INTERNATIONAL STANDARD

ISO 13082

IDF 218

First edition
2011-11-15

Milk and milk products — Determination of the lipase activity of pregastric lipase preparation

Lait et produits laitiers — Détermination de l'activité de lipase de la préparation de lipase prégastrique

Reference numbers
ISO 13082:2011(E)
IDF 218:2011(E)

ISO copyright office
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
Silver Building • Boulevard Auguste Reyers 70/B • 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

Published in Switzerland

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.

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.

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.

ISO 13082 | IDF 218 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.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. IDF shall not be held responsible for identifying any or all such patent rights.

ISO 13082 | IDF 218 was prepared by the International Dairy Federation (IDF) and Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*. It is being published jointly by IDF and ISO.

All work was carried out by the Joint ISO-IDF Project Group *Lipase activity* of the Standing Committee on *Analytical methods for processing aids and indicators* under the aegis of its project leaders, Mrs M. Harboe (DK) and Dr J. Jacobsen (DK).

Introduction

Lipases (EC 3.1.1.3) are the group of esterases that hydrolyse emulsified triacylglycerolesters, which are the main component of milk fat.

Commercial pregastric lipase and some rennet preparations (paste or liquid) contain lipases from calf, kid-goat or lamb sources. These lipase preparations are used particularly in the production of Italian type cheeses, e.g. in Romano, Provolone, and Asiago and in other similar cheese varieties and in enzyme-modified dairy products as described in IDF Bulletin 294[6]. Lipase is not allowed in Feta, but it is often used in Feta-type cheese.

The method is based on the principle of the FCCIV method for forestomach lipase activity[7], but in its current form the FCCIV method is not sufficiently developed. As such, it does not provide adequate details in several critical areas, most notably in sample and substrate preparation. However, the FCCIV method served as a useful model for the development of this International Standard.

Milk and milk products — Determination of the lipase activity of pregastric lipase preparation

1 Scope

This International Standard specifies a method for the determination of the lipase activity. It is intended for the preparation of pregastric lipase and rennet paste, both of animal origin.

NOTE No reference method was used to check this method as no stable standard can be found. On the other hand, a reference method can be omitted as the substrate is reproducible and well defined.

2 Terms and definitions

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

2.1
international lipase unit
ILU
amount of lipase activity that releases butanoic acid, also known as butyric acid, at a rate of 1,25 µmol/min under specified conditions

NOTE 1 Lipase activity is expressed either in international lipase units (ILU) per gram of product or ILU per millilitre of product.

NOTE 2 The definition is based on the direct consumption of titrant while not considering that a small molar fraction of the butyric acid (4 %) is not dissociated and thus cannot be titrated. As such, that creates a small error in the definition.

3 Principle

Triglyceride esters are hydrolysed by lipase. The free fatty acids (as butyric acid) released from the substrate tributyrin are titrated in a pH-stat with sodium hydroxide. The amount of sodium hydroxide consumed within a defined period is used to calculate the activity in ILU per millilitre or ILU per gram.

Due to the non-existence of a reference standard, it is recommended that a control (known) sample be included in the test.

4 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and distilled water or demineralized water or water of equivalent purity.

The brand of chemicals can affect the result. Therefore, before using a brand other than the one mentioned, verify whether it gives the same result.

4.1 Tributyrin (glycerintributyrate or glyceryl tributyrate), e.g. Merck No. 1.01958.0100[1)] or similar.

4.2 Sodium caseinate, e.g. Sigma C8654[1)] or similar.

4.3 Lecithin, from soya bean, e.g. BDH Prod. 29863[1)] or similar.

4.4 Liquid paraffin. Use paraffin which is highly liquid (or similar light mineral oil), e.g. Merck No. 7174.1000[1)], or similar.

4.5 Soda lime granules [Carbosorb[1)]], e.g, BDH no 331104[1)] or similar.

4.6 Sodium hydroxide solution, c(NaOH) = 0,025 mol/l, which can either be purchased or be prepared as follows.

Using a pipette (5.1), add 25,00 ml of 1 mol/l sodium hydroxide with an accurately known titre into a container. Dilute with water to 1 000 ml.

