INTERNATIONAL STANDARD

ISO 12824

First edition
2016-09-15

Royal jelly — Specifications

Gelée royale — Spécifications

Reference number
ISO 12824:2016(E)

ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

Contents

Page

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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information.

The committee responsible for this document is ISO/TC 34, *Food products*.

Royal jelly — Specifications

1 Scope

This International Standard specifies the production and sanitary requirements for royal jelly and establishes a series of organoleptic and chemical test methods to control royal jelly quality. It also specifies the requirements of transport, storage, packaging and marking for royal jelly. This International Standard applies to the royal jelly production (collecting, preliminary processing and packaging) and trade links. This International Standard is not applicable to royal jelly products in which other foods are mixed.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 4833-1, *Microbiology of the food chain — Horizontal method for the enumeration of microorganisms — Part 1: Colony count at 30 °C by the pour plate technique*

ISO 21528-2, *Microbiology of food and animal feeding stuffs — Horizontal methods for the detection and enumeration of Enterobacteriaceae – Part 2: Colony-count method*

ISO 6579, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Salmonella spp.*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
royal jelly
mixture of secretions from hypopharyngeal and mandibular glands of worker bees, free from any additive

Note 1 to entry: It is the food of larval and adult queens. It is a raw and natural food, unprocessed except for filtration which does not undergo addition of substances. The colour, the taste and the chemical composition of royal jelly are determined by absorption and transformation by the bees fed with the following two types of foods during the royal jelly production time:

— type 1: only bee's natural foods (pollen, nectar and honey);

— type 2: bee's natural food and other nutrients (proteins, carbohydrates, etc.).

3.2
10-HDA
10-hydroxy-*2*-decenoic acid
characteristic material of royal jelly

4 Requirements

4.1 Description

Royal jelly is milky white, pale yellow, with luster. It is pasty or jelly-like at room temperature with fluidity and shall be free from bubbles and foreign substances. Minor crystallization phenomena can occur naturally in royal jelly during storage.

4.2 Odour and taste

It is pungent, unfermented and shall not be rancescent. It is acerb, spicy and it brings acrid taste to palate and throat.

4.3 Chemical requirements

Royal jelly shall comply with the requirements given in Table 1.

Table 1 — Chemical requirements of royal jelly

Characteristic		Requirement		Analysis method
		Type 1	Type 2	
Moisture content (%)	min.	62,0		Annex A
	max.	68,5		
10-HDA(%)	min.	1,4		Annex B
Protein %	min.	11		Annex C
	max.	18		
Total sugar %	min.	7		Annex D
	max.	18		
Fructose %		2–9		Annex D
Glucose %		2–9		Annex D
Sucrose %		<3,0	Na[a]	Annex D
Erlose %		<0,5	Na[a]	Annex D
Maltose %		<1,5	Na[a]	Annex D
Maltotriose %		<0,5	Na[a]	Annex D
Total acidity [(1 mol/l NaOH) ml/100g]	min.	30,0		Annex E
	max.	53,0		
Total lipid (%)		2		Annex F
		8		
C13/C12 Isotopic ratio (δ ‰)		−29 to −20	−29 to −14	Annex G

[a] Na = Not applicable.

Furosine is an additional, optional quality parameter which shows freshness of royal jelly (see informative method in Annex H).

NOTE A value is to be specified in the next revision of this International Standard.

Pollen screening may be used to determine geographical origin of royal jelly (see informative method in Annex I).

4.4 Hygienic requirements

Royal jelly shall comply with the requirements given in Table 2.

Table 2 — Hygienic requirements of royal jelly

Characteristic		Requirement	Analysis method
Colony count (cfu/g)	max.	500	ISO 4833-1
Pathogenic bacteria:			
Enterobacteriaceae (cfu/g)		absent in 10 g	ISO 21528-2
Salmonella (cfu/g)		absent in 25 g	ISO 6579

5 Test methods

5.1 General

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

5.2 Sample collection

Sample collector shall use stainless steel bar, tube or spoon. Put the sample into the sterile sample bottle, stir sufficiently in order to mix it evenly, and put it aside as the sample to be tested. Each sample shall not be less than 20 g.

The sample shall be tested immediately or it shall be stored in a refrigerator below 5 °C.

5.3 Test methods of chemical requirements

Samples shall be tested according to the test methods specified in Annex A, Annex B, Annex C, Annex D, Annex E, Annex F and Annex G or any other test methods with performances recognized as at least equivalent according to recognized standards.

6 Packaging, marking, storage and transportation

6.1 Packaging

Packaging in contact with royal jelly shall be of food grade.

