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Milk and milk products — Ovine and caprine rennets — Determination of total milk-clotting activity

Lait et produits laitiers — Présures ovines et caprines — Détermination de l'activité totale de coagulation du lait

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Contents

Page

Foreword

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ISO 23058 | IDF 199 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 worldwide federation of the dairy sector with a National Committee in every member country. Every National Committee has 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.

Draft International Standards adopted by the Action Teams and Standing Committees are circulated to the National Committees for voting. Publication as an International Standard requires approval by at least 50 % of the 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 23058 | IDF 199 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 Action Team on *Enzymes in cheesemaking*, of the Standing Committee on *Milk components and characterization of physical properties*, under the aegis of the project leaders Mrs M. Harboe (DK) and Mr C. Repelius (NL).

Introduction

Animal rennets comprise all milk-coagulating products derived from ruminants, but until now only bovine rennets have been characterized according to the ISO/IDF standards on composition and milk-clotting activity. Ovine and caprine rennets are also available in the market and usually follow the same specifications as for bovine rennets. Each of these enzymes has its own characteristics as far as milk-clotting activity and cheese-making properties are concerned. There are differences in temperature and pH sensitivity and especially in the organoleptical properties of the cheese produced. A limited number of manufacturers produce these specific rennets and in different presentations (liquid, powder or rennet paste). No internationally recognized reference methods specific to the characterization and isolation of samples of these products have been available until now.

Milk and milk products — Ovine and caprine rennets — Determination of total milk-clotting activity

1 Scope

This International Standard specifies a method for the determination of the total milk-clotting activity of an ovine or caprine rennet, including rennet paste, containing only chymosin and pepsin as the active coagulating enzymes on a standard milk substrate prepared using a calcium chloride solution of 0,5 g per litre (pH ≈ 6,5).

The method allows analysis of ovine and caprine rennets in accordance with the relative milk-clotting activity test (REMCAT) for bovine rennets given in ISO 11815 | IDF 157.

2 Normative references

The following referenced documents are indispensable for the application 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 648, *Laboratory glassware — One-mark pipettes*

ISO 1042, *Laboratory glassware — One-mark volumetric flasks*

IDF 110B, *Calf rennet and adult bovine rennet — Determination of chymosin and bovine pepsin contents (chromatographic method)*

3 Terms and definitions

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

3.1
total milk-clotting activity of a standard milk substrate at pH 6,5
amount of activity set relative to the international calf rennet reference standard powder and the adult bovine rennet reference standard powder

NOTE 1 For the first batch of both the calf rennet reference standard powder and the adult bovine rennet reference standard powder (also used for the analysis of bovine and caprine rennets), this was defined as 1 000 International Milk-Clotting Units per gram (IMCU/g). Future preparations of reference standards will be set relative to the previous reference standards.

NOTE 2 The total milk-clotting activity of the microbial coagulant reference standard powder is approximately 1 000 IMCU/g, but the real activity with respect to the international calf rennet control powder is indicated on the glass ampoules.

NOTE 3 The total proteolytic (milk-clotting) activity of the microbial coagulant reference standard powder is checked on a synthetic hexapeptide substrate every second year by NIZO [1)].

1) Netherlands Institute for Dairy Research (NIZO), P.O. Box 20, 6710 BA Ede, The Netherlands. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO or IDF of these products.

4 Principle

The time needed for visual flocculation of a standard milk substrate prepared with a calcium chloride solution of 0,5 g per litre (pH ≈ 6,5) is determined. The clotting time of the ovine (or caprine) rennet sample is compared to that of a bovine rennet reference standard with a defined enzyme composition of 75:25 and with known milk-clotting activity.

It is measured relative to a reference standard having 75 % calf chymosin and 25 % bovine pepsin because the composition of the test samples is unknown and a reference standard with a ratio 75/25 should cover most samples with enough accuracy. It is not worthwhile to measure the true chymosin/pepsin composition of ovine and caprine rennets as their composition is generally close to the above ratio.

NOTE The composition of ovine and caprine liquid or powder rennets may be determined using IDF 110B, but the accuracy for these rennet types has not been validated.

