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BSI Standards Publication

Milk and milk products — Calf rennet and adult bovine rennet — Determination by chromatography of chymosin and bovine pepsin contents

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National foreword

This British Standard is the UK implementation of ISO 15163:2012.

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Milk and milk products — Calf rennet and adult bovine rennet — Determination by chromatography of chymosin and bovine pepsin contents

Lait et produits laitiers — Présure de veau et coagulant issu de bovin adulte — Détermination des teneurs en chymosine et en pepsine bovine par chromatographie

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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 15163 | IDF 110 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 IDF and ISO.

This first edition of ISO 15163 | IDF 110 cancels and replaces IDF 110B:1997, which has been technically revised.

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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ISO 15163 | IDF 110 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 an ISO-IDF Project Group on *Chymosin and bovine pepsin determination,* of the Standing Committee on *Analytical methods for processing aids and indicators,* under the aegis of its project leader, Prof. A. Andrén (SE).

This first edition of ISO 15163 | IDF 110 cancels and replaces IDF 110B:1997, which has been technically revised.

Introduction

Calf rennet and adult bovine rennet preparations contain both chymosin and bovine pepsin in various amounts as main clotting enzymes. The proportion of chymosin decreases relative to pepsin in the abomasum (the fourth “true” stomach) with age and at weaning of the calf.

The ratio of abomasa from young cattle to that of old cattle in the raw material for rennet production thus highly influences the composition of chymosin and pepsin in the final rennet. The higher the abomasa from young milk-fed calves, the higher the proportion of chymosin and vice versa[5][6].

Both chymosin and pepsin have special characteristics relevant to milk-clotting activity and suitability for cheese making. The milk-clotting activity of pepsin is, for example, much more pH-dependent than chymosin and pepsin also has a more general proteolytic activity than chymosin.

Therefore, it is very important to analyse the content of chymosin and pepsin in addition to the strength (total milk-clotting activity) of the rennet[6][7].

INTERNATIONAL STANDARD

ISO 15163:2012(E)
IDF 110:2012(E)

Milk and milk products — Calf rennet and adult bovine rennet — Determination by chromatography of chymosin and bovine pepsin contents

1 Scope

This International Standard specifies a reference method for the determination of the amounts of chymosin and bovine pepsin present in a test sample of calf rennet and adult bovine rennet. In addition, it can be used for mixtures of calf/bovine rennet with fermentation-produced bovine chymosin (FPC).

2 Normative references

The following referenced document is 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 11815 | IDF 157:2007, *Milk — Determination of total milk-clotting activity of bovine rennets*

3 Principle

As a first step, the rennet sample is desalted and the enzymes chymosin and bovine pepsin separated on an anion exchange column[8][9]. In a second step, the milk-clotting activity of each of the separated two enzymes is determined by ISO 11815 | IDF 157 (reconstituted milk with pH 6,5). The enzymatic composition of the rennet sample is expressed in percentage chymosin activity and percentage pepsin activity of the sum of the activities in International Milk-Clotting Units (IMCU) of both components, or the results are expressed in milligrams per litre of active chymosin and milligrams per litre of active pepsin.

The total milk-clotting activity of the first batch of calf rennet reference standard powder and the first batch of adult bovine rennet reference standard powder has once and for all been set at 1 000 IMCU/g. Future preparations of reference standards shall be set relative to the previous reference standards (see ISO 11815 | IDF 157).

This International Standard specifies both a manual set-up of the anion exchange chromatography and an alternative automated set-up.

This is a reference method and changes may therefore only be made if confirmed to give the same result, and repeatability and reproducibility at least as high as the original standard method. Any change to what is stated in this International Standard method shall also be mentioned in the test report (see Clause 10).

4 Reagents

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

4.1 Resin, Fractogel® EMD DEAE (M) (Merck cat. no. 1.16883)[1] or Mono Q® 1 ml prepacked column (HR 5/5 or 5/50 GL from GE Healthcare)[2] or equivalent resin.

NOTE 1 Fractogel® EMD DEAE (M) is a suitable resin for manual chromatography and Mono Q® is suitable for the automated chromatography.

NOTE 2 If the Fractogel® or Mono Q resins are substituted by another resin, it will most likely be necessary to change the buffers in 4.12, resulting in a need to re-evaluate the method.

4.2 Piperazine hexahydrate ($C_4H_{10}N_2 \cdot 6H_2O$).

4.3 Sodium chloride (NaCl).

4.4 Thymol, optional preservative.

4.5 Sodium hydroxide (NaOH).

4.6 Hydrochloric acid solution, $c(HCl) = 1$ mol/l.

4.7 Ethanol (C_2H_5OH), with a volume fraction of at least 96 %.

4.8 Ethanol (C_2H_5OH), with an approximate volume fraction of at least 20 %.

Add 105 ml ethanol 96 % (4.7) to 400 ml water and mix. If a sterile filtration is desired, filter the water before mixing it with the ethanol.

4.9 Urea, $c(N_2H_4CO) = 8$ mol/l.

Dissolve 48 g urea in water and fill to a total volume of 100 ml.

4.10 Dialysis tubing, of diameter approximately 1 cm (Union Carbide)[3] or equivalent (optional).

NOTE The quality of the dialysis tubing is not critical.

4.11 Desalting columns, Bio-Rad – Econopac 10DG (cat. no. 732-2010)[4] or equivalent (optional).

Use either the dialysis tubing (4.10) or the desalting columns for desalting the rennet.

