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**Milk and cheese — Determination
of hen's egg white lysozyme
content by high performance liquid
chromatography**

*Lait et fromages — Détermination de la teneur en lysozyme de blanc
d'oeuf par chromatographie liquide haute performance*

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Forewords

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).

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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*, Subcommittee SC 5, *Milk and milk products* and the International Dairy Federation (IDF) and it is being published jointly by ISO and IDF.

This first edition of ISO 27105:2016/IDF 216:2016 cancels and replaces ISO/TS 27105:2009/IDF/RM 216:2009, which has been technically revised.

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 27105|IDF 216 was prepared by the IDF Standing Committee on *Analytical Methods for Additives and contaminants* and ISO Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*.

All work was carried out by the ISO/IDF Project Group on *Determination of hen's egg white lysozyme content by high performance liquid chromatography* of the Standing Committee on *Analytical Methods for Additives and Contaminants* under the aegis of the project leaders, T. Berger (CH) and Prof. L. Pellegrino (IT).

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Introduction

Lysozyme (EC 3.2.1.17, muramidase) is an enzyme widely dispersed in nature; it appears, e.g. in hen's egg white (approximately 3 % to 4 %), saliva and in tear liquid. Lysozyme has a preservative effect because of the lytic activity on the cell wall of some bacteria. Hen's egg white lysozyme is used in cheese making to prevent late blowing of semi-hard and hard cheeses.

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Milk and cheese — Determination of hen's egg white lysozyme content by high performance liquid chromatography

1 Scope

This International Standard specifies a method for the quantitative determination of hen's egg white lysozyme content in milk and cheese.

The method is suitable for measuring low levels of hen's egg white lysozyme with a quantification limit of 10 mg/kg.

2 Terms and definitions

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

2.1

hen's egg white lysozyme content

mass fraction of substance determined by the procedure specified

Note 1 to entry: The lysozyme content is expressed as milligram per kilogram.

3 Principle

Casein and denatured whey proteins from milk and cheese are precipitated isoelectrically at pH 4,3 (cheese) or at pH 2,2 (milk). Acid-soluble hen's egg white lysozyme is then determined by reversed-phase high-performance liquid chromatography (HPLC) and fluorescence detection. The lysozyme peak can be verified by LC/MS (see [Annex A](#)).

4 Reagents and reference substances

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

4.1 Reagents and materials

4.1.1 Sodium chloride solution, $c(\text{NaCl}) = 1 \text{ mol/l}$.

Dissolve 58,44 g of sodium chloride in 1 l water.

4.1.2 Hydrochloric acid, $c(\text{HCl}) = 1 \text{ mol/l}$.

Dissolve 4,0 ml of hydrochloric acid with mass fraction 37 % in a 50 ml one-mark volumetric flask. Dilute to the mark with water.

4.1.3 Sodium hydroxide, $c(\text{NaOH}) = 1 \text{ mol/l}$.

Dissolve 2,6 ml of sodium hydroxide with a mass fraction of 50 % in a 50 ml one-mark volumetric flask. Dilute to the mark with water.

4.1.4 Trifluoroacetic acid (CF_3COOH), analytical grade.

4.1.5 **Acetonitrile** (CH₃CN), HPLC grade.

4.1.6 **Water**, HPLC grade.

4.2 Lysozyme

Pure hen's egg white lysozyme¹⁾. Sufficiently pure and characterized lysozyme is difficult to obtain. A control on lot-to-lot variability is needed.

5 Apparatus

Usual laboratory equipment and, in particular, the following.

5.1 **pH-meter**, accurate to 0,1 unit.

5.2 **Fluted filter**, diameter 15 cm²⁾.

5.3 **Membrane filter**, pore size 0,22 µm³⁾.

5.4 **Balance**, capable of weighing to the nearest 100 mg, with a readability of 10 mg.

5.5 **Analytical balance**, capable of weighing to the nearest 0,1 mg, with a readability of 0,01 mg.

5.6 **Magnetic stirrer**.

5.7 **Homogenizer** ⁴⁾, capable of spinning at a rotational frequency of 3 000 rpm to 3 500 rpm.

5.8 **HPLC equipment**.

5.8.1 **Gradient pumping system**, capable of operating with a speed of 1,0 ml/min.

5.8.2 **Manual or automatic injector**, capable of injecting amounts of 50 µl.

5.8.3 **Column heater**, capable of maintaining a column temperature of 45 °C ± 2 °C.

5.8.4 **Column**, reversed phase⁵⁾, 5 µm, 250 mm × 4,6 mm.

1) Lysozyme SIGMA L-6876 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 either ISO or IDF of this product.

2) Fluted filter Schleicher&Schuell 595 ½ 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 either ISO or IDF of this product.

3) Millipore Millex-GV PVDF 0,22 µm 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 either ISO or IDF of this product. Equivalent products may be used if they can be shown to lead to the same results.

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5) PLRP-S 300 Å (Polymer Laboratories, UK) 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 either ISO or IDF of this product.

5.8.5 Fluorescence detector, capable of operating at 280 nm excitation and at 340 nm emission.

6 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 707|IDF 50.^[4]

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

7 Procedure

7.1 Preparation of lysozyme standard solution

7.1.1 Lysozyme standard stock solution

Weigh to the nearest 0,01 mg, 10 mg of lysozyme (4.2) into a 10 ml one-mark volumetric flask and wait for complete dissolution. Dilute to the mark with sodium chloride solution (4.1.1).

Prepare fresh the standard stock solutions daily.

7.1.2 Lysozyme standard working solution

Pipette 80 µl of the lysozyme standard stock solution (see 7.1.1) into a 10 ml one-mark volumetric flask. Dilute to the mark with sodium chloride solution (4.1.1).

The obtained lysozyme standard working solution contains 8,0 mg of lysozyme per litre.

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7.2 Test portion <https://standards.iteh.ai/catalog/standards/sist/63448434-66bb-46eb-b163-126fe49d0c75/iso-27105-2016>

7.2.1 Milk

Weight to the nearest 0,01 g, 10,00 g of test sample into a 100 ml beaker.

7.2.2 Cheese

Before weighing, grate the test samples of cheese. Weight to the nearest 0,01 g, 2,00 g of test sample into a 100 ml beaker.

NOTE Soft cheese can be grated after freezing.

7.2.3 Preparation of test solution

Add 20 ml of sodium chloride solution (4.1.1) to the test portion (see 7.2.1 or 7.2.2) and mix. Adjust the pH of the obtained solution drop wise with sodium hydroxide solution (4.1.3) to pH 6,0.

Homogenize the test solution for 30 s by using the homogenizer (5.7) at a speed rate of 2 500 rpm to 3 000 rpm. Rinse the homogenizer in a separate 100 ml beaker using 10 ml of sodium chloride solution (4.1.1). Add the rinsing to the test solution.

Stir the beaker containing the test solution on a magnetic stirrer at room temperature for 1 h. Adjust the pH of the test solution obtained from the test portion of milk (see 7.2.1) to pH 2,2 or that obtained from the test portion of cheese to pH 4,3 by using hydrochloric acid (4.1.2).

Transfer the test solution from the 100 ml beaker to a 50 ml one-mark volumetric flask. Use sodium chloride solution (4.1.1) to rinse the 100 ml beaker and add the rinsing to the test solution. Dilute to the mark with the sodium chloride solution (4.1.1) and mix.