5 Reagents and materials

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

5.1 Buffer solution, pH 5,5.

Add, using a pipette (6.1), 10,0 ml of 1 mol/l acetic acid (CH_3COOH) to 10,0 g of sodium acetate trihydrate ($CH_3COONa \cdot 3H_20$) and mix. Dilute with water to 1 000 ml. Adjust the pH to 5,5 if necessary.

5.2 Calcium chloride stock solution, $c(CaCl_2)$ = 500 g/l.

Calcium chloride solutions with the required accurate concentration of 500 g/l of calcium chloride and the actual density stated are commercially available[2)]. Store the solution as described by the manufacturer.

Prior to use, bring the calcium chloride stock solution to room temperature (18 °C to 22 °C). Check the concentration of the solution by titration with EDTA (ethylenediaminetetraacetic acid) every year.

5.3 Calcium chloride working solution, $c(CaCl_2)$ = 0,5 g/l.

Use the density of the calcium chloride stock solution (5.2) to calculate the mass of calcium chloride needed to obtain a final amount of 0,5 g/l of calcium chloride in the calcium chloride working solution.

The mass of the solution should be equivalent to the addition of 2,00 ml of the stock solution with the exact concentration required, $c(CaCl_2)$ = 500 g/l; in that case, the solution mass is ≈ 2,70 g.

Weighing the calcium chloride stock solution (5.2) is recommended in order to be able to prepare the calcium chloride working solution, as the viscous solution is difficult to pipette.

Weigh, to the nearest 0,01 g, about 2,70 g of the calcium chloride stock solution (5.2) of exactly known concentration at room temperature (18 °C to 22 °C) in a 2 000 ml one-mark volumetric flask. Dilute to the mark with water and mix. The calcium chloride solution shall be freshly prepared on the day of use.

Alternatively, an intermediate calcium chloride solution of 50 g/l may be prepared and further diluted before use.

2) Ordering address: Chr. Hansen A/S, 1-27 Jernholmen, 2650 Hvidovre, Denmark (Fax: +45 36 86 77 76). This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO or IDF of these products.

5.4 Low-heat, low-fat, spray-dried milk powders, of good renneting and bacteriological quality.

NOTE Low-heat, low-fat, spray-dried milk powders meeting the requirements are commercially available[2), 3)].

5.5 Calf rennet reference standard powder[4)], in pouches containing 2,7 g of powder, containing > 98 % chymosin and < 2 % bovine pepsin in terms of enzyme activity, as determined according to IDF 110B.

The exact total milk-clotting activity is indicated on the certificate of analysis and should be about 1 000 IMCU/g.

The calf rennet reference standard powder is a primary reference standard; a secondary liquid standard may be made and used if it has been ensured that the same result is obtained.

Store the calf rennet reference standard powder in the dark at −18 °C, protected against moisture. For short periods, for example during transport, it may be kept at ambient temperatures.

5.6 Adult bovine rennet reference standard powder[4)], in pouches containing 2,7 g of powder, containing < 2 % chymosin and > 98 % bovine pepsin in terms of enzyme activity, as determined according to IDF 110B.

The exact total milk-clotting activity is indicated on the certificate of analysis and should be about 1 000 IMCU/g.

The adult bovine rennet reference standard powder is a primary reference standard; a secondary liquid standard may be made and used if it has been ensured that the same result is obtained.

Store the adult bovine reference standard powder in the dark at −18 °C, protected against moisture. For short periods, for example during transport, it may be kept at ambient temperatures.

6 Apparatus

Usual laboratory equipment and, in particular, the following.

6.1 Micropipette, or any other pipette capable of delivering 0,5 ml in less than 1 s with a repeatability of 0,2 % or better.

6.2 One-mark pipettes, in accordance with ISO 648, to deliver appropriate amounts.

Alternatively, a dilutor (e.g. a Hamilton diluter) with the same high precision may be used for diluting the coagulants. For measuring the substrate, a syringe or a dispenser delivering the appropriate amount with a repeatability of 0,4 % may also be used.

6.3 One-mark volumetric flasks, in accordance with ISO 1042, of the required capacities.

6.4 Thermometer, calibrated, graduated between 20 °C and 45 °C, with a precision of ± 0,1 °C.

6.5 pH-meter, capable of measuring the pH in 0,01 units.

