TECHNICAL SPECIFICATION

ISO/TS 19046-1

IDF/RM 233-1

First edition
2017-05

Cheese — Determination of propionic acid level by chromatography —

Part 1: Method by gas chromatography

Fromages — Détermination de la teneur en acide propionique par chromatographie —

Partie 1: Méthode par chromatographie en phase gazeuse

Reference numbers
ISO/TS 19046-1:2017(E)
IDF/RM 233-1:2017(E)

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Forewords

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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).

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This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products* and the International Dairy Federation (IDF). It is being published jointly by ISO and IDF.

A list of all parts in the ISO/TS 19046 | IDF/RM 233 series can be found on the ISO website.

IDF (the International Dairy Federation) is a non-profit private sector organization representing the interests of various stakeholders in dairying at the global level. IDF members are organized in National Committees, which are national associations composed of representatives of dairy-related national interest groups including dairy farmers, dairy processing industry, dairy suppliers, academics and governments/food control authorities.

ISO and IDF collaborate closely on all matters of standardization relating to methods of analysis and sampling for milk and milk products. Since 2001, ISO and IDF jointly publish their International Standards using the logos and reference numbers of both organizations.

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ISO/TS 19046-1 | IDF/RM 233-1 was prepared by the IDF Standing Committee on *Analytical Methods for Composition* and ISO Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products.*

The IDF Reviewed method is equal to an ISO Publicly Available Specification (ISO/PAS) or an ISO Technical Specification (ISO/TS) and is therefore published jointly under ISO conditions.

The work was carried out by the IDF/ISO Project Group on Propionic acid (C25) of the Standing Committee on *Analytical Methods for Composition* under the aegis of its project leader P. Trossat (FR).

A list of all parts in the ISO/TS 19046 | IDF/RM 233 series can be found on the ISO website.

Cheese — Determination of propionic acid level by chromatography —

Part 1:
Method by gas chromatography

WARNING — This document can involve the use of products and implementation of procedures and equipment of a hazardous nature. This document does not aim to address all the risks related to its use. It is the responsibility of the user of this document to establish appropriate hygiene and safety practices before using it, and to determine the applicability of any other restrictions.

1 Scope

This document specifies a method for the determination of propionic acid level in cheese, using gas chromatography.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

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

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

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

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

3.1
level of propionic acid
mass fraction of propionic acid determined following the procedure described in this document

Note 1 to entry: The level of propionic acid is expressed in mg/100 g of cheese.

4 Principle

Preparation of the test sample by addition of the internal standard and homogenization in the presence of sulfuric acid. Continuous extraction of the mixture in a liquid-liquid extractor by a mixture of ethers. Separation of the volatile fatty acids from the fatty phase in the form of their sodium salts (soaps) after neutralization in the presence of phenolphthalein (or other equivalent indicator) and drying of the soaps recovered in the aqueous phase. Separation of the propionic acid using gas chromatography and quantification by reference to an internal standard.

5 Reagents

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

5.1 Valeric acid (internal standard) [$CH_3(CH_2)_3COOH$] of purity ≥ 99 % mass fraction.

5.2 Sulfuric acid solution [H_2SO_4] containing a mass/volume fraction of 10 %.

5.3 Petroleum ether, with any boiling range between 45°C and 65°C.

5.4 Diethyl ether [$C_2H_5O\ C_2H_5$].

5.5 Mixed solvent, prepared shortly before use by mixing equal volumes of diethyl ether (5.4) and petroleum ether (5.3).

5.6 Ethanol [C_2H_5OH] containing a volume fraction of ethanol of approximately 77 %.

Into a 50 ml volumetric flask, add 40 ml ± 2 ml of ethanol 96 % not denaturated and make up to the mark with water.

5.7 Sodium hydroxide, *c*(NaOH) around 1 mol/l.

5.8 Indicator solution. Phenolphthalein solution [$C_{20}H_{14}O_4$] containing a mass fraction of 1 % (in ethanol 95 % to 96 %) or other indicator with equivalent turning zone.