4.12 Buffer solutions

4.12.1 Buffer solution I, piperazine $[(CH_2)_4 (NH)_2]$, $c[(CH_2)_4 (NH)_2] = 0{,}025$ mol/l.

1) Fractogel® EMD DEAE (M) is an example of a suitable product available commercially. This information is given for the convenience of the users of this document and does not constitute an endorsement of the product by ISO or IDF.

2) Mono Q® 1 ml prepacked column is an example of a suitable product available commercially. This information is given for the convenience of the users of this document and does not constitute an endorsement of the product by ISO or IDF.

3) Union Carbide 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 of the product by ISO and IDF.

4) Bio–RAD - Econopac 10DG 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 of the product by ISO or IDF.

Weigh 4,85 g of piperazine (4.2) and 42,8 g of hydrochloric acid solution (4.6) in a beaker and mix. Transfer quantitatively the contents of beaker to a 1 000 ml one-mark volumetric flask (5.5), dilute with water to the 1 000 ml mark, and mix. The pH shall be 5,30 ± 0,05. If not, adjust with piperazine or hydrochloric acid. Before use, degas and preserve the buffer solution as described in 4.12.5.

4.12.2 Buffer solution II, c(NaCl) = 0,25 mol/l.

Weigh 14,6 g of NaCl into a 1 000 ml one-mark volumetric flask (5.5). Add the buffer solution I (4.12.1) to the 1 000 ml mark and mix. Do not adjust the pH. Buffer solution II is used only for the manual method. Before use, degas and preserve the buffer solution as described in 4.12.5.

4.12.3 Buffer solution III, c(NaCl) = 0,50 mol/l.

Weigh 29,2 g of NaCl into a 1 000 ml one-mark volumetric flask (5.5). Add buffer solution I (4.12.1) to the 1 000 ml mark and mix. Do not adjust the pH. Buffer solution III is used only for the manual version. Before use, degas and preserve the buffer solution as described in 4.12.5.

4.12.4 Buffer solution IV, c(NaCl) = 1,0 mol/l.

Weigh 58,4 g of NaCl into a 1 000 ml one-mark volumetric flask (5.5). Add buffer solution I (4.12.1) to the 1 000 ml mark and mix. Do not adjust the pH. Before use, degas and preserve the buffer solution as described in 4.12.5.

4.12.5 Degassing and preservation

Before use, degas the buffer solutions I to IV (4.12.1 to 4.12.4) under vacuum or by use of an ultrasound water bath. Preserve buffer solutions I to IV for use in the manual method by adding a few thymol crystals and in the automated method by sterile filtering using a filter of 0,2 µm.

Buffer solutions I to IV can be kept for at least 5 days at room temperature or for 2 months in a refrigerator.

5 Apparatus

Usual laboratory apparatus and, in particular, the following.

5.1 Multiway peristaltic pump or **other suitable pump** (for manual set-up only).

5.2 pH-meter, of sensitivity ±0,01 pH unit.

5.3 Chromatographic column, of diameter ~1,0 cm and length 10 cm, with one flow adaptor or equivalent column suitable for a gel bed height of ~5 cm (for manual set-up only).

5.4 Magnetic stirrer.

5.5 One-mark volumetric flasks, of the capacities required, ISO 1042[2].

5.6 FPLC®[5], **ÄKTA®**[6] or **HPLC equipment**, suitable for the purpose, used for the automatic set-up only.

5.7 Laboratory equipment, for the determination of the clotting time (see ISO 11815 | IDF 157).

5) FPLC® 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 of the product by ISO or IDF.

6) ÄKTA® 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 of the product by ISO or IDF.

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[1].

NOTE 1 Sampling of liquid rennet is given in ISO 707 | IDF 50:2008, Clause 9, and of powdered rennet in ISO 707 | IDF 50:2008, Clause 13.

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

NOTE 2 Powdered products can separate rapidly.

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

7 Procedure

7.1 Check

Prior to determination, check the rennet for absence of main milk-clotting enzymes of non-bovine origin by using a suitable method (see Annex A). However, checking can be skipped if the rennet is known to contain chymosin and pepsin of bovine origin only.

7.2 Preparation of a fresh column with Fractogel

After degassing under vacuum, pour a suspension of the ready-to-use Fractogel resin (4.1) directly from the supplier's bottle, or by use of an ultrasound water bath, into the column (5.3), fixed in a vertical position, with the outlet open, until the layer of settled Fractogel resin is 4,5 cm to 5,5 cm high. The gel bed shall not run dry during the whole operation.

Close the outlet tube. Immerse the end of the intake tube of the peristaltic pump into a beaker containing buffer solution I (4.12.1). Connect the tube of the adaptor to the exit tube of the pump. Adjust the flow rate to $(1{,}3 \pm 0{,}1)$ ml/min. Fill the tube of the adaptor with buffer solution I (4.12.1), not exceeding a total internal tubing volume of 1,5 ml.

Close the column with the adaptor, as described by the supplier of the column. Compress the gel bed a few millimetres with the adaptor in order to avoid free space above the gel bed. Avoid any air bubble entering the column. Rinse the column with buffer solution I (4.12.1) for 5 min at a flow rate of 1,3 ml/min.

7.3 Regeneration and equilibration of the Fractogel resin in the column

After the preparation of a new column and after each run, regenerate and equilibrate the Fractogel resin (4.1) in the column by the following procedure.

Regenerate the Fractogel resin in the column at a flow rate of 1,3 ml/min using at least 15 ml of buffer solution IV (4.12.4) (~11,5 min). Equilibrate the column with at least 40 ml of buffer solution I (4.12.1) (~30 min). The column is now ready for loading the test sample.

