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Bee propolis — Specifications

Propolis d'abeille — Spécifications

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Contents

Page

Foreword ..... **iv**

Introduction ..... **v**

1 Scope **1**

2 Normative references **1**

3 Terms and definitions **1**

4 Requirements **3**
4.1 Classification of raw propolis types 3
4.1.1 Temperate, Mediterranean and boreal, brown, *Populus spp.* propolis 3
4.1.2 Tropical, green, *Baccharis dracunculifolia* propolis 3
4.1.3 Tropical, red, Dalbergia and Clusia propolis 3
4.1.4 Other types of propolis 3
4.2 Physical and chemical requirements 3
4.3 Traceability requirements 4

5 Test methods **4**
5.1 Reagents 4
5.2 Sample collection 5
5.3 Sample preparation 5
5.4 Test methods for physical and chemical requirements 5

6 Packaging, marking, labelling, storage and transportation **5**
6.1 Packaging 5
6.2 Marking (label and/or certificate) 5
6.3 Labelling 5
6.4 Storage and transportation 6

Annex A (normative) **Ethanol extractables of raw propolis (as dry matter)** **7**

Annex B (normative) **Loss on drying determination** **9**

Annex C (normative) **Ash content in raw propolis** **11**

Annex D (normative) **Petroleum ether extractables of raw propolis (as dry matter)** **13**

Annex E (normative) **Total phenolic content** **15**

Annex F (Normative) **Total flavonoids content (aluminium chloride method)** **18**

Annex G (Normative) **Total flavonoids content (polyamide method)** **20**

Annex H (normative) **Chemical characterization of polyphenols in poplar propolis — HPLC/MS** **24**

Annex I (normative) **Chemical characterization of polyphenols in green propolis — HPLC/PDA** **38**

Annex J (normative) **Chemical characterization of polyphenols in red propolis by HPLC-PDA and HPLC-MS** **43**

Annex K (normative) **Determination of antioxidant capacity by DPPH** **47**

Foreword

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This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 19, *Bee products*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Propolis is a resinous substance produced by worker bees combining plant resins and/or fragments of newly formed buds with their salivary and wax gland secretions.

The chemical composition of propolis is quite complex. Hundreds of natural compounds such as flavonoids and phenolic acids have been identified in propolis. Different geographical and plant sources, bee species, production methods, etc., have a significant influence on the chemical composition of propolis.

For the purposes of this document, propolis is divided into *Populus, Baccharis* and *Dalbergia* types (the primary sources) and opens the opportunity in the future to cover other types, such as *Araucaria* spp., *Betula* spp., *Castanea* spp., *Clusia* spp., *Cupressus* spp., *Eucalyptus* spp., *Macaranga* spp., *Symphonia* spp., and the mixed plant source propolis (this list is not exhaustive). Only propolis produced by *Apis mellifera* bees is covered in this document.

Scientific literature predominantly relates to three main propolis types (brown, green and red) of which brown (*Populus*) and green (*Baccharis*) propolis are the main types traded internationally. This document considers the complex chemical composition of propolis, and the influences that geographical and plant species variation, and honey bee sub-species have on the proximate, flavonoid and phenolic composition of propolis. Propolis is rich in polyphenols, in particular flavonoids, phenolic acids and derivatives, which can be involved in the biological activities. The decisions made about the types, methodologies and requirements included in this document were based on the scientific literature available at the time.

This document sets out the terms, definitions, classification, quality requirements, authenticity requirements, test method procedures, transportation, storage conditions and packing marks. It aims to provide a document for the classification and quality control for the international trade of raw propolis.

Bee propolis — Specifications

1 Scope

This document specifies the quality requirements, analytical methods, and packaging, marking, labelling, storage and transportation conditions for bee propolis.

This document is applicable to propolis collected from beehives of *Apis mellifera* colonies, i.e. raw propolis.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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.