The 0,025 mol/l NaOH solution can be kept in a closed container, protected against carbon dioxide in the air by use of a CO_2 trap with soda lime (4.5) at room temperature for at least 1 month. If necessary, seek advice from the supplier of the equipment or reagent. Change the soda lime at least once a year.

When changing the sodium hydroxide batch, check the actual stability of the titre by comparing the old and new titrant, e.g. using a control sample.

For samples with low activity and manual titrations, use a 0,010 mol/l NaOH instead of a 0,025 mol/l NaOH solution. As such, the 0,010 mol/l NaOH solution gives a higher and more useful consumption of titrant. Prepare the 0,010 mol/l NaOH solution freshly before use (unless the titre has been checked) as it is unstable. If using the 0,010 mol/l NaOH solution, correct the calculation according to the formulae in 8.1.

4.7 Lecithin solution, with a mass per volume fraction of 10 %. Weigh 10,0 g of lecithin in a suitable bottle. Use magnetic stirring to dissolve it in approx. 95 ml of liquid paraffin, which may take between 1 day and 2 days of mixing. When the lecithin is completely dissolved, make it up to a total volume of 100 ml with the liquid paraffin.

When stored in a refrigerator, the lecithin solution is stable for 1 year.

4.8 Control sample. Include a control sample of known activity in each series of test for lipase samples. Collect the results and use them for the evaluation of the variation of the test.

The control sample can be the last sample analysed or another well-known sample.

When carrying out the method for the very first time, use a control sample obtained from another laboratory or the first sample analysed being kept as control sample for the next series of analyses. If needed, store the control sample(s) in a freezer.

NOTE It can be difficult to get a suitable control sample for rennet paste.

5 Apparatus

Usual laboratory equipment and, in particular, the following.

The laboratory equipment can be substituted by other equipment verified as giving similar results.

5.1 Micropipette or **any other pipette**, of capacities 1 ml and 10 ml with a repeatability of 0,5 % or higher.

1) Example of a suitable product available commercially. This information is given for the benefit of users of this document and does not constitute an endorsement by ISO of this product.

5.2 One-mark volumetric flasks, of required capabilities, ISO 1042[3] class A.

5.3 Water bath, capable of circulating the water externally and of maintaining a constant temperature in the reaction vessel of 42 °C ± 0,5 °C.

5.4 Blender, Warren[1)], Ultraturax[1)] or any equivalent apparatus.

5.5 pH stated equipment, including the following components:

a) a thermostated reaction vessel capable of stirring effectively, e.g. mechanical or magnetic stirring;

b) a burette for titration;

c) a recorder, printer or computer.

A Metrohm 718 Stat Titrino[1)] is suitable for the purpose. A manual titration set-up may also be used but that can reduce the precision of the method.

For control purposes, therefore, mention the equipment used in the test report.

5.6 Stomacher and **stomacher bags**, for dissolving rennet paste, e.g. standard bags BA 6041 from Seward[1)] or equivalent.

6 Sampling

A representative sample should have been sent to the laboratory. It should not have been damaged or changed during transport or storage.

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 707 | IDF 50[2].

Test samples may be stored at a temperature of 5 °C or lower for 2 months. In case of a long storage period, store the test samples frozen, e.g. at −18 °C, as that will significantly improve the stability of the lipase powder.

7 Procedure

7.1 Substrate

Disperse 600 mg of sodium caseinate (4.2) in 95 g water in the blender vessel. Add 0,5 ml lecithin solution (4.3) and 1,0 ml tributyrin (4.1). Blend for 60 s at low speed. Pour the substrate into a flask or beaker and keep it at room temperature on a magnetic stirrer using slow speed.

Use the substrate within 4 h.

7.2 Preparation of lipase test solution

7.2.1 Liquid lipase sample

Accurately pipette the required amount of the liquid lipase sample or control into a 100 ml one-mark volumetric flask (5.2) to obtain a 100 ml lipase solution with a concentration of (4 ± 1) ILU/ml. Make up to the mark with water.

NOTE Volumetric flasks of different capacities can be used or the sample can be analysed undiluted if the lipase activity is 5 ILU or below.