5.9 Trifluoroacetic acid [CF_3COOH] dissolved in ethanol 95 % to 96 % (6,67 % in volume fraction).

5.10 Propionic acid [$CH_3\ CH_2COOH$] of purity ≥ 99,5 % mass fraction.

5.11 Calibration solution.

Weigh to the nearest 0,1 mg about 400 mg of propionic acid (5.10) and about 100 mg of valeric acid (5.1). Dissolve up to 100 g with ethanol (5.6).

6 Apparatus

WARNING — The determination involves the use of volatile flammable solvents. All electrical apparatus employed shall be safe relating to the hazards in using such solvents.

Usual laboratory equipment and, in particular, the following.

6.1 Grinding or **grating device**.

6.2 Homogenizer/mixer (e.g. Ultraturrax type 251[1)]), equipped with a rotor.

6.3 Mixer type vortex.

6.4 Soxhlet-type extractor apparatus (for light solvents), equipped with flask heater.

See Annex A.

6.5 Separating funnel, of capacity 500 ml.

6.6 Rotary evaporator and controlled vacuum device.

1) Ultraturrax type T251 is an 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 or IDF of this product.

6.7 Drying oven, capable of maintaining at a temperature around 60 °C.

6.8 Analytical balance, capable of weighing to the nearest 1 mg, with a readability of 0,1 mg.

6.9 Measuring cylinders, of capacity 50 ml and 100 ml.

6.10 Volumetric flasks, of capacity 50 ml.

6.11 Glass flasks, of capacity around 125 ml.

6.12 Glass flasks, of capacity around 250 ml.

6.13 Dispensers, of capacity 2,5 ml and 10 ml.

6.14 Gas chromatograph, with the following equipment.

6.14.1 Carrier gas, hydrogen, helium or nitrogen, purity ≥ 99,999 7 %.

6.14.2 Semi-capillary column, 30 m length, 0,53 mm internal diameter, 2 µm film thickness, with a modified polyethylene glycols polar stationary phase [phase free fatty acid phase (FFAP), Carbowax™ 2)] or equivalent.

6.14.3 Gas chromatographic conditions.

The following conditions have been found suitable to obtain a correct separation of propionic acid:

— carrier gas flow: 7 ml/min;

— oven temperature program: initial temperature of 80 °C, maintained for 0,5 min, raised at a rate of 20 °C min^{-1} up to 170 °C, maintained at this temperature for 6,5 min.

An example of a GC profile obtained with these conditions is shown in Annex B.

6.14.4 Flame ionization detector, set at a temperature of 250 °C.

6.14.5 On column injector, set at a temperature of 250 °C.

6.14.6 Injection syringe, of capacity 10 µl.

6.14.7 Integration system, preferably being computerized.

6.15 Glass tubes (with stoppers).

7 Sampling

Sampling is not part of the method specified in this document. A recommended sampling method is given in ISO 707 | IDF 50.

It is important that the laboratory receives a sample that is representative and has not been damaged or changed during transport or storage.

2) Carbowax™ is an 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 or IDF of this product.

8 Preparation of test sample

Prior to analysis, grind or grate the test sample by using an appropriate grinding or grating device (6.1), if necessary, after removing the rind, the smear or the mouldy surface layer of the cheese.

Weigh, to the nearest 0,1 mg, about 20 g of cheese in a 125 ml flask (6.11) and record the mass (m). Add about 50 mg of valeric acid to the test sample and record the mass of valeric acid to ± 0,000 1 g. Add 50 ml ± 2 ml of sulfuric acid solution (5.2) and crush for 1 min using the homogenizer (6.2).

9 Procedure

9.1 Extraction system

Pour 100 ml of the mixed solvent (5.5) into the glass flask (6.12) and put the extraction unit (6.4) on the flask. Introduce the cheese prepared (see Clause 8) in the extraction unit (6.4) and rinse slightly with water. Place the adapter in the extraction unit (6.4), install the condenser and switch on the flask heater. Perform extraction for 6 h (example of extraction system is presented in Annex A).

