

ICS 67.120.30

English Version

**Foodstuffs - HPLC method for the determination of xanthophylls
in fish flesh - Part 1: Determination of astaxanthin and
canthaxanthin**

Produits alimentaires - Méthode de dosage des
xanthophylles dans la chair de poisson par CLHP - Partie 1:
Dosage de l'astaxanthine et de la canthaxanthine

Lebensmittel - HPLC-Verfahren für die Bestimmung von
Xanthophyllen in Fischfleisch - Teil 1: Bestimmung von
Astaxanthin und Canthaxanthin

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Foreword

This document (CEN/TS 16233-1:2011) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

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1 Scope

This Technical Specification specifies a method for the determination of canthaxanthin and astaxanthin in fish flesh by high performance liquid chromatography (HPLC). The method can be applied at a range above 0,02 mg/kg. The method should not be applied to the determination of canthaxanthin in poultry tissues, egg yolks and shrimp tissues due to a possible interference of canthaxanthin with cryptoxanthin and xanthophyll esters sometimes present in these matrices.

2 Normative references

The following referenced documents are indispensable for the application 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 ISO 3696:1995, *Water for analytical laboratory use – Specification and test methods (ISO 3696:1987)*.

3 Principle

Extract fish flesh by homogenising the tissue in acetone. Filter the extract and evaporate at reduced pressure using a rotary evaporator or a flow of nitrogen at 50 °C. Dissolve the residue in a mixture of *n*-heptane and acetone and analyse by an isocratic normal-phase HPLC.

The HPLC system described effectively separates astaxanthin and canthaxanthin and these both xanthophylls from other carotenoids found in fish flesh as e.g. β -carotene, lutein and zeaxanthin. The main geometrical isomers of astaxanthin are separated from each other and from oxidation products of astaxanthin, astacene and semi-astacene. In contrast, the isomers of canthaxanthin are not separated.

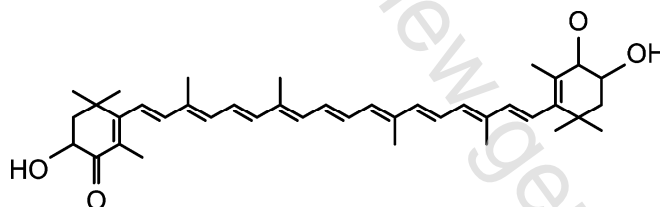


Figure 1 — all-E-Astaxanthin

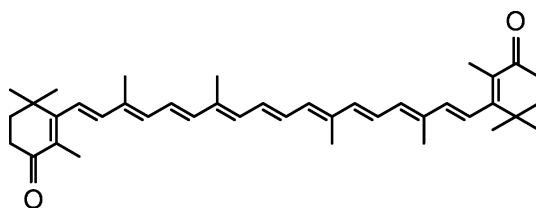


Figure 2 — all-E-Canthaxanthin

4 Reagents

During the analysis, unless otherwise stated, use only water complying with grade 1 of EN ISO 3696:1995 and reagents of recognized analytical grade, e.g. pro analysis (p.a.).