SLOVENSKI STANDARD
SIST ISO 13685:1998

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Ingverjevi oleorezini - Doloļ evanje glavnih pekoļ ih sestavin (gingerols in shogaols) - Metoda s tekoļ insko kromatografijo visoke loļ ljivosti

Ginger and its oleoresins -- Determination of the main pungent components (gingerols and shogaols) -- Method using high-performance liquid chromatography

Gingembre et oléorésines de gingembre -- Dosage des principaux composés piquants (gingérols et shogaols) -- Méthode par chromatographie en phase liquide à haute performance

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Ginger and its oleoresins — Determination of the main pungent components (gingerols and shogaols) — Method using high-performance liquid chromatography

Gingembre et oléorésines de gingembre — Dosage des principaux composés piquants (gingérols et shogaols) — Méthode par chromatographie en phase liquide à haute performance

Reference number
ISO 13685:1997(E)

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 13685 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 7, *Spices and condiments.*

Annexes A to E of this International Standard are for information only.

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Introduction

This method, initially based on Wood's publications [Overseas Development Natural Resources Institutes (ODNRI)], enables one to know without any ambiguity the identity of the peaks obtained by HPLC, for the quality control of ginger extracts. It is particulary useful in this case as the pungent compounds of interest are very sensitive to heat.

The method has been validated by two international interlaboratory tests carried out by seven laboratories.

After examination of the results, it is estimated that the method may be considered as the best that can be achieved for the moment but it could be improved, in particular for the precision data.

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Ginger and its oleoresins — Determination of the main pungent components (gingerols and shogaols) — Method using high-performance liquid chromatography

1 Scope

This International Standard describes a method for the determination of gingerols [6]-G, [8]-G and [10]-G and the corresponding shogaols [6]-S, [8]-S and [10]-S in dried ginger or in oleoresins of ginger, by high-performance liquid chromatography (HPLC) in the reverse phase.

NOTE - Chemical structures of gingerols and shogaols are shown in annex A.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of the publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on the International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 939:1980,	*Spices and condiments - Determination of moisture content - Entrainment method.*
ISO 2825:1981,	*Spices and condiments - Preparation of a ground sample for analysis.*

3 Principle

From ground and dried ginger, extraction of the pungent components by methanol at atmospheric temperature and concentration at reduced pressure of part of the extract. The oleoresins are dissolved in the methanol.

The resulting solutions are analysed directly (without any cleaning) by reverse-phase HPLC on a column filled with octadecylsilyl silica, with a mixture of acetonitrile and aqueous acetic acid as the mobile phase and by UV detection at 280 nm.

Quantification is carried out using an external standard, nonanoic acid vanillylamide, which has a retention time comparable to that of gingerol-[6] (and a similar UV absorption spectrum).

4 Reagents and products

Use only reagents of recognized analytical grade, unless otherwise specified, and water of HPLC grade.

4.1 External standard: Nonanoic acid vanillylamide (NVA), with melting point between 42 °C and 44 °C and giving primarily a unique peak (at least 98 % of the total area of the peak between 3 min and 8 min) when analysed by HPLC under the conditions described in this International Standard.

WARNING: Nonanoic acid vanillylamide is a product which can cause irritation.

NOTE — NVA is also known as nonyl vanillylamide or pelargonic acid vanillylamide.

4.2 Solvents

WARNING: Acetonitrile is a toxic product which must be used with caution. Studies are underway to replace it by another product.

4.2.1 Methanol, HPLC grade, for preparation of the NVA standard.

4.2.2 Methanol, analytical grade, for dilution and extraction of the test samples.

4.2.3 Acetonitrile, HPLC grade.

4.2.4 Glacial acetic acid.

5 Apparatus

Usual laboratory equipment and, in particular, the following.

5.1 Chromatograph

Apparatus equipped for HPLC analysis:

- an injection system capable of delivering portions of 20 µl of the sample solution with considerable precision;
- an ultraviolet (UV) detector measuring absorbance at a wavelengh of 280 nm;
- a recorder/integrator capable of precisely measuring the area of the peaks with appropriate baseline corrections, with a sensitivity of 0,25 to 0,5 a.u.f.s.

5.2 Stainless-steel column, of 250 mm length and 4,6 mm diameter (or of comparable dimensions) filled with type C_{18} stationary phase with particles of 5 µm.[1]

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[1] ODS, distributed by Phase Separations Ltd., UK, is an example of a suitable product available commercially.This information is given for the convenience of users of International Standard and does not constitute an endorsement by ISO of this product.

5.3 Rotary vacuum evaporator, fitted with a thermostatically controlled water bath.

5.4 Vacuum filtration apparatus

5.5 FH-type Millipore filters[2], with 0,5 µm pores, for organic solvents.

5.6 HA-type Millipore filters[2], with 0,45 µm pores, for aqueous solvents.

5.7 Pipettes, with two marks, class A, of capacity 20 ml.

5.8 Pasteur pipette

5.9 Volumetric flasks, of 5 ml, 25 ml and 100 ml capacities.

5.10 Round-bottom flask, of 100 ml capacity.

5.11 Analytical balance, capable of weighing to the nearest ± 0,001 g.

6 Preparation of test sample

6.1 Dried ginger

Unless it is already in fine powder form, the dry ginger shall be ground as described in ISO 2825, to allow it to pass through a sieve of 1 mm aperture.

The moisture content of the ground product shall be determined in accordance with ISO 939.

6.2 Oleoresins in ginger

Stir well and homogenize the oleoresin before taking test samples.

7 Procedure

7.1 Preparation of standard solutions of NVA

Weigh, to the nearest 0,001 g, 0,1 g of NVA (4.1) and dissolve in methanol (4.2.1). Dilute the solution to 100 ml. Preserve this solution (about 1,0 mg/ml) at –10 °C and when required, prepare the standard solutions (about 0,2 mg/ml and 0,4 mg/ml) by diluting the initial solution (5 ml and 10 ml, respectively, diluted to 25 ml) with methanol (4.2.1). Note the exact concentrations.

7.2 Preparation of sample solutions of NVA

7.2.1 Dried ginger

In a 100 ml volumetric flask (5.9), weigh to the nearest 0,001 g, 1 g of ground ginger. Dilute to the mark with methanol (4.2.2). Plug the flask and shake it vigorously. After 2 h, again shake it vigorously, and then leave it to rest for at least 12 h. With the help of a pipette (5.7) and without

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[2] Millipore filters are examples of suitable products available commercially.This information is given for the convenience of users of International Standard and does not constitute an endorsement by ISO of these products.