Determination of Astaxanthin tel quel by Photometry at the Isobestic Wavelength of 431 nm

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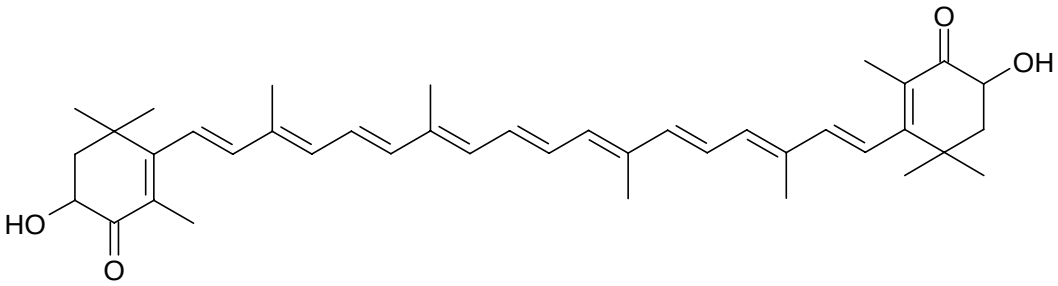
Introduction

This method is part of a collection of methods suitable for the determination of carotenoids [1] in products tel quel which are designed for the use in the feed industry. In this products the carotenoids are stabilized via powdery or water dispersible formulations. The determination is performed at the isobestic wavelength of 431 nm in the flank of the Astaxanthin spectrum. This allows a quantification independently of the ratio of geometrical isomers [2].

Structure:



all-E Astaxanthin

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1 Scope

The method is suited for the analysis of powdery or water dispersible product forms containing chemically synthesized Astaxanthin. The method is not suited for products based on natural sources as e.g. shrimp meal or oil, algae or other microorganisms containing considerable amounts of other pigments besides the main carotenoid.

2 Terms and Definitions / Keywords

Astaxanthin, products tel quel, all-trans isomer, cis isomers, photometry, isobestic wavelength.

3 Principle

The powdery or water dispersible formulations are digested in water with protease. After dilution with acetone the mass fraction of Astaxanthin is determined photometrically at the isobestic wavelength of 431 nm. This analytical method is designed for the determination of the content of Astaxanthin in the range of 5 to 25 g/100 g. Because of measuring at an isobestic wavelength the result for the content of Astaxanthin does not depend on the ratio of the geometrical isomers present in the product forms.

4 Safety Notes

The method described involves the handling of hazardous substances. Attention is therefore drawn to the various provisions governing the handling of potentially dangerous materials. Protective measures of a technical, organizational and personal nature have to be observed.

5 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water or water of equivalent purity.

Pronase E, protease from streptomyces griseus, 4000000 PU/g, e.g. from Merck in Darmstadt, Germany, Art. No. 107433.

Alternative to Pronase E: Protex 6L, bacterial alkaline protease enzyme preparation in water, synonym: Subtilisin, e.g. from Genencor International.

Water, distilled or deionized.

Acetone for spectroscopy, e.g. from Merck in Darmstadt, Germany, Art. No. 100022.

6 Apparatus

Semimicro balance, e.g. AT 261 Delta range from Mettler (resolution 0.01 mg).

Heatable ultrasonic bath, e.g. Elma Transsonic T 460/H from Hans Schmidbauer KG, Singen.

Spectrophotometer, e.g. model UV4-200 from UNICAM.

Disposable polypropylene syringe, volume 20 ml, with adapting cone, e.g. from B. Braun Melsungen AG.

Disposable filter holder, pore size 0.2 µm, for nonaqueous solutions, e.g. Rezist 30/0.2 from Schleicher & Schuell.

Two matched cuvettes made of quartz glass, path length 1.000 cm.

Usual laboratory equipment, low actinic glassware must be used.

7 Sample Preparation / Procedure

A test portion equivalent to 6.5-7.5 mg of Astaxanthin is accurately weighed to 0.01 mg into a 250 ml measuring flask. After adding approx. 25 mg of Pronase E or Protex 6L in the same amount as test portion, the measuring flask is filled to about 1/2 of its volume with deionized water. Care has to be taken that the entire sample is wetted with deionized water and no substance remains clinging to the walls of the flask.

The mixture is then treated in an ultrasonic bath for approx. 10 minutes at 60 °C. During this time it should be ensured by occasional shaking of the flask that the matrix is completely digested. After this the solution is cooled to room temperature. The measuring flask is filled up to the calibration mark with deionized water and then treated in an ultrasonic bath for approx. 10 minutes at room temperature. After shaking thoroughly 5.00 ml of the aqueous suspension are pipetted into a 50 ml measuring flask. Then the flask is filled to the calibration mark with acetone and the contents are intensively mixed.

The solution obtained is filtered through a 0.2 µm disposable filter holder into a quartz glass cuvette. Its absorbance at the isobestic wavelength of 431 nm is measured on the spectrophotometer. A mixture of acetone and of deionized water in the ratio of 9:1 (by volume) is used as reference solution.

Two weighings are performed for each sample.

Due to the sensitivity of carotenoids e.g. against oxygen it is necessary to perform the single steps in a timely manner and without time delay between them.

8 Spectrophotometer Control

Verify the wavelength adjustment by comparison with an international calibration standard. If necessary, correct the working wavelength according to the equation:

$\lambda w = 431.0 + \lambda f - \lambda t$

where λw is the working wavelength used for analysis, λf the wavelength found for the reference peak at approx. 360.8 nm of Holmium oxide spectrum, and λt the target wavelength certified for the peak at approx. 360.8 nm of Holmium oxide spectrum.

9 Calculation

The mass fraction of Astaxanthin is calculated by the formula:

$$w(Astaxanthin) = \frac{Abs\,(431nm) * D}{Iw * A_{1cm}^{1\%}}$$

where

w(Astaxanthin) = mass fraction of Astaxanthin in g/100 g

Abs (431 nm) = absorbance of the test solution at 431 nm

D = dilution factor, 2500 in this case

Iw = initial weight of sample in g

$A_{1cm}^{1\%}$ = specific absorbance of Astaxanthin at 431 nm is 1120

The mean value of a double determination is taken as the result in each case.

10 Repetition of Analyses

All analyses are conducted as duplicates. The results of the two determinations are compared by calculating the relative standard deviation RSD_r. The mean of the double determination is reported if the RSD_r is below the repetition limit of 2,5 %. If the RSD_r exceeds this limit a further double determination is performed. The mean of all four determinations is then reported, except one of the four values is clearly identifiable as outlier. In this case, the outlaying value is not taken into account and the mean is calculated from the three values left.

11 Measurement Uncertainty

The measurement uncertainty of results generated with the present method may be estimated from the predicted relative standard deviation RSD_R according to Horwitz:

RSD_R (%) = $2C^{-0,15}$

Doubling this concentration-dependant value results in an expanded measurement uncertainty of approx. 5 % for a confidence level of 95 %.

Bibliography

[1] Carotenoids, Volume 1B: Spectroscopy, G.Britton, S.Liaaen-Jensen and H.Pfander, Birkhäuser Verlag (Basel, Boston, Berlin), 1995.

[2] J.Schierle, T.Schellenberger, C.Fizet and R.Betz, A simple spectrophotometric determination of total β-carotene in food additives with varying E/Z-isomer ratios using an isobestic wavelength, Eur Food Res Technol, Vol. 215, pp. 268-274 (2002).