SLOVENSKI STANDARD
SIST EN 18197:2026

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Alge in izdelki iz alg - Ugotavljanje aminokislinskega profila mikro- in makroalg

Algae and algae products - Determination of the amino acid profile of micro- and macroalgae

Algen und Algenprodukte - Bestimmung des Aminosäuren Profils von Mikro- und Makroalgen

Algues et produits à base d'algues - Détermination du profil en acides aminés des micro et macroalgues

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Algae and algae products - Determination of the amino acid profile of micro- and macroalgae

Algues et produits à base d'algues - Détermination du profil en acides aminés des micro et macroalgues

Algen und Algenprodukte - Bestimmung des Aminosäuren-Profils von Mikro- und Makroalgen

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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 18197:2026) 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of an identical text or by endorsement, at the latest by November 2026, and conflicting national standards shall be withdrawn at the latest by November 2026.

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.

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Introduction

This document has been prepared by the experts of CEN/TC 454 'Algae and algae products'.

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, 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 textile, cosmetics, biopolymers, biofuel and fertilizer/biostimulants. Standardization was identified as having an important role in order to promote 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 commercialisation of the European algae industry.

Determining amino acids is important for the industry to measure specific amino acids and establish the whole amino acid profile of algae and algae products. Moreover, total amino acids can be used to calculate the protein content of a sample with accuracy.

The goal of this document is to give algae producers and algae products industries a recommendation for an analysis method for amino acids which can be utilized for algae. The document relies strongly on standards ISO 4214, *Milk and milk products — Determination of amino acids in infant and adult/paediatric nutritional formulas and dairy products* [1], AOAC 2018.06, *Total Amino Acids in Infant Formulas and Adult Nutritionals* [2], and AACC 07-50.01, *Total Amino Acids by UHPLC-UV* [3], and adds the possibility to use mass spectrometry for the determination of amino acids.

1 Scope

This document describes a method for determining the amino acid profile of micro- and macroalgae.

It specifies a method for the quantitative determination, in one single analysis, of the following amino acids: alanine, arginine, aspartic acid (combined with asparagine), cystine (dimer of cysteine, combined with cysteine), glutamic acid (combined with glutamine), glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine and valine.

This method does not apply to the determination of tryptophan.

2 Normative references

The following document is 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 — Vocabulary*

EN 17605:2022, *Algae and algae products — Methods of sampling and analysis — Sample treatment*

3 Terms and definitions

For the purposes of this document, the terms and definitions in EN 17399 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/

4 Principle

Proteins are hydrolysed in 6 mol/l hydrochloric acid (HCl) for 24 h at 110 °C in the presence of phenol and 3-3′-dithiodipropionic acid (DDP). Phenol is added to prevent halogenation of tyrosine. DDP is added to convert cystine and cysteine to S-2-carboxyethylthiocysteine (XCys) as described in Reference [4].

After neutralization, amino acids and XCys are derivatized with 6-aminoquinolyl-N-hydroxy-succinimidyl carbamate. Derivatized amino acids are separated using reversed phase UHPLC and detected by UV at 260 nm or by mass spectrometry. For UV detection, norvaline is added as an internal standard, while heavy labelled amino acids are used for MS detection.

NOTE 1 Fluorescence detection can be used provided equivalence has been demonstrated.

NOTE 2 UV and MS detection methods have been shown to yield comparable results (Annex C).

During acid hydrolysis, glutamine (Gln) and asparagine (Asn) are converted to glutamic acid (Glu) and aspartic acid (Asp), respectively. Thus, Glu values represent the combined values of Glu and Gln, and Asp values represent the combined values of Asp and Asn. Cys2 values represent the combined values of cysteine and cystine since both are converted to XCys by DDP.

5 Reagents

Use only reagents of recognized analytical grade.

Reagents can be either prepared in the laboratory or purchased in ready-to-use formats. Commercial references are only a guideline. Equivalent chemicals or materials can be used provided their equivalence has been demonstrated.

Before using chemicals, refer to the safety data sheets and ensure that the safety precautions are applied. Phenol is toxic if swallowed, in contact with skin or if inhaled. It can cause severe skin burns and eye damage, is suspected of causing genetic defects, may cause damage to organs through prolonged or repeated exposure, and is toxic to aquatic life with long lasting effects. Due to its toxicity and corrosive properties, strict preventive measures are required when storing and using phenol and its aqueous solutions.

5.1 AccQ·Tag™ Ultra Derivatization Kit (Waters 186003836[1]).

As an alternative derivatizing buffer, di-sodium tetraborate decahydrate (CAS Registry Number®[2] 1303-96-4) can be used.

As an alternative derivatizing reagent, 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (CAS 148757-94-2) can be used.

5.2 AccQ·Tag™ Ultra Eluent A concentrate (Waters 186003838[1]).

As alternative reagents, acetonitrile (CAS 75-05-8), ammonium formate (CAS 540-69-2) and formic acid (CAS 64-18-6) can be used.

NOTE This reagent is only required for UV detection.

5.3 AccQ·Tag™ Ultra Eluent B (Waters 186003839[1]).

As alternative reagents, acetonitrile (CAS 75-05-8) and formic acid (CAS 64-18-6) can be used.