6.2 Marking

At least the following information shall be marked on each package or on a label:

a) the name of the product, and trade name or brand name, if any;

b) the name and address of the producer or packer;

c) the net weight;

d) the harvesting country/countries;

e) the harvesting year;

f) the date of minimum durability;

g) the storage mode and instructions;

h) the freezing month if any;

i) the type, according to this International Standard;

k) the batch number.

6.3 Storage and transportation

The temperature for storage shall be between +2 °C and +5 °C or, preferably, less than −18 °C for long-term storage.

Royal jelly produced in different areas and times should be stored separately in giving them different batch numbers (in bottle or in box).

It shall be transported at low temperature and shall not be stored and transported with toxic, corrosive material or material with peculiar smell or that might cause contamination.

Annex A
(normative)

Determination of moisture content

A.1 Vacuum drying oven method (Reference method)

A.1.1 Apparatus

A.1.1.1 Vacuum drying oven.

A.1.1.2 Weighing dish, of height 25 mm to ~30 mm, of diameter 35 mm to 50 mm.

A.1.1.3 Analytical balance, capable of weighing to the nearest 0,000 1 g.

A.1.2 Procedure

Weigh approximately 0,5 g of the royal jelly sample, put it in the weighing dish which is dried to constant weight, spread evenly, weigh accurately and put it in the vacuum drying oven, dry for 4 h at 75 °C and under the pressure between 0,000 MPa and 0,005 MPa, take out the weighing dish and put it in the drying oven or desiccator, weigh after it has been cooled for 30 min, redry for 2 h and repeat the process until the weight difference between two consecutive times is no more than 2 mg, namely, until a constant weight is achieved.

A.1.3 Calculation

The moisture content in royal jelly, X_1, expressed as a percentage by mass, is given by Formula (A.1):

$$X_1 = \frac{m_1 - m_2}{m_1 - m_3} \times 100 \qquad \text{(A.1)}$$

where

X_1 is the moisture content in royal jelly, %;

m_1 is the mass of the weighing dish and the sample, in grams;

m_2 is the mass of the weighing dish and the sample that is dried until a constant weight is achieved, in grams;

m_3 is the mass of the weighing dish, in grams.

A.1.4 Precision

Relative deviation of parallel experiments shall not be more than 0,8 %.

A.2 Karl Fisher

A.2.1 Apparatus

A.2.1.1 Karl Fischer titration system, Mettler DL 18 titrator[1)] or equivalent.

A.2.1.2 Analytical balance, capable of weighing, to the nearest 0,000 01 g.

A.2.1.3 Hydranal Composite 5 R.D.H.[1)], as titrating solution or equivalent.

A.2.1.4 Methanol, UV purity or analytical purity, as solvent.

A.2.2 Procedure

Prior to titration of a sample, each working day, the titre of the employed one-component reagent [e.g. Hydranal(R)-Composite 5] is determined. A suitable water standard [e.g. Hydranal(R) -Water Standard 10,0, ultrapure water or terpine hydrate with a moisture content well defined at 10,46 %] is determined in triplicate in the employed titration medium.

Weigh a 1 ml syringe. Weigh approximately 30 mg of the royal jelly sample in the syringe.

Introduce the sample into the titration cell of the titrator containing about 40 ml of methanol.

Weigh again the syringe.

The weighing of royal jelly exactly introduced in the titration cell in calculated by the difference of the two weighings of the syringe.

After 600 s of stirring, the moisture content is determined and automatically calculated by the titrator in % and mg/kg.

The determined titre shall be taken into account for the calculation of the water content in the sample.

A.2.3 Precision

Each sample shall be analysed twice and the relative deviation between both measures shall not be more than 0,4 %.

A.3 Lyophilization

See Reference [1].

A.3.1 Apparatus

A.3.1.1 Analytical balance, capable of weighing to the nearest 0,000 1 g.

A.3.1.2 Centrifuge tubes.

A.3.1.3 Lyophilizer.

A.3.1.4 Freezer.

1) 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 ISO of this product.

A.3.2 Procedure

Weigh a centrifuge tube with its cap. Weigh exactly around 1 g of royal jelly in it. Lyophilize at least 36 h, without the cap. As soon as the lyophilization process is stopped, put the cap and weigh the sample immediately.

A.3.3 Calculation

The percentage of dry matter is calculated using Formula (A.2):

$$\% \text{ dry matter} = 100 \times (m_1 - m_0)/m \quad \text{(A.2)}$$

where

m_1 is the mass of the tube after the lyophilization process with the cap, in grams;

m_0 is the mass of the empty tube with its cap, in grams;

m is the mass of the sample, in grams.

The moisture content in royal jelly is calculated using Formula (A.3):

$$\% \text{ moisture content} = 100 - \% \text{ dry matter} \quad \text{(A.3)}$$

A.3.4 Precision

Relative deviation of parallel experiments shall not be more than 0,8 %.

