SLOVENSKI STANDARD
SIST EN 1141:1996

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Sadni in zelenjavni sokovi - Spektrometrijsko ugotavljanje vsebnosti prolina

Fruit and vegetable juices - Spectrometric determination of proline content

Frucht- und Gemüsesäfte - Spektralphotometrische Bestimmung des Prolingehalts

Jus de fruits et de légumes - Dosage de la proline par spectrométrie

Ta slovenski standard je istoveten z: **EN 1141:1994**

ICS:

67.160.20 Brezalkoholne pijače Non-alcoholic beverages

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EUROPEAN STANDARD

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English version

Fruit and vegetable juices - Spectrometric determination of proline content

Jus de fruits et de légumes - Dosage de la proline par spectrométrie

Frucht- und Gemüsesäfte - Spektralphotometrische Bestimmung des Prolingehalts

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CEN

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

Central Secretariat: rue de Stassart,36 B-1050 Brussels

Foreword

This European Standard has been prepared by the Technical Committee CEN/TC 174 "Fruit and vegetable juices - Methods of analysis", the secretariat of which is held by AFNOR.

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of an identical text or by endorsement, at the latest by April 1995, and conflicting national standards shall be withdrawn at the latest by April 1995.

Annexes designated "informative" are given only for information. In this standard annexes A and B are informative.

According to the CEN/CENELEC Internal Regulations, the following countries are bound to implement this European Standard: Austria, Belgium, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland, United Kingdom.

1 Scope

This european standard specifies a spectrometric method for the determination of proline in fruit and vegetable juices and related products.

2 Normative references

This european standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

ISO 5725:1986 Precision of test methods - Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests

ISO 3696:1987 Water for analytical laboratory use - Specification and test methods

3 Symbols

For the purposes of this standard, the following symbols apply :

c	Substance concentration ;
ρ	Mass concentration ;
ω	Mass fraction.

4 Principle

Proline forms a coloured complex with ninhydrin. This is extracted with n-butyl acetate and the absorbance at 509 nm of the coloured extract is measured spectrometrically.

5 Reagents

5.1 General

Use only reagents of recognized analytical grade and only water in accordance with at least grade 3 of ISO 3696:1987.

5.2 **Ninhydrin** solution in ethyleneglycol monomethyl ether.

ρ (Ninhydrin) = 30 g/l.

Prepare once a week and store in a dark container at 4 °C ± 2 °C.

5.3 n-Butyl acetate

5.4 Formic acid ω (CH_2O_2) = 98 % (m/m) ± 1 % (m/m).

5.5 Sodium sulfate (anhydrous).

5.6 L-Proline

6 Apparatus

Usual laboratory apparatus and, in particular, the following :

6.1 Glass cuvettes, of 10 mm optical path length, and which do not have significant absorption at 509 nm.

6.2 Spectral-line photometer with mercury lamp and filters for measuring at 509 nm.

6.3 Spectrometer (variable wavelength) for measuring at 509 nm (alternative to 6.2).

6.4 Test tubes, stoppered, approximately 25 ml capacity. The stoppered test tubes used shall have similar wall thickness.

6.5 Funnels, approximately 65 mm diameter.

6.6 Filter papers, hydrophobic silicone coated, 110 mm diameter.

6.7 Laboratory timer.

6.8 Water bath, temperature control ± 2 °C.

7 Procedure

7.1 Preparation of the test sample

Normally products shall not be pretreated and their analysis by this method shall be on a volumetric basis, results beeing expressed per litre of sample. The analysis of concentrated products may also be carried out on a volumetric basis, after dilution to a known relative density. In this case, the density shall be indicated. Based on a weighed sample and taking the dilution factor for analysis into account, the results may also be expressed per kilogram of product. In products with high viscosity and/or very high content of cells (for example pulp), determination on the basis of a weighed test sample is the usual procedure.

Samples with proline content 50 mg/l and over should be diluted with water as follows :

50 mg/l	-	499 mg/l	dilute 1 part sample	+ 9 parts water ;
500 mg/l	-	1000 mg/l	dilute 1 part sample	+ 19 parts water.