NOTE This reagent is only required for UV detection.

5.4 Phenol (CAS 108-95-2).

5.5 3,3′-Dithiodipropionic acid (CAS 1119-62-6).

5.6 Amino acid standard solution

An amino acid standard solution that contains the following 17 amino acids at 2,5 mmol/l each (except L-cystine at 1,25 mmol/l) in 0,1 mol/l HCl: L-alanine, L-arginine, L-aspartic acid, L-cystine, L-glutamic acid, L-glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine and L-valine.

5.7 Heavy-labelled amino acid standard solution

A heavy-labelled amino acid standard solution that contains the following 17 heavy-labelled amino acids at 2,5 mmol/l each (except L-cystine at 1,25 mmol/l) in 0,1 mol/l HCl: L-alanine ($^{13}C_3$, ^{15}N), L-arginine ($^{13}C_6$, $^{15}N_4$), L-aspartic acid ($^{13}C_4$, ^{15}N), L-cystine ($^{13}C_6$, $^{15}N_2$), L-glutamic acid ($^{13}C_5$, ^{15}N), L-glycine ($^{13}C_2$, ^{15}N), L-histidine ($^{13}C_6$, $^{15}N_3$), L-isoleucine ($^{13}C_6$, ^{15}N), L-leucine ($^{13}C_6$, ^{15}N), L-lysine ($^{13}C_6$, $^{15}N_2$), L-methionine ($^{13}C_5$, ^{15}N), L-phenylalanine ($^{13}C_9$, ^{15}N), L-proline ($^{13}C_5$, ^{15}N), L-serine ($^{13}C_3$, ^{15}N), L-threonine ($^{13}C_4$, ^{15}N), L-tyrosine ($^{13}C_9$, ^{15}N) and L-valine ($^{13}C_5$, ^{15}N). This standard solution is provided by Euristop (MSK-A2-1.2)[1)]. If

[1] This 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 the product named. The alternative reagents listed in this document have been shown to lead to the same results.

[2] CAS Registry Number® is a trademark of CAS corporation. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of the product named. Equivalent products may be used if they can be shown to lead to the same results.

another supplier is selected, verify that the labelling is similar to that described in this clause. If this is not the case, the masses of the internal standards shall be adapted accordingly in Table 8.

NOTE This reagent is only required for MS detection.

5.8 L-cystine (CAS 56-89-3).

5.9 Heavy-labelled L-cystine ($^{13}C_6$, $^{15}N_2$) (CAS 1252803-65-8).

NOTE This reagent is only required for MS detection.

5.10 Norvaline (CAS 6600-40-4).

NOTE This reagent is only required for UV detection.

5.11 Sodium hydroxide pellets, reagent grade (CAS 1310-73-2).

5.12 Sodium hydroxide solution (CAS 1310-73-2), substance concentration c = 1 mol/l.

5.13 Sodium hydroxide solution (optional) (CAS 1310-73-2), c = 6 mol/l.

5.14 Hydrochloric acid fuming 37 % (CAS 7647-01-0), c = 12 mol/l, GR grade for analysis.

5.15 Hydrochloric acid solution (CAS 7647-01-0), c = 1 mol/l.

5.16 Hydrochloric acid solution (CAS 7647-01-0), c = 0,1 mol/l.

5.17 Laboratory water, with a resistivity of 18,2 MΩ-cm (ultra-pure water).

NOTE Use gradient grade for liquid chromatography (LC) for UV detection or LC-MS grade for MS detection.

5.18 Acetonitrile (CAS 75-05-8).

NOTE Use gradient grade for liquid chromatography (LC) for UV detection or LC-MS grade for MS detection.

5.19 Formic acid (CAS 64-18-6).

5.20 Ammonium formate (CAS 540-69-2).

NOTE This reagent is only required for UV detection.

6 Reagents and standard preparation

6.1 Reagents common to UV and MS detection

6.1.1 Sodium hydroxide (NaOH) solutions, *c* = 6 mol/l, *c* = 0,2 mol/l and *c* = 0,05 mol/l.

For the *c* = 6 mol/l solution, weigh out 24 g of sodium hydroxide (5.11) into a 100 ml volumetric flask. Dissolve in about 80 ml of water. Allow to cool down and dilute to the mark with water. Optional: use a commercially available equivalent (5.13).

For the *c* = 0,2 mol/l solution, pipet 20 ml of 1 mol/l NaOH (5.12) into a 100 ml volumetric flask and make up to the mark with water.

For the *c* = 0,05 mol/l solution, pipet 5 ml of 1 mol/l NaOH (5.12) into a 100 ml volumetric flask and make up to the mark with water.

6.1.2 Hydrochloric acid (HCl) solution, c = 0,2 mol/l.

Pipet 20 ml of 1 mol/l HCl (5.12) into a 100 ml volumetric flask and make up to the mark with water.

6.1.3 DDP solution, mass concentration ρ = 20 g/l (in NaOH, c = 0,2 mol/l).

Into a 50 ml volumetric flask, weigh out 1 000 mg of DDP and make up to the mark with 0,2 mol/l NaOH (6.1.1).