The same column can be used over 20 times. Regenerate the column more extensively after frequent use by rinsing with 0,1 mol/l NaOH for 10 min followed by water for 10 min. Then follow the normal regeneration and equilibration procedure as described in the preceding.

7.4 Storage of the column with Fractogel

If the column has to be stored for more than 1 week, rinse it with at least 15 ml of 20 % ethanol (4.8) with the help of the peristaltic pump (5.1). Store the column with the inlet and the outlet tubes tightly closed.

The column can be stored for several months at room temperature avoiding direct sunlight.

7.5 Preparation of test sample

7.5.1 General

Liquid samples may be used as they are; just proceed to 7.5.2.

A powder rennet sample is prepared as follows. Dissolve a powder rennet sample so as to give, for example, 200 IMCU/ml in a suitable amount of buffer solution I (4.12.1) or by using a buffer solution with pH 5,5 (see ISO 11815|IDF 157) before desalting.

Determine the clotting time of the dissolved rennet sample in accordance with ISO 11815|IDF 157 before desalting. Make a rough estimate of the strength of the sample, in IMCU/ml, by measuring it relative to the calf rennet reference standard in order to determine the amount of sample to apply on to the column (7.6.1 or 7.6.2).

If the results are expressed in milligrams per litre, determine the clotting time of the rennet sample at least twice in accordance with ISO 11815|IDF 157. In this case, measure the clotting times simultaneously or in rapid succession “before” and "after" desalting.

Before applying the test sample on to the column, desalt it by dialysis (7.5.2) or by gel filtration (7.5.3).

7.5.2 Desalting by dialysis

Immerse the dialysis tubing (4.10) in boiling water for about 5 min and rinse the inside and the outside of the tubing with water.

Dialyse 5 ml of the prepared test sample (7.5) under examination (~900 IMCU) against 500 ml of buffer solution I (4.12.1) at 4 °C for at least 5 h but no longer than 20 h. Compress the dialysis tubing very firmly by hand on closing it in order to reduce the dilution, which occurs during dialysis. Stir the buffer with a magnetic stirrer (5.4) during the dialysis.

If the results are expressed in milligrams per litre, determine the clotting time of the dialysed rennet sample at least twice in accordance with ISO 11815|IDF 157.

7.5.3 Desalting by gel filtration

Follow the instructions of the supplier.

Equilibrate the desalting column (4.11) with buffer solution I (4.12.1). Apply 3,3 ml of the prepared test sample (7.5) on to the column. Elute the sample with 4,0 ml of buffer solution I (4.12.1).

For test samples with a low amount of either chymosin or pepsin, it is sometimes necessary to run the aforementioned procedure twice in order to have enough activity to be able to determine the low component adequately.

If the results are expressed in milligrams per litre, determine the clotting time of the gel-filtered rennet sample at least twice in accordance with ISO 11815|IDF 157.

7.5.4 Column lifetime

In order to prolong the lifetime of the columns and to avoid clog-up, the following procedure is recommended for regular cleaning of the desalting columns.

After each day of use, rinse the whole column in clean tap or distilled water. Apply approximately 5 ml of 8 mol/l urea on the column and drain. Rinse the column once in tap water and equilibrate by letting a full reservoir volume of buffer solution I (4.12.1) run through the column.

Store equilibrated columns in a cool place with some buffer above the gel surface. For long storage, it is recommended that the column be stored in buffer or distilled water with preservative added (see supplier's instruction). When following this procedure, each column can be used for up to 2 years or until the flow rate reduces considerably.

7.6 Analysis of the desalted rennet

7.6.1 Separation of chymosin and pepsin by ordinary column — Manual set-up

After equilibration of the column in 7.3, introduce on to the column a known amount of desalted test sample (7.5.2 or 7.5.3) using the pump (5.1), by immersing the end of the intake tube of the pump into the sample test tube. Set the flow rate at 1,3 ml/min.

The amount of test sample should be sufficient to give, after separation, a clotting time for the weakest fraction of 350 s to 550 s whenever possible, but not so much that activity can be detected in the intermediate fraction or in the eluate after the second fraction. A volume of 3 ml up to a maximum of 5 ml of test sample after desalting is sufficient in most cases. However, the total amount of IMCU applied on the column shall not exceed 900 IMCU. If the results are expressed in milligrams per litre, it is necessary to know exactly the amount added to the column. This is not necessary if the results are expressed as percentages only.

When the sample has entered the intake tube almost completely, wash the test tube with 1 ml of buffer solution II (4.12.2). Avoid any air bubbles entering the tube. Repeat the washing procedure when the first wash has entered the intake tube.

Immerse the end of the intake tube into a beaker containing at least 50 ml of buffer solution II (4.12.2). Collect the first elution fraction in a 50 ml one-mark volumetric flask (5.5). Collect either exactly to the 50 ml mark or, for convenience, to a volume of about 47 ml. In the latter case, make up to the 50 ml mark with buffer solution II (4.12.2).

Then collect an amount of 3 ml in a small beaker, which is designated as the intermediate fraction.

Subsequently, immerse the end of the intake tube of the peristaltic pump (5.1) into a beaker containing at least 50 ml buffer solution III (4.12.3). Collect the second elution fraction in another 50 ml one-mark volumetric flask (5.5). Collect either exactly to the 50 ml mark or, for convenience, to a volume of about 47 ml. In the latter case, make up to the 50 ml mark with buffer solution III (4.12.3).

Then collect again an amount of 3 ml in a small beaker, which is designated as the after-fraction.

NOTE The first elution fraction contains the chymosin. The intermediate fraction is used to check that the separation of the two enzymes has been correctly achieved. The second elution fraction contains the bovine pepsin. The after-fraction is used to check whether all pepsin has been eluted.