6.6 Analytical balance, capable of weighing to the nearest 1 mg.

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3) Institut national de la Recherche agronomique, France, which is dependent on the Station expérimentale laitière, P.P. No. 94, 39800 Poligny, France. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO or IDF of these products.

4) AMAFE. Ordering address: Chr. Hansen A/S, 1-27 Jernholmen, 2650 Hvidovre, Denmark (Fax: +45 36 86 77 76). This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO or IDF of these products.

6.7 Stopwatch, capable of reading in seconds.

6.8 Flasks or **test tubes**, for milk-clotting testing, with a suitable capacity (see 6.9.1 and 9.5.1).

6.9 Water bath, capable of maintaining a temperature of 32 °C ± 1 °C but also capable of keeping the maintained temperature constant within limits of ± 0,2 °C throughout the bath (see also 9.5.1), with the following attachments.

6.9.1 Electric motor, provided with a rotating spindle to which the flask or test tube (6.8) can be attached, capable of rotating at a suitable angle of about 30° with the water surface of the water bath.

NOTE The rotation speed is not very important for this method; a speed of between 2 r/min and 4 r/min is suitable.

6.9.2 Electric lamp, placed in such a position as to illuminate the flask or test tube (6.8) effectively.

A screen with a dark background, placed in the water bath, may be used to improve the determination of the flocculation point in the flask or test tube.

6.10 Stomacher, for dissolving rennet paste, e.g. from Seward[5)].

7 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. Sampling of liquid rennet (8.1) and powdered rennet (8.3) should be carried out following the instructions given in ISO 707 | IDF 50 for “Milk and liquid milk products” and for “Dried milk and dried milk products”, respectively.

Store the test samples in the dark at a temperature of between 0 °C and 5 °C.

8 Preparation of test sample

8.1 Liquid ovine (or caprine) rennet

Mix the test sample by swirling while avoiding the formation of foam. Bring the sample to room temperature (18 °C to 22 °C) prior to starting preparation of the coagulant test solution (9.4).

8.2 Ovine (or caprine) rennet paste

Mix the rennet paste test sample to obtain a homogeneous paste. Dissolve a sample of known mass of 8 g ± 2 g in a plastic bag with 200 ml of buffer solution (5.1) by using a stomacher (6.10) set at a recommended speed of 230 r/min for 60 s.

Alternatively, the paste dissolution in the plastic bag may be done manually in the stomacher bag for 60 s.

Record the exact amount of sample taken, in grams, to three significant digits. Filter the extraction solution through gauze or a Buchner filter.

5) Seward Ltd., London, United Kingdom. (www.seward.co.uk). This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO or IDF of this product.

Set the dilution factor of the paste to the liquid extract at (200 g + mass of the paste) divided by the mass of paste to be used in the calculation of the final dilution factor (see 9.4).

NOTE Alternatively, a Bagfilter®[6] can be used to allow extraction and filtration in one operation.

8.3 Powdered ovine (or caprine) rennet

Mix the test sample thoroughly to obtain a homogeneous powder. Bring the sample to room temperature (18 °C to 22 °C) prior to starting the preparation of the rennet test solution (9.4).

NOTE 1 Note that powdered products can separate rapidly.

NOTE 2 Consider the amount of test sample to be taken out. Often sample amounts of 3 g to 5 g are sufficient, but when testing inhomogeneous samples or when very accurate results are desired, then larger sample sizes such as 10 g are needed for analysis.

9 Procedure

9.1 Preparation of substrate

Measure 1 000 ml of calcium chloride working solution (5.3) into a 1 000 ml volumetric flask (6.3). Weigh, to the nearest 0,1 g, 110 g of low-heat, low-fat, spray-dried milk powder (5.4) into a 2 000 ml beaker. Add about 100 ml of calcium chloride working solution (5.3) to the powder in the beaker. Stir manually to obtain a homogeneous mixture. Add the remaining 900 ml of calcium chloride working solution to the contents of the beaker, allowing the 1 000 ml volumetric flask to drain. Stir the substrate thus obtained with a magnetic stirrer for 30 min, taking care to avoid the formation of foam.

Leave the substrate at room temperature for 30 min. The substrate may be kept at room temperature for no longer than 4 h, but it may be kept refrigerated during the day of preparation.

The pH of the prepared substrate will be approximately 6,50 and should not be adjusted.