ISO 22005, *Traceability in the feed and food chain — General principles and basic requirements for system design and implementation*

3 Terms and definitions

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

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
antioxidant capacity
ability of a substance that retards deterioration of oxidation

3.2
ash content
incombustible component remaining after a sample of *raw propolis* (3.17) is completely burnt

3.3
authenticity requirement
requirement that the addition of resins, extracts or any compounds, and/or bioactive substances in raw propolis is not allowed

3.4
balsamic
relating to or containing balsam

3.5
beeswax
honey bee secretions including mixtures of substituted long-chain aliphatic hydrocarbons, containing alkanes, alkyl esters, fatty acids, primary and secondary alcohols, diols, ketones and aldehydes

3.6
contaminant
substance not intentionally added to propolis, which is present as a result of a process such as production to harvesting, manufacture, processing, preparation, treatment, packing, packaging, transport or holding of the product, or because of environmental contamination

3.7
ethanol extractables of propolis (as dry matter)
content obtained after an exhaustive extraction process using ethanol (80 % volume/volume) as the extractor solvent

Note 1 to entry: Also known as genuine, crude, balsamic and dry extract of propolis.

3.8
petroleum ether extractables of propolis (as dry matter)
content obtained after an exhaustive extraction process using petroleum ether as an extraction solvent

3.9
flavonoids
class of plant and fungus secondary metabolites that have the general structure of a 15-carbon skeleton (abbreviated C6-C3-C6), consisting of two phenyl rings (A and B) and a heterocyclic ring (C) that includes subgroups of flavones, flavonols, flavanones, flavanonols, flavanols or catechins, anthocyanins and chalcones

3.10
harvesting
mechanical process used to remove raw propolis from the hive where it is deposited by the *Apis mellifera*

3.11
loss on drying
amount of all volatile substances present in propolis sample, including moisture, that is lost in drying at 105 °C for 1 h

3.12
phenolic acids
carboxylic acids derived from either benzoic or cinnamic acid skeletons

3.13
plants of propolis source
plants of different geographical and botanical origin, which produce resinous balsamic exudates that are collected and converted into *propolis* (3.15) in beehives by *Apis mellifera* bees

3.14
polyphenols
natural organic molecules widely present in the plant kingdom, which are the main active ingredients of propolis, and are characterized by the presence of multiple phenolic groups associated in complex structures, some of them with high molecular mass

3.15
propolis
resinous balsamic mixture, exclusively of natural and plant origin, harvested by worker bees of the species *Apis mellifera* from newly formed buds, flower buds and exudates of specific *plants of propolis source* (3.13), to which the bees add their own secretions, mostly from their salivary and wax glands, and use it to protect the honey bee and colony health

3.16
propolis extract
components, derived from *raw propolis* (3.17) devoid of foreign matter, that are soluble in solvents generally recognized as safe (GRAS) for human consumption

3.17
raw propolis
propolis produced by *Apis mellifera* without any external intervention, except the removal from the hive

3.18
total phenolic content
total amount of any compound with a hydroxyl group linked directly to a benzene ring

3.19
traceability
ability to follow the movement of propolis through specified stage(s) of production, processing and distribution

4 Requirements

4.1 Classification of raw propolis types

4.1.1 Temperate, Mediterranean and boreal, brown*, Populus spp.* propolis

Poplar propolis can be brownish yellow, brownish red, brown, yellowish-brown, greyish-brown, greenish-brown or grey-black. The main botanical source is *Populus spp.* However, several other botanical sources can be present. At 20 °C to 24 °C, it appears as a lump shape or broken grains shape, and becomes soft, malleable and sticky with the increase of temperature above 30 °C. There is a characteristic balsamic and resinous aroma of Poplar propolis. The taste is slightly bitter, a little astringent and micro-tingly.

4.1.2 Tropical, green, *Baccharis dracunculifolia* propolis

Baccharis propolis is yellowish-green, green, greenish, greenish-brown and brown. The main botanical source is *Baccharis dracunculifolia*. At 20 °C to 24 °C, it appears strip shape and broken grains shape, and becomes malleable at about 25 °C. It has a characteristic resinous, woody, spicy aromatic odour, and a strong bitter and spicy taste.

4.1.3 Tropical, red, Dalbergia and Clusia propolis

Dalbergia and *Clusia* propolis are red, yellowish-red and brownish-red. The main botanical sources are *Dalbergia ecastaphyllum*, *Symphonia globulifera* and *Clusia* spp. At temperatures higher than 20 °C they appear malleable. It has a characteristic resinous and aromatic odour. The taste is aromatic and slightly bitter.

4.1.4 Other types of propolis

The current scientific literature does not provide complete data to fully characterize the chemical and biological properties of other floral types of propolis for inclusion in this document. Some examples of other types of propolis are *Araucaria spp., Betula spp., Castanea spp.*, Cupressaceae family, *Macaranga tanarius*, Salicaceae, Pinaceae and others (the list is not exhaustive).