If the sample is known to contain a very low amount of chymosin or pepsin, elution fractions 1 and 2 can be collected in smaller volumetric flasks, for example of 25 ml.

7.6.2 Separation of chymosin and pepsin by FPLC®/ÄKTA®/HPLC — Automatic set-up

7.6.2.1 Follow the general instructions for the equipment (5.6) and column Mono Q (4.1) using buffer solutions I (4.12.1) and IV (4.12.4) only.

Draw up a programme for the analysis. Check the correctness of the separation and whether the results obtained are in line with the manual reference method. In the automatic set-up, the chromatography is scaled down by a factor of 5, meaning 10 ml fractions are collected instead of the 50 ml fractions collected in the manual method. Further, it is not necessary to collect the intermediate fraction, because the chromatogram shows whether the chymosin and pepsin are fully separated into fractions with one enzyme in each.

If the column has not been used during the last 24 h, perform a blind run using buffer I (4.12.1) as test sample.

7.6.2.2 The following guidelines for a programme are suitable for the application of 0,5 ml or 1,0 ml of test sample.

a) Start (at 0,0 ml buffer) with 100 % of buffer I (4.12.1) using a flow rate of 2 ml/min and record at 280 nm. Inject the sample, which was pre-injected into a loop of 0,500 ml or 1,000 ml, on to the column.

b) After 0,60 ml of buffer I (4.12.1) has been eluted, start collecting fraction 1 (chymosin fraction).

c) After 1,50 ml of buffer I has been eluted, change buffer I to a buffer mixture of 80 % of buffer I (4.12.1) and 20 % of buffer IV (4.12.4) to elute the chymosin fraction.

d) After a total of 10,60 ml of buffer I and the buffer IV mixture have been eluted, stop collecting fraction 1 (volume is 10 ml) and change the mixture of buffers I and IV to 50 % of buffer I (4.12.1) and 50 % of buffer IV (4.12.4) to elute fraction 2 (pepsin fraction) while starting to collect it.

e) After a total of 20,60 ml of buffer mixture has been eluted, stop collecting fraction 2 (volume is 10 ml) and change the mixture of 50 % each of buffers I and IV to 100 % of the buffer IV (4.12.4) to wash the column.

f) After a total of 25,60 ml of buffer mixture has been eluted, change buffer IV to 100 % of buffer I (4.12.1) to equilibrate the column.

g) After a total of 32,60 ml of buffer has been eluted, end the programme.

7.6.2.3 Chromatography of a test sample is carried out as follows.

Pre-inject the desalted rennet into a loop of 0,500 ml or 1,000 ml, applying as much IMCU as possible, but not exceeding 180 IMCU in total. Start the chromatography and collect the two fractions. Determine the clotting time of the first and second fraction in accordance with ISO 11815 | IDF 157.

If the sample is known to contain a very low amount of either chymosin (adult bovine rennet) or pepsin (calf rennet), the fraction size can be changed to 5 ml, since a small amount of one of the enzymes is eluted in the first 5 ml fraction, which can be checked on the chromatogram. If all of an enzyme is eluted in the first 5 ml fraction, then the activity is measured in that fraction only and the calculation adjusted accordingly by dividing the IMCU/ml in question by two. Check whether the other 5 ml fraction has a clotting time higher than 1 800 s.

In general, it is recommended that a sample of known composition be included at regular intervals, in particular, when using the automated method.

Adjust the programme if the separation of chymosin and pepsin fraction is not complete.

7.7 Determination of the clotting times

Mix the contents of each fraction obtained in either 7.6.1 or 7.6.2 thoroughly before taking a test portion of each. Make the required dilutions with buffer solution II (4.12.2) for the first fraction and with buffer solution III (4.12.3) for the second fraction.

Determine the clotting times of the first and second elution fraction in accordance with ISO 11815 | IDF 157 while considering the following.

Determine the first fraction together with the calf rennet reference working solution and the second fraction together with the adult bovine rennet reference working solution. Determine the clotting times twice in rapid succession for each pair of “fraction-reference working solutions” using the average time for the calculations.

Furthermore, for the milk-clotting assay in ISO 11815|IDF 157, up to five times more volume of sample may be added to the milk, when needed, in order to obtain a clotting time within the range 350 s to 550 s.

However, under these circumstances, dilute the corresponding rennet reference working solution in the same buffer as used to elute the particular fraction, in order to maintain the same coagulation conditions for the sample and the rennet reference working solution. That means that if three times more volume of the test sample is needed (1,5 ml instead of 0,5 ml), the rennet reference working solution should also be diluted three times more (1,5 ml instead of 0,5 ml) for the clotting test (see ISO 11815|IDF 157:2007, 9.5.1).

In sample fractions with a very low level of either chymosin or pepsin, the use of clotting times longer than 550 s are allowed after having added five times more volume of sample to the test.

Determine the clotting time of the intermediate and final fractions by adding five times the normal volume (2,5 ml per 25 ml milk). These clotting times should exceed 1 800 s because a lower clotting time indicates that the separation was unsatisfactory.

Perform all analysis as soon as possible after the separation of chymosin and pepsin due to possible denaturation of the enzymes in the diluted solutions.

8 Calculation and expression of results

8.1 Calculation of the activity of chymosin and pepsin, expressed as a percentage

The chymosin activity and the bovine pepsin activity, expressed in IMCU per millilitre, as described in ISO 11815|IDF 157, can be converted into the percentage of chymosin, a_c, and the percentage of bovine pepsin, a_p, using Equations (1) and (2), respectively:

$$a_c = \frac{n_c \times 100}{n_c + n_p} \quad (1)$$

$$a_p = 100 - a_c \quad (2)$$

where

n_c is the chymosin content, expressed in IMCU per millilitre;

n_p is the pepsin content, expressed in IMCU per millilitre.