9.2 Preparation of the calf and adult bovine rennet reference solutions

9.2.1 Preparation of the calf rennet reference standard solution

Bring the pouch with the calf rennet reference standard powder (5.5) to room temperature (18 °C to 22 °C) before opening it, to avoid moisture getting into the powder.

Open the pouch and weigh, to the nearest 1 mg, 2,500 g of calf rennet reference standard powder (5.5) into a 50 ml volumetric flask (6.3). Add 15 ml to 20 ml of buffer solution (5.1) and mix by swirling, while avoiding foam formation, to dissolve the powder. Dilute to the 50 ml mark with buffer solution (5.1) and mix well again.

9.2.2 Preparation of the adult bovine rennet reference standard solution

Repeat the preparation in 9.2.1 by replacing the calf rennet reference powder (5.5) with the adult bovine rennet reference standard powder (5.6).

6) Bagfilter® is available from Interscience, 30, chemin du Bois-des-Arpents, 78860 St-Nom-la-Bretèche, France (www.interscience.fr). This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO or IDF of this product.

9.3 Preparation of the calf and adult bovine rennet reference working solutions

9.3.1 Preparation of the calf rennet reference working solution

In order to obtain a proper clotting time, use a one-mark pipette (6.2) to add 3 ml of calf rennet reference standard solution (9.2.1) into another 50 ml volumetric flask (6.3). Dilute to the mark with buffer solution (5.1) and mix well.

The final dilution factor is 333,33 times. The clotting time for the calf rennet reference working solution should be in the range of 350 s to 550 s.

Keep the calf rennet reference working solution at room temperature during the day of its preparation. It may be stored at between 0 °C and 5 °C for 2 days.

9.3.2 Preparation of the adult bovine rennet reference working solution

Repeat the operations in 9.3.1 but replacing the calf rennet reference standard solution (9.2.1) with the prepared adult bovine rennet reference standard solution (9.2.2).

The final dilution factor is 333,33 times. The clotting time for the adult bovine rennet reference working solution should be in the range of 350 s to 550 s.

Keep the adult bovine rennet reference working solution at room temperature during the day of its preparation. It may be stored at between 0 °C and 5 °C for 2 days.

9.4 Preparation of the ovine (or caprine) rennet test solution

Take an appropriate test portion (3 g to 5 g of powder, or 3 ml to 5 ml of extracted paste solution) from the prepared test sample (8.1, 8.2 or 8.3). Dilute the test portion with the buffer (5.1) until a test solution is obtained with a clotting time that is similar to the calf or adult bovine rennet reference working solution used in 9.3.1 or 9.3.2 with a tolerance of $\pm$ 40 s. Record the final dilution factor of the test solution for use in the calculation (10.1). Take care to include the dilution factor noted under 8.2 in the case of a paste rennet.

9.5 Clotting

9.5.1 Use a one-mark pipette (6.2) to add 25 ml $\pm$ 0,1 ml of substrate (9.1) to a dry flask or test tube (6.8). Pre-heat the substrate, while rotating the flask or test tube in the water bath (6.9) set at 32 °C for at least 12 min, but no longer than 20 min.

Then quickly add, using the micropipette (6.1), 0,5 ml of calf rennet reference working solution (9.3.1) to the substrate. At the same time, activate the stopwatch (6.7). Mix by swirling while avoiding the formation of foam, and immediately attach the flask or test tube to the rotating spindle.

Read the clotting time from the stopwatch when the first flocculation is observed in the substrate film on the wall of the flask or test tube.

9.5.2 Repeat the procedure in 9.5.1 without delay but replacing the calf rennet reference working solution with the ovine (or caprine) rennet test solution (9.4) and then repeat with the adult bovine rennet reference working solution (9.3.2).

This procedure gives relative results. It is most important, therefore, to maintain the same temperature for the test samples and the reference samples. Check the temperature by measuring the temperature of the samples at different positions in the bath. If the maximum allowed variation of temperature in the water bath ($\pm$ 0,2 °C) cannot be reached, then the design of the bath or water-circulating system should be improved. It is also recommended to always keep test samples as close as possible to the reference samples in the bath in order to obtain identical conditions for both.