6.1.4 Phenol/HCl solution, ρ = 1 g/l (in HCl, c = 12 mol/l).

Into a 100 ml volumetric flask, weigh out 100 mg of phenol and make up to the mark with 12 mol/l HCl (5.14).

NOTE In some matrices, the addition of phenol was shown to have negligible impact on amino acids, including tyrosine. Phenol can be omitted from this solution provided equivalence between the two methods (with or without phenol) is demonstrated in the matrix of interest.

6.1.5 AccQ·Tag™ Ultra Derivatization kit[1)]

Prepare the reagents included in the kit following the manufacturer's instructions.

6.1.5.1 AccQ·Tag™ Ultra Borate buffer (reagent 1)[1)]

Ready-to-use solution. An alternative reagent is sodium tetraborate in water solution (ρ = 50 g/l). Into a 100 ml volumetric flask, weigh 5 g of sodium tetraborate decahydrate, dissolve and make up to the mark with water.

6.1.5.2 AccQ·Tag™ Ultra reagent (vial 2A and 2B)[1)]

Reconstitute AccQ·Tag™ Ultra reagent (vial 2A) according to the manufacturer's instructions as follows:

a) Preheat a heating block to 55 °C.

b) Tap vial 2A lightly before opening to ensure all AccQ·Tag™ Ultra reagent powder is at the bottom of the vial.

c) Rinse a clean micropipette by drawing and discarding 1 ml of AccQ·Tag™ Ultra reagent diluent from vial 2B (ready-to-use solution). Repeat twice.

d) Draw 1,0 ml from vial 2B and transfer it to the AccQ·Tag™ Ultra reagent powder in vial 2A. Cap the vial tightly.

e) Vortex mix for approximately 10 s.

f) Heat vial 2A on top of the preheated heating block until the AccQ·Tag™ Ultra reagent powder is dissolved. Do not heat the reagent for longer than 10 min.

Once reconstituted, the AccQ·Tag™ Ultra reagent concentration is approximately 10 mmol/l. Store the reconstituted AccQ·Tag™ Ultra reagent in a desiccator at room temperature for up to one week.

CAUTION — AccQ·Tag™ Ultra reagent reacts with atmospheric moisture. Seal the container tightly when not in use. Do not refrigerate. Do not use discoloured reagent, especially if it is yellow or green.

The following alternative reagent can be used. Into a 4 ml vial, weigh out approximately 3,0 mg to 4,0 mg of 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate. Continue with step c) above using LC-grade acetonitrile instead of the AccQ·Tag™ Ultra reagent diluent.

6.1.6 Cystine calibration standards

Check purity of cystine by MS (full scan) or UV prior to using it as a standard.

6.1.6.1 Cystine stock solution, c = 10 mmol/l.

Weigh out 240 mg cystine into a 100 ml volumetric flask and make up to the mark with 0,05 mol/l NaOH (6.1.1). Store this solution at −20 °C for up to one month as 1 ml aliquots.

6.1.6.2 Cystine solution, c = 1 mmol/l.

Add 900 µl of 0,05 mol/l NaOH (6.1.1) to 100 µl of cystine stock solution (6.1.6.1). Prepare this solution freshly for each analysis.

6.1.7 Amino acid (AA) calibration standards (with exception of cystine)

6.1.7.1 AA stock solution, c = 2,5 mmol/l.

Amino acid standard solution is ready-to-use and contains 2,5 mmol/l of each amino acid (although present in this solution, cystine is not used for quantification and is prepared separately, see 6.1.6).

Store this calibration standard stock solution at −20 °C for up to six months as 60 µl aliquots.

6.1.7.2 AA solution 1, c = 0,5 mmol/l.

Add 200 µl of 0,1 mol/l HCl (5.16) to 50 µl of AA stock solution (6.1.7.1). Prepare this solution freshly for each analysis.

6.1.7.3 AA solution 2, c = 0,05 mmol/l.

Add 180 µl of 0,1 mol/l HCl (5.16) to 20 µl of AA solution 1 (6.1.7.2). Prepare this solution freshly for each analysis.

6.1.8 Wash solvents.

Use gradient grade for liquid chromatography (LC) reagents.

a) The weak needle wash solvent is 50 ml/l acetonitrile in water.

b) The strong needle wash solvent is 950 ml/l acetonitrile in water.

c) The seal wash solvent is 500 ml/l acetonitrile in water.

6.2 Reagents specific for UV detection

6.2.1 Norvaline (Nva) internal standards

Norvaline internal standards are only used for UV detection.

6.2.1.1 Nva stock solution, c = 10 mmol/l.

Weigh out 117,16 mg Nva into a 100 ml volumetric flask and make up to the mark with 0,1 mol/l HCl (5.16).

6.2.1.2 Nva solution, c = 2,5 mmol/l.

Pipet 2,5 ml of Nva stock solution (6.2.1.1) into a 10 ml volumetric flask and make up to the mark with 0,1 mol/l HCl (5.16).

Store both Nva solutions at −20 °C for up to six months as 2 ml aliquots.