4.2 Physical and chemical requirements

The physical and chemical requirements of propolis shall be as given in Table 1, except total flavonoids that are a normative parameter, but the corresponding procedure shall be selected between Annex F or G.

Table 1 — Physical and chemical requirements for bee propolis and test methods for each characteristic

Characteristic	Min. or max.	Requirements (on a dry basis)			Test method
		Brown propolis (4.1.1)	Green propolis (4.1.2)	Red propolis (4.1.3)	
Ethanol extractables of propolis (as dry matter), in % mass fraction	min.	30,0	30,0	30,0	Annex A
Loss on drying, in % mass fraction	max.	10,0	10,0	10,0	Annex B
Ash content, in % mass fraction	max.	5,0	5,0	5,0	Annex C
Petroleum ether extractables of propolis (as dry matter), in % mass fraction	max.	65,0	30,0	60,0	Annex D
Total phenolic compounds (Folin), in % mass fraction, as gallic acid[a]	min.	10,0	7,0	7,0	Annex E
Total phenolic compounds (Folin), in % mass fraction, as galangin[a]	min.	17,0	12,0	12,0	Annex E
Total flavonoids ($AlCl_3$), in % mass fraction, as quercetin	min.	3,0	1,0	0,5	Annex F
Total flavonoids (polyamide method), in % mass fraction, as rutin	min.	6,0	2,0	1,0	Annex G
Total polyphenolics by high-performance liquid chromatography (HPLC) (poplar, green and red propolis)	—	Presence of: apigenin, caffeic acid, CAPE, p-coumaric acid, chrysin, ferulic acid, galangin, pinobanksin and pinocembrin	Presence of: caffeic acid, p-coumaric acid, 3,5-dicaffeoyilquinic acid, 4,5-dicaffeoyilquinic acid, cinnamic acid, drupanin, artepellin C and baccharin	Presence of: calycosin, isoliquiritigenin, formononetin and biochanin	Annex H Annex I Annex J
Total antioxidant capacity (DPPH) – EC50, in µg/ml	max.	25,0	40,0	50,0	Annex K

[a] Total phenolic can be expressed as gallic acid or galangin equivalents. To convert from gallic acid to galangin, multiply the value obtained using the conversion factor of 1,7. To convert from galangin to gallic acid, multiply the value by 0,59.

4.3 Traceability requirements

ISO 22005 shall be followed to guarantee the traceability of propolis.

5 Test methods

5.1 Reagents

Use only analytical grade reagents unless otherwise specified. Distilled water should be in accordance with the first-grade water or water with the same purity given in ISO 3696.

5.2 Sample collection

Propolis is a very heterogeneous product; therefore, at least 1 % of the batch (minimum of 1 kg of sample for batches less than 100 kg) shall be collected. A minimum of 10 representative points shall be sampled to take into consideration the diversity of the propolis. Pack them in a food-grade container and store them below –18 °C.

Sampling tools shall be clean and shall not add any foreign matter or contaminants to the samples.

5.3 Sample preparation

Combine the representative points samples and crush in a pulveriser, while still frozen, until they pass through a 10 mesh (2 mm) sieve. If there are visible impurities before the pulverization, they shall be removed. Transfer to a food-grade container and mix until homogenous. Take an appropriate subsample sufficient for testing, seal in an airtight container and store at below –18 °C, if required, until analysis.

5.4 Test methods for physical and chemical requirements

The sample should be tested according to the test methods specified in Annexes A to K.

6 Packaging, marking, labelling, storage and transportation

6.1 Packaging

Raw propolis packaging should protect the product from light. Propolis loss on drying shall be lower than 10 %.

6.2 Marking (label and/or certificate)

The information listed in Table 2 shall be used on each package or label/certificate. Additional information can be included.

6.3 Labelling

Labelling requirements shall be as given in Table 2.

Table 2 — Labelling requirements

Requirement	Beekeeper	Company B2B	Company B2C
Product name and brand (if exists), and/or trademark	✓	✓	✓
Name, complete address of the producer and packer	✓	✓	✓
Net mass	✓	✓	✓
Country or countries of origin (in order of proportional content: highest to lowest)	✓	✓	✓
Propolis type according to 4.1 of this document	✓	✓	✓
Ethanol extractables of propolis[a]	—	—	✓

Key

B2B: business to business

B2C: business to consumers

[a] Raw propolis for consumers: The information described on the label, or accessed by certificate of analysis using a QR code, or other equivalent (see Annex A).