8.2 Calculation of active chymosin and active bovine pepsin, in milligrams per litre

Express the concentration of the active chymosin and bovine pepsin in milligrams per litre if a standardized milk powder is used for the preparation of the substrate. The coefficients K_c, K_p, f_c and f_p for the standardized milk powder are provided by the supplier (Cecalait)[7)]. Determine the concentration as given in ISO 11815|IDF 157 using Equation (3):

$$\rho_x = \frac{K_x}{t_x - f_x} \frac{V}{V_p} d \frac{t_2}{t_1} \tag{3}$$

where

ρ_x is the concentration, expressed in milligrams per litre, of either chymosin, ρ_c, or bovine pepsin, ρ_p, in the enzyme preparation;

K_x is either the K_c or the K_p coefficient used to calculate the concentration, in milligrams per litre, of active chymosin and bovine pepsin, respectively;

t_x is the clotting time, in seconds, obtained with 0,5 ml of fraction 1, t_c, or 2, t_p, diluted or concentrated F times (cf. 7.7 and below);

f_x is either the f_c or the f_p correction factor, expressed in seconds, for the lag time of the coagulation;

V_p is the volume, expressed in millilitres, of the desalted preparation used for analysis (7.6);

V is the volume, expressed in millilitres, of the collected fraction ($V = 50$ ml);

d is the dilution or concentration factor of the analysed fraction (for example $d = 3$ when 1 ml of the fraction has been diluted with 2 ml of buffer before the clotting test and $d = 0{,}25$ when 2 ml instead of 0,5 ml have been used for 25 ml of milk in the clotting test);

t_1 is the clotting time, expressed in seconds, obtained with the diluted enzyme preparation (7.5);

t_2 is the clotting time, expressed in seconds, obtained with the desalted enzyme preparation at the same dilution (7.5.2 or 7.5.3).

For a given milk powder and a given enzyme, obtain f by plotting clotting times versus $1/\rho$ (see Example). According to Holter's equation, $t = f + b/c$, where f is the extrapolated value of t for $b/c = 0$. The supplier of standardized milk powder also gives the factor f.

EXAMPLE

To determine the constant f for the chymosin fraction, prepare 100 ml of concentrated pure chymosin solution in the buffer solution II (4.12.2). This preparation can be obtained from one or several chromatographic separations of calf rennet (first fraction). Then, prepare at least six different dilutions of this preparation with the buffer solution II (4.12.2).

Determine the clotting times of the different dilutions in accordance with the method in ISO 11815|IDF 157. The clotting times should be evenly distributed between 240 s and 1 200 s.

The value of ρ for the lowest dilution (clotting time close to 300 s) is set to 1,00 and the values for the other dilutions are set in relation to that, for example 0,50 (1/2), 0,33 (1/3), 0,25 (1/4), etc. Plot $t = d\ (1/\rho)$ and calculate the correlation coefficient, r. If r is equal to or higher than 0,999, it is possible to determine the constant f; if r is below 0,999, make new dilutions and repeat the measurements of the clotting times.

7) Cecalait, rue de Versailles, B.P. 70129, 39802 POLIGNY, FRANCE (e-mail: secretariat@cecalait.fr). This information is given for the convenience of users of this document and does not constitute an endorsement by ISO or IDF of the supplier named.

To determine the constant f for the bovine pepsin fraction, prepare 100 ml of concentrated pure bovine pepsin solution in the buffer solution III (4.12.3). This preparation can be obtained from one or several chromatographic separations of adult bovine rennet (second fraction). Then, prepare at least six different dilutions of this preparation with the buffer solution III (4.12.3).

Determine the clotting times within the same time limits as described for the chymosin fraction and calculate r. If r is equal to or higher than 0,999, it is possible to determine the constant f.

8.3 Expression of results

Express the test result to whole significant figures.

9 Precision

9.1 Interlaboratory test

The values for the precision parameters are derived from the results of an interlaboratory study on liquid rennets carried out in 1995 according to ISO 5725[8]. The results of the interlaboratory test were statistically reanalysed in March 2009 according to ISO 5725-1[3] and ISO 5725-2[4]. The values obtained might not be applicable to concentration ranges and matrices other than those given.

The study results are shown in Annex B.

NOTE 1 The values for repeatability and reproducibility are derived from the standard deviations, s_D, 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 can be expected in 95 % of the cases when two results are compared. If, in the long run, significantly less than 95 % of cases are within the values given below (9.2 and 9.3), then it is recommended that the execution of analysis be improved.

NOTE 2 Due to some difference in solubility and a certain degree of inhomogeneity of rennet powders, the percentage values for the precision parameters, repeatability and reproducibility mentioned in the following can be somewhat higher when analysing rennet powders.

9.2 Repeatability

The standard deviation, s_r, and the coefficient of variation of repeatability, $C_{V,r}$, both expressing the variability of independent analytical results obtained by the same operator, using the same apparatus under the same conditions on the same test sample and in a short interval of time, will in not more than 5 % of cases for liquid rennets be greater than a) or b):

a) 0,7 % s_r in absolute value when expressing the chymosin and pepsin activity as a percentage;

b) 3,6 % $C_{V,r}$ relative to the arithmetic mean of the chymosin content, expressed in milligrams per litre, and 8,4 % $C_{V,r}$ relative to the arithmetic mean of the pepsin content, expressed in milligrams per litre.