Cool the flask 15 min in a beaker of cold water until ambient temperature approximately without disconnecting the assembly on the extraction unit (6.4). Transfer the contents of the flask into the separating funnel, containing 40 ml of ethanol solution (5.6), 10 ml of water, 2,5 ml of sodium hydroxide (5.7) and 2 or 3 drops of indicator solution (5.8).

Add a sufficient quantity of sodium hydroxide (5.7) so that the solution turns pink. Mix and allow the separating funnel to stand for at least 15 min and collect the hydroalcoholic solution lower phase of soaps in a glass flask (6.12).

Evaporate in the rotary evaporator under controlled vacuum (water bath at 80 °C to 90 °C) and complete drying in the oven at 60 °C.

Scrape off the soaps using a spatula and keep them in tube (6.15) until determination.

9.2 Preparation of the soap

Weigh approximatively 30 mg of the soap prepared as in 9.1 in a tube (6.15) and add 1 ml ± 0,2 ml of the trifluoroacetic acid solution (5.9). Mix until dissolved using a mixer vortex (6.3).

The solution thus prepared shall be injected immediately and shall not be stored.

9.3 Quantitative determination by gas chromatography

9.3.1 Calculation of response factor

Inject into the gas chromatograph 1,0 µl of the calibrating solution (5.11). Determine the area of the peaks of propionic and valeric acid and calculate the response factor of propionic acid (Rf_i) using Formula (1):

$$Rf_i = \frac{m'_i \times A'_0}{m'_0 \times A'_i} \quad (1)$$

where

m'_i is the mass fraction of propionic acid in the calibration standard solution in mg/100 g (5.11);

A'_o is the peak area of valeric acid in the calibration standard solution chromatogram;

m'_o is the mass of valeric acid in the calibration standard solution in mg (5.11);

A'_i is the peak area of propionic acid in the calibration standard solution chromatogram.

The variation of Rf_i between three injections is optimal when coefficient of variation is less than 2 %.

9.3.2 Determination of the test portion

Inject 1 µl of the test portion prepared as in 9.2 into the gas chromatograph applying the same conditions as used for the calibration solution.

10 Calculation and expression of results

10.1 Calculation of propionic acid in cheese

Calculate the mass fraction of propionic acid, w_i, expressed in mg/100 g of cheese using Formula (2):

$$w_i = \frac{m_o \cdot A_i \cdot Rf_i \cdot 100}{A_o \cdot m} \quad (2)$$

where

m_o is the mass of valeric acid internal standard, in milligrams, added to the test sample (see Clause 8);

A_i is the peak area of propionic acid in the sample chromatogram;

Rf_i is the response factor, calculated according to 9.3.1;

A_o is the peak area of valeric acid internal standard in the sample chromatogram;

m is the mass of test portion, in g.

10.2 Expression of results

The results are expressed in mg/100 g of cheese, without decimals.

11 Precision

11.1 Interlaboratory test

The values for the repeatability and reproducibility are derived from the results of an interlaboratory test carried out in accordance with ISO 5725-1 and ISO 5725-2 by three laboratories (see Annex C) and therefore shall be considered as indicative only.

11.2 Repeatability

An indicative value of absolute difference between two single test results obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time of 47 mg of propionic acid/100 g of cheese should be considered.

12 Test report

The test report shall specify:

a) all information necessary for the complete identification of the sample;

b) the sampling method used, if known;

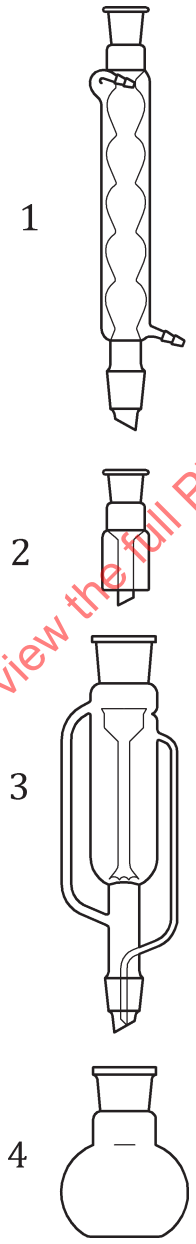
c) the test method used, with reference to this document, i.e. ISO/TS 19046-1 | IDF/RM 233-1;

d) all operating details not specified in this document, or regarded as optional, together with details of any incident which might have influenced the result(s);

e) the test result(s) obtained and, if the repeatability has been checked, the final quoted result obtained.