[b] In the case of total phenolic, identify if gallic acid or galangin equivalent is being used (see Annex E).

[c] In the case of total flavonoids, identify quercetin (see Annex F, $AlCl_3$) or rutin (see Annex G, polyamide) equivalents.

Table 2 *(continued)*

Requirement	Beekeeper	Company B2B	Company B2C
Harvesting time: month(s)/year(s)	✓	✓	—
Best before or expiry date	—	✓	✓
Storage information	—	✓	✓
Batch number	✓	✓	✓
Total phenolics[b]	—	—	✓
Total flavonoids[c]	—	—	✓

Key

B2B: business to business

B2C: business to consumers

[a] Raw propolis for consumers: The information described on the label, or accessed by certificate of analysis using a QR code, or other equivalent (see Annex A).

[b] In the case of total phenolic, identify if gallic acid or galangin equivalent is being used (see Annex E).

[c] In the case of total flavonoids, identify quercetin (see Annex F, $AlCl_3$) or rutin (see Annex G, polyamide) equivalents.

6.4 Storage and transportation

Storage and transportation shall take into consideration the type of propolis, protection from light, elevated temperature (keep < 25 °C) and humidity conditions of the room to avoid the degradation of the genuine characteristics of propolis and prevent the growth of microorganisms on the surface.

Raw propolis shall not be stored and shipped with articles that are odorous, poisonous and corrosive, and potentially polluting products.

Annex A
(normative)

Ethanol extractables of raw propolis (as dry matter)

A.1 Principle

Propolis is partially soluble in an ethanol/water solution. The dry mass of ethanol/water extract is calculated as a percentage of the mass of the sample, after complete removal of the solvent.

A.2 Reagents and materials

A.2.1 Ethanol, $\varphi(CH_3CH_2OH)$ = 80 % (volume/volume).

A.2.2 Iron (III) chloride in methanol = 5 % (mass/volume).

A.3 Apparatus and equipment

A.3.1 Analytical balance, capable of weighing to the nearest 0,000 1 g.

A.3.2 Vacuum dryer or oven.

A.3.3 Erlenmeyer or beaker, 100 ml.

A.3.4 Magnetic stirrer.

A.3.5 Glass funnel, Φ = 60 mm.

A.3.6 Quantitative filter paper, Φ = 12,5 cm.

A.3.7 Magnetic rod.

A.3.8 Volumetric flask, 100 ml

A.4 Procedure

Use the following procedure:

a) The extraction procedure given in steps b) to f) shall be done in triplicate.

b) Weigh 1 g (accurate to 0,001 g) of the propolis sample (m_1) into a 100 ml Erlenmeyer or beaker (A.3.3) and add 30 ml of 80 % ethanol.

c) Keep the mixture under mechanical or manual agitation, at 50 °C, for 3 h and protected from light.

d) Then, filter the mixture through quantitative filter paper.

e) To confirm the absence of phenolics in the remaining residue, add a few drops of $FeCl_3$ (5 % in methanol). If a colour development is observed, the residue shall be reextracted (following steps a) to c)), until no colour development is observed (no more than three extractions).

f) Combine all extracts in a 100 ml volumetric flask and make up to volume with ethanol 80 % volume/volume.

g) For evaluation of the dry extract, weigh a glass drying dish (m_2), combine 2 ml of each extraction solution (3 x 2) ml in a glass drying dish and dry to constant mass (m_3) in an oven set at 105 °C for 90 min. Place the dish in a desiccator at room temperature (15 min) between weighings (maximum of 2 mg of difference between two consecutive weighings).

A.5 Calculation

The content of ethanol/water extract in the sample (expressed as dry matter), E_E, expressed as a percentage of mass, is given by Formula (A.1):

$$E_E = \frac{m_3 - m_2}{m_1} \times \left(\frac{50}{3}\right) \times 100 \quad \text{(A.1)}$$

where

m_1 is the average mass of the propolis sample, in g;

m_2 is the mass of the glass drying dish, in g;

m_3 is the mass of the dry extract and glass drying dish, in g.

NOTE (50/3) is the dilution factor.

A.6 Precision

The relative deviation of parallel experiments shall not be more than 5 %.