Annex B
(normative)

Determination of 10-HDA

B.1 HPLC-UV External Standard (Reference method)

B.1.1 Reagents

Use only ultrapure water.

B.1.1.1 Methanol, UV purity or analytical purity with light transmittance above 30 % at detection wavelength.

B.1.1.2 10-HDA reference standard, of purity above 99,0 % (certificate of analysis of supplier).

B.1.1.3 10-HDA S_0 standard stock solution, c = 0,13 mg/ml.

For example, accurately weigh approximately 6,50 mg reference substance into 50 ml measuring flask, dissolve in methanol and fill up to volume with methanol.

For dilution: factor x (standard S_0) = 0,13 (mg/ml)/calculated concentration S_0 (mg/ml). The calculated concentration shall be corrected for the purity of the reference material.

B.1.1.4 25 mm phosphate buffer pH 2,5, as Eluent A, for extraction solution and sample solvent).

For example, weigh 6,90 g sodium dihydrogen phosphate monohydrate (M = 137,99 g/mol) into 2l measuring flask, dissolve in approximately 1 800 ml H_2O, adjust pH to 2,5 with 85 % H_3PO_4 and fill up to volume with H_2O.

B.1.1.5 Extraction solution, 55 % 25 mm phosphate buffer pH 2,5/45 % methanol (v:v).

For example, mix 550 ml 25 mm phosphate buffer pH 2,5 with 450 ml methanol, equilibrate to room temperature.

B.1.1.6 Sample solvent, 70 % 25 mm phosphate buffer pH 2,5/30 % methanol (v:v).

For example, mix 700 ml 25 mm phosphate buffer pH 2,5 with 300 ml methanol, equilibrate to room temperature.

B.1.2 Apparatus

B.1.2.1 HPLC with ultraviolet detector, recorder or microprocessor.

B.1.2.2 Chromatographic column, Zorbax SB-CN 150 × 3,0 mm; 3,5 µm or equivalent.

B.1.2.3 Ultrasonic bath.

B.1.2.4 Homogenizer, Ultraturrax or equivalent.

B.1.2.5 Analytical balance, capable of weighing to the nearest 0,000 01 g.

B.1.3 Procedure

B.1.3.1 Sample treatment

Exactly weigh approximately 80,00 mg lyophiliated royal jelly or 200 mg fresh royal jelly into a 50 ml centrifuge tube.

Add 40,0 ml extraction solution. Homogenize for approximately 10 s to 20 s using an ultraturrax at 15 000 rpm until all royal jelly material is emulsified. Treat for 10 min in ultrasonic bath.

Pipette 1 ml of the homogeneous extract into a 10 ml measuring flask and fill up to volume with sample solvent. Filter an aliquot of the diluted extract through membrane filter (0,45 µm).

B.1.3.2 Chromatography conditions

Detection wavelength: 216 nm

Eluent A: 25 mm phosphate buffer pH 2,5

Eluent B: Methanol

Gradient:	34 % B,	0-2,0 min
	34-43 % B,	2,0-9,0 min
	43-80 % B,	9,0-10,0 min
	34 % B	10,1 min-16,0 min

B.1.3.3 External calibration

Realize an external standard calibration curve (solutions corresponding to concentration of 10-HDA: 1,0 g/100 ml, 1,5 g/100 ml, 2,0 g/100 ml, 2,5 g/100 ml).

The calibration curve shall be linear by visual assessment with a coefficient of correlation $r > 0,99$.

Standard dilutions for calibration curve:

Standard 10-HDA 1,0 g/100 ml:
c = 5 µg/ml
(corresponds to 1,0 g/100 g in sample)

Pipette (x * 385) µl 10-HDA-S_0 standard stock solution into a 10 ml measuring flask and dilute to volume with sample solvent

Standard 10-HDA 1,5 g/100 ml:
c = 7,5 µg/ml
(corresponds to 1,5 g/100 g in sample)

Pipette (x * 578) µl 10-HDA-S_0 standard stock solution into a 10 ml measuring flask and dilute to volume with sample solvent

Standard 10-HDA 2,0 g/100 ml:
c = 10 µg/ml
(corresponds to 2,0 g/100 g in sample)

Pipette (x * 770) µl 10-HDA-S_0 standard stock solution into a 10 ml measuring flask and dilute to volume with sample solvent

Standard 10-HDA 2,5 g/100 ml:
c = 12,5 µg/ml
(corresponds to 2,5 g/100 g in sample)

Pipette (x * 963) µl 10-HDA-S_0 standard stock solution into a 10 ml measuring flask and dilute to volume with sample solvent

x = factor of 10-HDA-S_0 stock solution (see B.1.1.3)