Strongly coloured products with less than 50 mg proline/l should be diluted 1 part sample + 1 part water or 1 part sample + 4 parts water according to their colour intensity.

7.2 Measurement of extinction coefficient

Dissolve L-proline in water to give a stock solution, r (L-proline) = 100 mg/l. Dilute this stock solution to give solutions with L-proline concentrations = 5 mg/l, 10 mg/l, 25 mg/l, 40 mg/l, and 50 mg/l. Treat 1,0 ml portions of these solutions as described in section 7.3 below.

Plot the absorbance values obtained against L-proline content (mg/l). This should give a straight line passing through the origin. Since the extinction coefficient is subject to variation, it should be determined with each set of samples as follows.

Make three separate measurements of the absorbance at different points on the standard curve (e.g. 10 mg/l, 25 mg/l and 40 mg/l). Calculate the extinction coefficient (ε) using the equation :

$$\varepsilon = \frac{\rho_S}{A}$$

where :

ρ_S is the proline concentration of the standard solution, in mg/l;

A is the corresponding absorbance at that concentration.

The average extinction coefficient is calculated from three closely agreeing values.

7.3 Ninhydrin reaction and development of colour

Pipette 1,0 ml of either test sample or standard solution (see 7.2) into a stoppered test-tube, followed by 1 ml formic acid (5.4) and 2 ml of ninhydrin solution (5.2). Mix the contents well after adding each reagent. Then place the test-tubes in a boiling water bath in which the water level is higher than the level of the solutions in the tubes. The boiling water bath shall not be replaced by any other source of heat (e.g. Thermoblock). Ensure that water bath remains boiling. Leave the tubes in the boiling water bath for exactly 15 min. Then cool the tubes for 5 min to 10 min in water at about 20 °C. Add 10 ml of n-butyl acetate to each tube, insert the stopper and shake thoroughly to extract the colour into the organic phase.

Pour the whole solution into a folded hydrophobic filter paper (6.6) containing 2 g to 3 g of anhydrous sodium sulfate and collect the filtrate. After allowing to stand for about 15 min the absorbance of the organic phase (filtrate) is measured in a 10 mm cuvette at 509 nm against a blank solution prepared in the same way from 1 ml of sample solution but using only ethyleneglycol monomethyl ether instead of the ninhydrin solution, all other conditions remaining as described above.

8 Calculation

Calculate the proline content, ρ (Proline), using the value of the extinction coefficient determined in 7.2 and report in milligrams per litre, without a decimal place.

$$\rho \text{ (Proline)} = \varepsilon \times A$$

Take into account the dilution factor and the relation of the value to mass or volume. If a concentrated product has been diluted to single stength, report the relative density of the single strength sample.

9 Precision

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

9.1 Repeatability

The absolute difference between two single test results found on identical test material by one operator using the same apparatus within the shortest feasible time interval will exceed the repeatability value r in not more than 5 % of the cases.

The values are :

		ρ	≤	50 mg/l	r	=	2,0 mg/l ;
50 mg/l	<	ρ	≤	500 mg/l	r	=	11 mg/l ;
500 mg/l	<	ρ	≤	1000 mg/l	r	=	73 mg/l.

where :

ρ is the measured content, calculated as mean value from the two single test results.

9.2 Reproducibility

The absolute difference between two single test results on identical test material reported by two laboratories will exceed the reproducibility value R in not more than 5 % of the cases.

The values are :

		ρ	≤	50 mg/l	R	=	0,8 mg/l ;
50 mg/l	<	ρ	≤	500 mg/l	R	=	5,5 mg/l ;
500 mg/l	<	ρ	≤	1000 mg/l	R	=	3,5 mg/l.

where :

ρ is the measured content, calculated as mean value from the two single test results.

10 Test report

The test report shall contain the following data :

- all information necessary for the identification of the sample (kind of sample, origin of sample, designation) ;
- a reference to this European Standard ;
- the date and type of sampling procedure (if possible) ;
- the date of receipt ;
- the date of test ;
- the test results and units in which they have been expressed ;
- whether the repeatability of the method has been verified ;
- any particular points observed in the course of the test ;
- any operations not specified in the method or regarded as optional, which might have affected the results.