— transport shall be performed on dry ice;

— storage shall be at −80 °C. Also, macro-algae need to be freeze-dried.

Extensive storage times of the test sample should be avoided. In case this could not be avoided, the test sample should be freeze-dried again before usage.

Three test portions from the test sample shall be analysed as to be able to calculate the mean and standard deviation.

[2] Pyrex® is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product.

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8 Procedure

8.1 General

Some algae, cultivated under specific culture conditions, can have a high lipid content and/or a rigid cell envelope that makes that cell disruption is required prior to the extraction. Procedure 8.3 has been established for these cases. For a species or culture condition that has not been analysed before, both procedures 8.2 and 8.3 shall be performed and the results compared. In this case, first perform both procedure (8.2 without cell disruption and 8.3 with cell disruption) and compare the results. In case the total lipid content using procedure 8.3 is significantly (Level of significance: 0,01) higher than that using procedure 8.2, continue to use procedure 8.3 for that sample, otherwise stick to 8.2.

It is not appropriate to always incorporate this cell disruption step as for most samples it is not necessary and even leads to lower values (due to loss on beads) and more variability in the results. It also implies that special instrumentation should be available in the lab.

For all standard algae, cultivated under standard cultivation conditions, no cell disruption step is necessary to extract all the lipids and procedure 8.2 shall be used.

8.2 Procedure without cell disruption

8.2.1 General

The procedure without cell disruption shall be conducted through the following steps. The complete procedure, except for the weighing, should be conducted under the fumehood.

8.2.2 First extraction

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a) Weigh a test portion of approximately 100 mg of the test sample in a Pyrex® screw capped tube of minimum 10 ml (i.e. weight test portion). Record the weight to the nearest 0,01 mg (or 0,1 mg depending on the readability of your analytical balance);

b) Add 4 ml of methanol followed by 2 ml of chloroform;

c) Add 0,4 ml demineralized water;

d) Vortex at least 30 s until the different layers are mixed well;

e) Add 2 ml of chloroform followed by 2 ml of demineralized water (10 ml Pyrex® tube is now totally filled);

f) Vortex at least 30 s until the different layers are mixed well;

g) Centrifuge the tube at 450 g for 10 min;

h) After centrifugation two solvent phases are formed (often separated by the biomass pellet): remove the upper layer with a Pasteur pipette to a waste container;

NOTE This layer is waste and contains sugars, proteins and minerals.

i) Transfer the lower layer to a 20 ml Pyrex® screw capped tube using a Pasteur pipette. For each test portion another Pasteur pipette shall be used. This pipette should be kept in the 20 ml tube for the second extraction;

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It is recommended to tilt the tube about 45°. By doing this, the pellet will move and some space will be available to move the Pasteur pipette along the biomass pellet without touching it. It is possible that the pellet breaks in smaller parts during tilting, which is not a problem.

j) Leave the pellet in the 10 ml Pyrex® tube;

Add 4 ml of chloroform/methanol (1/1 by volume) to this pellet;

k) Vortex at least 30 s until the different layers are mixed well;

l) Centrifuge the tube at 450 g for 10 min;

m) After centrifugation, only one phase is observed. Transfer this solvent layer to the same 20 ml Pyrex® tube as before with the same Pasteur pipette as before.

8.2.3 Second extraction

Repeat step 8.2.2 a) from the first extraction on the same remaining biomass pellet. (Do not weigh new biomass). Then continue with the following steps:

a) Add 4 ml of methanol followed by 2 ml of chloroform;

b) Vortex at least 30 s until the different layers are mixed well;

c) Add 2 ml of chloroform followed by 2 ml of water (10 ml Pyrex® tube is now totally filled);

d) Vortex at least 30 s until the different layers are mixed well;

e) Centrifuge the tube at 450 g for 10 min;

f) After centrifugation two solvent phases are formed: remove the upper layer with a Pasteur pipette to a waste container;

g) Transfer the lower layer to the same 20 ml Pyrex® tube as before with the same Pasteur pipette that is still in the tube. After this step, the pipette can be thrown away;

h) Leave the pellet in the 10 ml Pyrex® tube;

i) Add 4 ml of chloroform/methanol (1/1 by volume) to this pellet;

j) Vortex at least 30 s until the different layers are mixed well;

k) Weigh the round-bottom flask (previously dried at 103 °C in the oven and then cooled to room temperature) on the analytical balance to the nearest 0,01 mg (i.e. weight empty flask) (or 0,1 mg depending on the readability of your analytical balance);

l) Equip the round-bottom flask with a funnel and filter paper nr. 1. Add 5 g of sodium sulphate in the filter paper;

It is very important that no sodium sulphate powder comes in the flask, otherwise the total lipid content will be overestimated. The presence of crystals after drying is an indication that sodium sulphate has been spilled into the flask. If this occurs, the method shall be started over with a new sample. Extra caution is recommended.

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