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**Cheese — Determination of nisin A
content by LC-MS and LC-MS-MS**

*Fromage — Détermination de la teneur en nisine A par CL-SM et
CL-SM-SM*



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Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

International Dairy Federation
Diamant Building • Boulevard Auguste Reyers 80 • B-1030 Brussels
Tel. + 32 2 733 98 88
Fax + 32 2 733 04 13
E-mail info@fil-idf.org
Web www.fil-idf.org

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. 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.

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An ISO/PAS or ISO/TS is reviewed after three years in order to decide whether it will be confirmed for a further three years, revised to become an International Standard, or withdrawn. If the ISO/PAS or ISO/TS is confirmed, it is reviewed again after a further three years at which time it must either be transformed into an International Standard or be withdrawn.

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ISO/TS 27106 | IDF/RM 217 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.

Foreword

IDF (the International Dairy Federation) is a non-profit organization representing the dairy sector worldwide. IDF membership comprises National Committees in every member country as well as regional dairy associations having signed a formal agreement on cooperation with IDF. All members of IDF have the right to be represented on the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO in the development of standard methods of analysis and sampling for milk and milk products.

The main task of Standing Committees is to prepare International Standards. Draft International Standards adopted by the Standing Committees are circulated to the National Committees for endorsement prior to publication as an International Standard. Publication as an International Standard requires approval by at least 50% of IDF National Committees casting a vote.

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All work was carried out by the Joint ISO-IDF Action Team on *Food additives and vitamins* of the Standing Committee on *Analytical methods for additives and contaminants* under the aegis of its project leader, Mr. T. Berger (CH).

Cheese — Determination of nisin A content by LC-MS and LC-MS-MS

1 Scope

This Technical Specification specifies a method for the quantitative determination of the nisin A content in cheese.

The method is suitable for measuring low levels of nisin A with a quantification limit of 1 mg/kg.

NOTE Nisin is a peptide produced by some bacteria (e.g. *Lactococcus lactis* subsp. *Lactis*) inhibiting or destroying other microorganisms. It is widely used as a natural preservative for foods, e.g. vegetables, cheese, meat, and cacao. In cheese making, nisin is used to prevent late blowing. Its use is restricted to maximum levels in the final product. Nisin appears in two forms, nisin A and nisin Z, which differ in one amino acid. This method is applicable to the determination of nisin A only.

2 Terms and definitions

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

2.1

nisin A content

mass fraction of substance determined by the procedure specified in this Technical Specification

NOTE The nisin A content is expressed in milligrams per kilogram.

3 Principle

The sample is grated and extracted with dilute formic acid at 60 °C. After ultracentrifugation, interfering proteins are separated by means of filtration through an ultrafiltration (UF) membrane. In the purified extract, nisin A is separated using a polymeric stationary phase and detected using mass spectrometry (with MS-MS as an option).

4 Reagents and reference substances

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

4.1 Bovine serum albumin (BSA) stock solution. Dissolve 10 mg of BSA (purity > 96 % mass fraction), in 10 ml water.

4.2 Bovine serum albumin (BSA) buffer solution. Mix 80 ml water with 20 ml of acetonitrile (4.6), 0,5 ml of formic acid (4.3), 0,01 ml of trifluoroacetic acid (4.5) and 1 ml of BSA stock solution (4.1).

4.3 Formic acid (HCOOH).

4.4 Formic acid solution, $\rho_{\text{HCOOH}} = 5 \text{ g/l}$. Pipette 0,41 ml of formic acid (4.3) into a 100 ml one-mark volumetric flask (5.12). Make up to the mark with water and mix.