9.5.3 Repeat operations 9.5.1 and 9.5.2 without delay to obtain duplicate values. Calculate the means of the clotting times for the calf and adult bovine rennet reference working solutions, respectively, and then for the ovine (or caprine) rennet test solution.

9.5.4 Instead of 25 ml of substrate and 0,5 ml of calf or adult bovine rennet reference working (or test) solution in 9.5.1 or 9.5.2, 10 ml of substrate plus 0,2 ml of working (or test) solution, or 50 ml of substrate plus 1,0 ml of working (or test) solution may be used. However, it is important that the ratio between the substrate and the working solution be 50:1.

10 Calculation and expression of results

10.1 Calculation

10.1.1 General

The milk-clotting activity is expressed in International Milk Clotting Units (IMCU) per gram or per millilitre. First, the milk-clotting activity of the test sample is calculated relative to both the activity of the calf reference standard powder (a_c, = 5,6) and the activity of the adult bovine rennet reference standard powder (a_a, = 5,7) (see 10.1.2). Then, the final calculation of the total milk-clotting activity is performed by interpolation (see 10.1.3)

10.1.2 Calculation of milk-clotting activity relative to both reference standards

Calculate the milk-clotting activity of the test sample relative to both reference standards (a_{tc} and a_{ta}) by using the following equations:

$$a_{tc} = \frac{t_{c,ref} \times w_{c,ref} \times V_1 \times d \times a_{c,ref}}{t_t \times V_2 \times V_3} \quad (1)$$

$$a_{ta} = \frac{t_{a,ref} \times w_{a,ref} \times V_1 \times d \times a_{a,ref}}{t_t \times V_2 \times V_3} \quad (2)$$

where

a_{tc} is the total milk-clotting activity of the test sample relative to the calf rennet reference standard;

a_{ta} is the total milk-clotting activity of the test sample relative to the adult bovine rennet reference standard;

$t_{c,ref}$ is the mean clotting time, in seconds, obtained with the calf rennet reference working solution (9.5.1 and 9.5.3);

$t_{a,ref}$ is the mean clotting time, in seconds, obtained with the adult bovine rennet reference working solution (9.5.2 and 9.5.3);

$w_{c,ref}$ is the mass, in grams, of the calf rennet reference standard weighed in 9.2.1;

$w_{a,ref}$ is the mass, in grams, of the adult bovine rennet reference standard weighed in 9.2.2;

V_1 is the volume taken, in millilitres, of either the calf (in 9.3.1) or the adult bovine (in 9.3.2) rennet reference standard solution (V_1 = 3 ml);

d is the noted final value of the dilution factor obtained with the test solution (9.4);

$a_{c,ref}$ is the milk-clotting activity (strength), in IMCU per gram, of the calf rennet reference standard powder (5.5); this value is indicated on the glass ampoule of the reference powder;

$a_{a,ref}$ is the milk-clotting activity (strength), in IMCU per gram, of the adult bovine rennet reference standard powder (5.6); this value is indicated on the glass ampoule of the reference powder;

t_t is the mean clotting time, in seconds, obtained with the ovine (or caprine) rennet test solution (9.5.2 and 9.5.3);

V_2 is the final volume, in millilitres, of either the calf (in 9.2.1) or the adult bovine (in 9.2.2) rennet reference standard solution (V_2 = 50 ml);

V_3 is the final volume, in millilitres, of either the calf (in 9.3.1) or the adult bovine (in 9.3.2) rennet reference working solution (V_3 = 50 ml);

Equations (1) and (2) can be simplified by introducing the following values: w_{ref} = 2,500 g; V_1 = 3 ml; V_2 = 50 ml; V_3 = 50 ml to obtain simplified Equations (3) and (4):

$$a_{tc} = \frac{t_{c,ref} \times 0{,}003 \times d \times a_{c,ref}}{t_t} \quad (3)$$

$$a_{ta} = \frac{t_{a,ref} \times 0{,}003 \times d \times a_{a,ref}}{t_t} \quad (4)$$

10.1.3 Final calculation of the total milk-clotting activity by interpolation

The total milk-clotting activity of the test sample (a_t) is finally calculated by interpolation relative to a bovine rennet reference standard with a composition of 75/25 by definition, and with an activity calculated on the basis of the clotting times found in 10.1.2 by using the following equation:

$$a_t = \frac{75}{100} \times a_{tc} + \frac{25}{100} \times a_{ta} \quad (5)$$

10.2 Expression of results

Express the results in International Milk-Clotting Units (IMCU) to whole units of grams or millilitres.