If two determinations are obtained under these conditions, the absolute difference, r, between the two results for liquid rennets should not exceed 2,0 % in absolute value of the percentage of the chymosin and pepsin activity.

8) ISO 5725:1986, now withdrawn and revised (in parts) by ISO 5725-1[3] and ISO 5725-2[4].

9.3 Reproducibility

The standard deviation, s_R, and the coefficient of variation of reproducibility, $C_{V,R}$, both expressing the variability of independent analytical results by operators in different laboratories, using different apparatus under different conditions for the analysis on the same test sample, will in not more than 5 % of cases for liquid rennets be greater than a) or b):

a) 1,2 % s_R in absolute value when expressing the activity of chymosin and pepsin as a percentage;

b) 6,9 % $C_{V,R}$ relative to the arithmetic mean of the chymosin content, expressed in milligrams per litre, and 12,0 % $C_{V,R}$ relative to the arithmetic mean of the pepsin content, expressed in milligrams per litre.

If two determinations are obtained under these conditions, the absolute difference, R, between the two results, for liquid rennets should not exceed 3,3 % in absolute value of the percentage of the chymosin and pepsin activity.

NOTE 1 If, for example, chymosin is determined to 80 % by one laboratory, the result of the other laboratory should not deviate more than 3,3 % in absolute from 80 %, i.e. the results should be between 76,7 % and 83,3 %.

NOTE 2 The values for precision parameters are valid when considering a broad range of laboratories. Experience has shown that highly trained laboratories are able to perform the analysis with reproducibility, R, between laboratories of 1,9 % absolute, when expressed as a percentage of chymosin activity.

10 Test report

The test report shall contain at least the following information:

a) all information necessary for the complete identification of the sample, including phase details (liquid or powder;

b) the sampling method used, if known;

c) the test method used with reference to this International Standard (ISO 15163 | IDF 110:2012);

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;

f) if the repeatability has been checked, the final quoted result obtained.

Annex A
(informative)

Qualitative determination by double immunodiffusion of milk-coagulating enzymes in commercial coagulants

A.1 General

The purpose of this method is to determine the possible occurrence of one or several of the following six coagulating enzymes: chymosin, bovine pepsin, pig pepsin, enzymes from *Rhizomucor (Mucor) miehei, Rhizomucor (Mucor) pusillus* and *Cryphonectria (Endothia) parasitica* in commercial rennets.

NOTE The method in this annex is complementary to the method for the determination of chymosin and bovine pepsin contents in calf rennet and adult bovine rennet, to the extent that this method makes it possible to ascertain only whether chymosin and bovine pepsin are present in such extracts or whether any of the most common milk-clotting enzymes (besides chymosin and bovine pepsin) are also present. In the latter case, the chromatography method is not applicable. Not all milk-clotting enzymes are identified by this method, but identification of the presence of chymosin and bovine pepsin, together with the absence of the most common rennet substitutes, gives a high certainty that the rennet being analysed is of pure bovine origin.

The method is applicable to: a) all coagulating enzymes obtained from bovine and porcine stomachs; and b) coagulating enzymes currently produced commercially from *Rhizomucor (Mucor) miehei, Rhizomucor (Mucor) pusillus* and *Cryphonectria (Endothia) parasitica*.

A.2 Principle

In an agarose medium, specific antigen-antibody precipitations provide visual evidence of the enzymes mentioned above in a rennet preparation[10]. The first step is to pour a layer of agarose on a plate. When the agarose has solidified, small round wells are bored into the agarose layer; some are filled with antigen at different concentrations and others with antiserum. Each of the antagonists migrates towards the others (double immunodiffusion) and precipitation occurs as a line when the relative concentrations of antigen and antiserum are optimal.

A.3 Apparatus

Usual laboratory equipment and, in particular, the following.

A.3.1 Glass plates (10 cm × 10 cm) or **agarose-coated polyester film**, “Gelbond”, available from FMC Corporation, Bio Products, Rockland, Maine 04841, USA[9)].

A.3.2 Micropipettes, of capacities 4 µl and 15 µl.

A.3.3 Hollow punches, of diameters 2,5 mm and 4,0 mm.

A.3.4 Plastic trays, for staining and rinsing the plates.

9) “Gelbond” 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 of the product by ISO or IDF.

A.4 Reagents

Use only reagents of recognized analytical grade and distilled or demineralized water or water of equivalent purity.

NOTE Any mention of, or information on, proprietary items in this International Standard is given for the convenience of the user of this International Standard and does not constitute an endorsement of such items by ISO or IDF.

A.4.1 Agarose, Indubiose A37 (IBF), type HSA (Litex)[10] or equivalent.

NOTE The choice of agarose is not critical.

A.4.2 Sodium chloride (NaCl).

A.4.3 Ethanol, 95 %.

A.4.4 Acetic acid, 100 % (glacial).

A.4.5 Monospecific antibodies and reference enzyme solutions.

Monospecific antisera and reference enzyme solutions with a leaflet giving the necessary instructions for use can be obtained from INRA (FR) or CHR. HANSEN (DK)[11].

A.4.6 Coomassie brillant blue, dye R-250 or Serva Blue R[12].

A.5 Procedure

A.5.1 Preparation of agarose plates

A.5.1.1 Sodium chloride, mass concentration 9 g/l.

Dissolve 9 g of sodium chloride in 1 l of distilled water.

A.5.1.2 Agarose solution

Add 1 g of agarose (A.4.1) to 100 ml of 0,9 % sodium chloride (A.5.1.1) and dissolve in a water bath set at 100 °C. Prepare the agarose gel fresh before use.