Annex A
(informative)

Example of extraction system



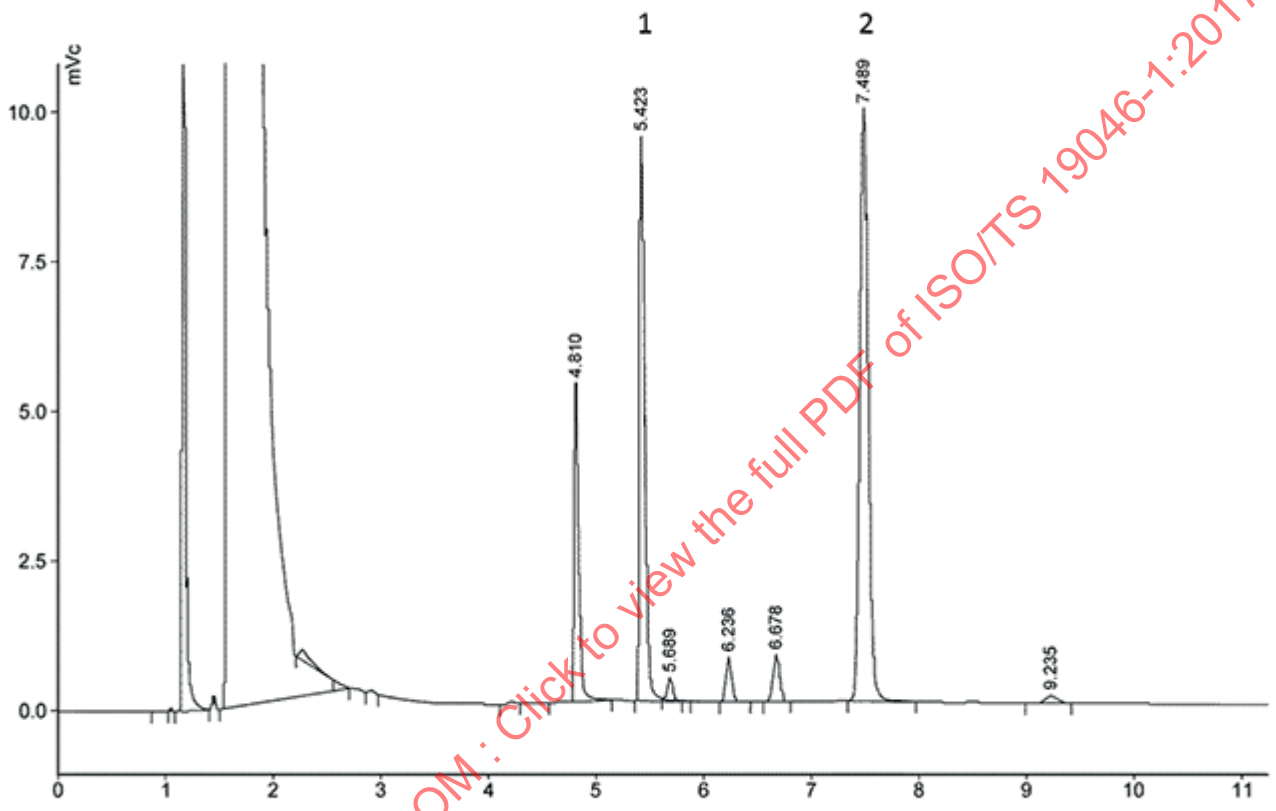
Key

1 condenser
2 adapter
3 extraction unit type “light solvents”
4 flask

Figure A.1 — Example of extraction system

Annex B
(informative)

Example of GC chromatogram



Key

1 propionic acid

2 internal standard

Figure B.1 — Example of GC chromatogram