11 Precision

11.1 Interlaboratory test

The results of an interlaboratory test on the precision of the method are give in Annex A. The values obtained may not be applicable to concentration ranges and matrices other than those given.

The values for repeatability and reproducibility are derived from the standard deviations which are estimates of the true standard deviation of the method. Each value given for the repeatability and reproducibility is the maximum difference between two test results which is expected in 95 % of the cases when two results are compared. If significantly less than 95 % of the cases are within the values given in 11.2 and 11.3, it is recommended to work on improving the execution of the method.

11.2 Repeatability

The relative standard deviation of repeatability [RSD(r)], which expresses the variability of analytical results obtained using 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, will in not more than 5 % of cases be greater than

— for liquid rennets: 2,1 % (relative),

— for paste rennets: 6,0 % (relative).

If two determinations are obtained under these conditions, the absolute difference [r(rel) %] between the two results will in not more than 5 % of cases be greater than

— for liquid rennets: 5,8 % (relative),

— for paste rennets: 17 % (relative).

11.3 Reproducibility

The relative standard deviation of reproducibility [RSD(R)], which expresses the variability of analytical results obtained using the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases be greater than

— for liquid rennets: 4,1 % (relative),

— for paste rennets: 13 % (relative).

If two determinations are obtained under these conditions, the absolute difference between two results [R(rel) %] will in not more than 5 % of cases be greater than

— for liquid rennets: 12 % (relative),

— for paste rennets: 36 % (relative).

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) a reference to this International Standard;

d) all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may 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)

Results of interlaboratory trial

A.1 General

An international collaborative test involving 12 laboratories from five countries was carried out on samples of both liquid and paste rennets of ovine and caprine origin. The test was organized by DSM Food Ingredients (NL). The test results were subjected to statistical analysis according to ISO 5725-1 and ISO 5725-2.

A.2 Liquid rennet samples

The liquid caprine rennet sample came from one batch, but was further diluted to yield samples of 100 %, 85 % and 75 % strength. In the same way, the liquid ovine rennet sample was diluted to 100 % and 85 % strength. In order to avoid a visual difference, all samples were matched in colour by the addition of caramel. The five test samples obtained were divided again into 10 blind duplicated samples. The milk-clotting activities are given in Table A.1.

The interlaboratory trial on the liquid rennets showed excellent results for relative repeatability and relative reproducibility. One laboratory was removed from the statistical analysis due to non-compliance with the test method. After testing for Cochran outliers on the remaining 11 laboratories, one outlier was detected and removed from the calculations. No single or double Grubbs outliers were subsequently detected.

Statistical evaluation of the results from the remaining 10 laboratories in the collaborative study on liquid caprine and ovine rennets is shown in Table A.2.

Table A.1 — Milk-clotting activities of liquid rennet samples (IMCU/ml)

Lab. No.	Coagulant (strength)									
	Cap (75 %)	Ov (85 %)	Cap (85 %)	Ov (100 %)	Cap (100 %)	Cap (75 %)	Ov (85 %)	Cap (85 %)	Ov (100 %)	Cap (100 %)
1	31,3	87,7	35,6	100,1	41,5	31,9	86,5	36,6	102,0	43,1
2	33,4	92,2	34,4	106,3	41,3	33,8	91,1	36,1	105,3	41,1
3[a]	27,4	57,9	31,8	97,4	35,8	31,6	60,5	40,1	92,2	44,9
4	35,2	87,3	40,1	103,0	47,0	35,4	85,0	39,4	101,9	46,5
5	31,9	86,8	36,0	101,6	41,2	31,5	86,1	36,0	100,8	41,6
6	29,5	85,8	35,8	99,4	38,5	26,5	87,5	34,3	99,8	40,0
7	33,4	91,3	36,5	105,1	42,6	33,1	91,8	34,6	97,7	42,8
8	31,2	86,5	35,6	101,9	41,1	34,8	88,5	36,0	102,3	40,9
9[b]	30,2	85,7	37,1	102,2	46,5	32,6	89,2	36,5	102,7	42,0
10	32,5	88,3	36,5	103,2	43,0	32,4	88,6	36,5	106,5	41,5
11	31,7	86,6	35,2	105,5	40,3	31,5	87,0	34,7	101,0	40,4
12	33,9	91,9	36,7	106,6	45,2	33,4	91,9	36,9	105,3	44,0

[a] Removed due to non-compliance.