A.5.1.3 Preparing agarose plates

After cleaning the glass plates with ethanol, apply a layer of agarose with a brush. Once the film of agarose has dried completely, place the plate horizontally, deposit a layer of agarose solution (A.5.1.2) at 60 °C on the plate with a pipette and let it solidify. The layer should have a thickness of 1,5 mm, e.g. for a 10 cm × 10 cm plate, 15 ml of agarose solution are required. Using agarose-coated polyester film, pour 15 ml of agarose solution on to the hydrophilic surface and allow it to solidify.

10) Indubiose A37 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 of this product by ISO or IDF.

11) INRA, place du Champ de Foire, 39800 POLIGNY, France and Chr. Hansen A/S, 1-27 Jernholmen, 2650 HVIDOVRE, Denmark (fax +45 36 86 77 76) are the organizations that supply these reference products.

12) Dye R-250 or Serva Blue R are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement of these products by ISO or IDF.

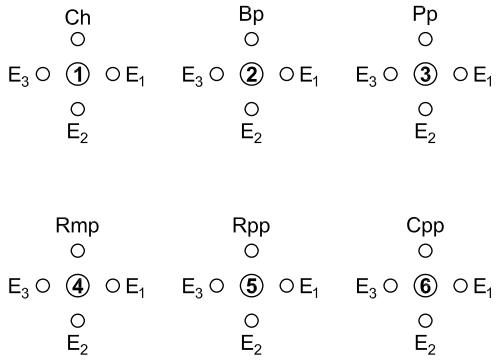
A.5.2 Diffusion

A.5.2.1 Preparation of samples

Samples can either be tested undiluted if their strength does not exceed 200 IMCU/ml or be tested in different dilutions (e.g. 1 to 20 and 1 to 100) with water. Dilute the reference enzyme solutions according to the manufacturer's instructions supplied with the antigens and antisera.

A.5.2.2 Preparation of the agarose plates

Just before starting diffusion, bore small cylindrical wells into the agarose, by sucking the agarose with a hollow punch (A.3.3). According to the sample volume (see the instructions), the wells should have a diameter of 2,5 mm or 4,0 mm. The centre of the wells containing the sample should be located 5 mm away from the well centre containing the antibody (see Figure A.1).



Key

1	rabbit antiserum to chymosin	4	rabbit antiserum to *Rhizomucor miehei* protease
2	rabbit antiserum to bovine pepsin	5	rabbit antiserum to *Rhizomucor pusillus* protease
3	rabbit antiserum to porcine pepsin	6	rabbit antiserum to *Cryphonectria parasitica* protease
Ch	reference sample of chymosin	Bp	reference sample of bovine pepsin
Pp	reference sample of pig pepsin	Rmp	reference sample of *Rhizomucor miehei* protease
Rpp	reference sample of *Rhizomucor pusillus* protease	Cpp	reference sample of *Cryphonectria parasitica* protease
E_1	sample for analysis (test sample)	E_2	sample for analysis (test sample)
E_3	sample for analysis (test sample)		

NOTE E_1, E_2 and E_3 can be three different rennets or be one rennet in three different dilutions, i.e. undiluted, diluted 20 and 100 times, respectively.

Figure A.1 — Example of set-up of the plate

A.5.2.3 Inserting the samples

Depending on the well diameter, use a micropipette (A.3.2) to pipette 4 µl or 15 µl of sample into the required well. Insert the antiserum in the central well, the reference enzyme in the upper well and the sample for analysis and the two dilutions in the three other wells (see Figure A.1). Thereafter, leave the plates in a water-vapour-saturated atmosphere at room temperature for 10 h to 15 h or follow the instruction from the supplier of antisera and reference enzyme solutions.

A.5.3 Staining the plates (optional)

Staining of the plates is recommended as the precipitate becomes clearer and more precipitates can appear.

A.5.3.1 Preparing the dye

Dissolve 5 g of Coomassie brilliant blue dye (A.4.6) in 1 l of solvent made up from ethanol (A.4.3), acetic acid (A.4.4) and water with volumes of 4,5 + 1,0 + 4,5 respectively. Heat the solution obtained to 60 °C and filter.

A.5.3.2 Washing and staining of plates

Each plate is covered with one layer of filter paper and several layers (for example 0,5 cm) of soft absorbing paper before it is pressed lightly (e.g. by using books) for a minimum of 10 min. Remove the paper. Soak the plate in 9 g/l sodium chloride (A.5.1.1) for >1 h. Repeat the pressing procedure for 10 min. Wash the plate in 9 g/l sodium chloride overnight at room temperature.

Press the plate and soak it in distilled water for 30 min. Press the plate and dry it in hot air.

Fill empty holes of the gels with distilled water in order to prevent the gels from cracking during pressing. Remove the filter paper from the gel after each pressing. The washing procedure may be modified by using a longer washing time and less or no pressing of the gel or by using shorter washing times and more frequent pressing of the gel (see also the instructions from the suppliers).

Soak the plate in the dye (A.5.3.1) for >15 min.

Destain the plate in the ethanol, acetic acid and water solvent with volumes of 4,5 + 1,0 + 4,5 respectively, used for preparation of the dye (A.5.3.1). After destaining, the plate may be rinsed in water and dried in hot air.

A.6 Interpretation of results

The precipitation curves can be noted directly after the incubation as an opaque-white precipitation line, but the precipitate often becomes more visible after staining.

The occurrence of a precipitation line, between the well containing the antibody against enzyme A and the well containing the sample for analysis E, shows the presence of enzyme A in sample E. There should be continuity between the precipitation line for the reference enzyme and the precipitation line for the test sample (see Figure A.2).