[b] Removed as Cochran outlier.

Table A.2 — Test results from remaining 10 laboratories for liquid rennet samples

Sample	Mean IMCU/ml	RSD(r)	r	r(rel) %	RSD(R)	R	R(rel) %	Outliers
Cap (75 %)	32,32	3,59	3,24	10,04	6,28	5,68	17,57	
Cap (85 %)	36,24	1,93	1,96	5,41	3,95	4,00	11,05	
Cap (100 %)	42,20	1,62	1,91	4,53	5,22	6,17	14,62	1 (Cochran)
Ov (85 %)	88,34	1,28	3,16	3,58	2,71	6,71	7,60	
Ov (100 %)	102,73	2,01	5,79	5,64	2,46	7,06	6,88	
Mean		2,09		5,84	4,12		11,54	

A.3 Paste rennet samples

Two different ovine paste rennet samples and two different caprine rennet samples which were not further diluted were tested. All test samples were divided into eight blind duplicated samples. The milk-clotting activities are given in Table A.3.

The results on the paste rennets showed much higher values for the relative repeatability and relative reproducibility. These values were obtained after removal of four laboratories from the participating 12 laboratories, partly on request of the laboratories themselves and partly for reasons of non-compliance with the test method. After the removal, eight laboratories remained and no Cochran or Grubbs outliers were found. The precision values obtained are relatively high but, in view of the rather inhomogeneous nature of these traditional paste rennets, the values obtained are considered to be acceptable.

Statistical evaluation of the results from the remaining eight laboratories in the collaborative study on paste caprine and ovine rennets are given in Table A.4.

Table A.3 — Milk-clotting activities of paste rennet samples (IMCU/g)

Lab. No.	Coagulant							
	Ov	Cap	Ov	Cap	Ov	Cap	Ov	Cap
1	158,0	141,8	132,1	115,6	154,0	124,1	137,9	114,0
2[a]	59,0	80,7	119,4	109,7	93,5	72,5	90,4	72,0
3[a]	99,9	81,8	88,8	79,7	91,3	81,3	81,9	81,5
4	95,6	107,5	99,3	100,1	94,3	106,4	96,9	101,7
5	108,3	102,9	94,8	80,3	85,9	95,3	84,6	102,7
6	144,9	125,0	119,1	106,7	145,4	125,3	132,9	109,6
7[a]	118,0	90,4	75,5	82,8	71,2	92,8	72,1	76,3
8	133,3	122,3	124,9	96,0	126,2	106,0	117,5	108,9
9	147,8	128,6	128,5	107,5	142,0	134,3	128,6	114,7
10	133,8	114,9	111,8	103,8	114,9	123,0	118,3	93,1
11[a]	74,9	105,3	87,5	76,9	99,7	93,6	98,5	63,0
12	132,8	121,5	119,4	104,9	121,4	116,1	124,6	103,9

[a] Removed due to non-compliance.

Table A.4 — Test results from remaining eight laboratories for paste rennet samples

Sample	Mean IMCU/ml	RSD(r)	r	r(rel) %	RSD(R)	R	R(rel) %	Outliers
Ovine P1/P5	127,4	6,50	23,19	18,20	17,96	64,09	50,30	
Caprine P2/P6	118,4	5,83	19,34	16,33	10,66	35,36	29,86	
Ovine P3/P7	116,9	4,57	14,96	12,80	13,66	44,74	38,26	
Caprine P4/P8	104,0	6,99	20,36	19,59	8,67	25,23	24,27	
Mean		5,97		16,70	12,74		35,70	

Bibliography

[1] ISO 707 | IDF 50, *Milk and milk products — Guidance on sampling*

[2] ISO 5725-1, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

[3] ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

[4] ISO 11815 | IDF 157, *Milk — Determination of total milk-clotting activity of bovine rennets*

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