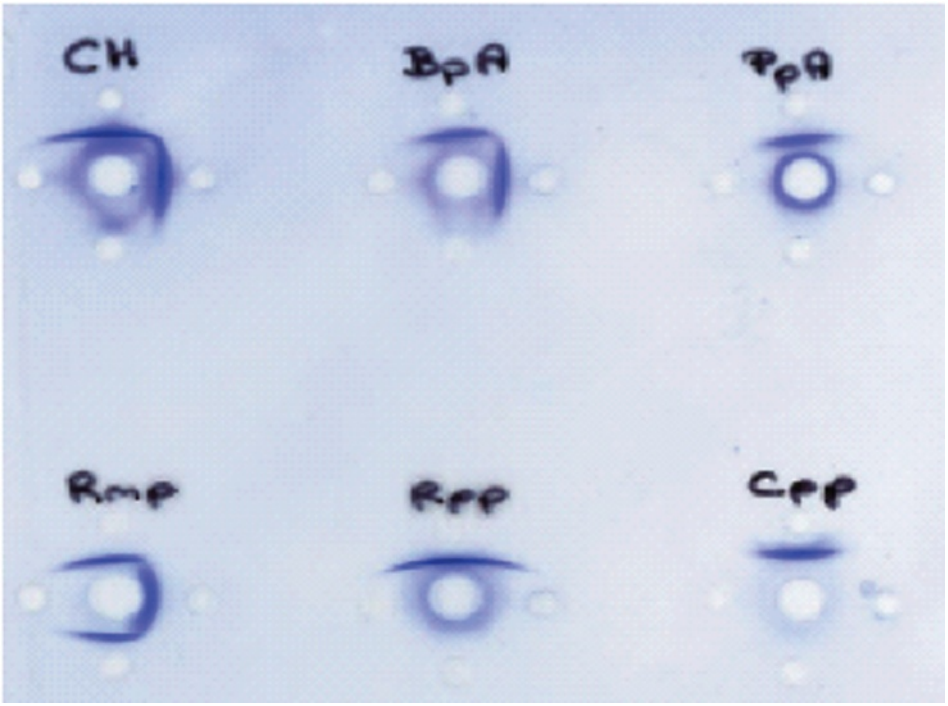


Figure A.2 — Example of results of double immunodiffusion

Interpretation of the plate is as follows.

— The precipitate between each antiserum and the corresponding reference sample (see A.5.2.2) shows that the test functions.

— Sample E_1 contains chymosin (CH), bovine pepsin (BpA) and *Rhizomucor miehei* protease (Rmp).

— Sample E_2 contains *Rhizomucor miehei* protease (Rmp) only.

— None of the samples contain porcine pepsin (PpA).

— No sample E_3 was applied on this plate.

A.7 Sensitivity

The sensitivity of the method varies with the antisera used, but in rennet of 200 IMCU/ml the presence of an enzyme corresponding to less than 5 % of total activity can easily be detected in a mixture. In general, a threshold value of 1 % of total activity can be achieved.

Annex B
(informative)

Interlaboratory test

B.1 General

An international collaborative test on IDF 110 involving 10 laboratories from six countries was carried out on bovine rennets in 1995. The interlaboratory study was organised by A. Andrén (SE) in autumn 1995 with the following participants: C. Repelius (NL), J.-C. Collin (FR), T. Sørhaug (NO), J.A. Jans (NL), M. Rampilli (IT), M. Harboe (DK), A. van Boven (NL), M. Stolz (FR), P. Molinari (IT) and A.-K. Levin (SE). The experimental design and the statistical analysis of the test results were realized according to ISO 5725-1[3] and ISO 5725-2[4].

B.2 Samples and results

The study was carried out using four different batches of liquid bovine rennets, covering high, medium and low activity ratios of chymosin and different levels of chymosin and pepsin content respectively. The four batches were divided into eight blind duplicated samples (1/4, 2/3, 5/7 and 6/8).

The test results were statistically re-analysed and updated in 2010 according to ISO 5725-1[3] and ISO 5725-2[4]. The results are shown in Tables B.1 to B.3. The results defined as outliers (Cochran: too high a difference between duplicates; Grubbs: too high a difference between laboratories) were excluded following statistical analysis. The interlaboratory trial of the rennet samples showed good results for repeatability and reproducibility, especially when expressed as a percentage of chymosin activity (see Table B.1).

Table B.1 — Statistical evaluation of the chymosin activity ratio, expressed as a percentage, from 10 laboratories

Sample	Mean %	s_r	$C_{V,r}$ %	r	r_{rel} %	s_R	$C_{V,R}$ %	R	R_{rel} %	Outliers
1/4	90,40	0,69	0,76	1,93	2,13	0,73	0,81	2,05	2,27	0
2/3	54,36	0,69	1,28	1,94	3,57	1,34	2,46	3,75	6,89	0
5/7	20,33	0,28	1,40	0,79	3,91	1,16	5,69	3,24	15,92	1 Cochran
6/8	72,55	0,94	1,29	2,62	3,61	1,41	1,94	3,94	5,43	1 Grubbs
Mean	—	**0,65**	1,18	**1,82**	3.31	**1,16**	2,72	**3,24**	7,63	—

Table B.2 — Statistical evaluation of the chymosin content, expressed in milligrams per litre, from 10 laboratories

Sample	Mean mg/l	s_r	$C_{V,r}$ %	r	r_{rel} %	s_R	$C_{V,R}$ %	R	R_{rel} %	Outliers
1/4	599,00	24,11	4,02	67,50	11,27	40,93	6,83	114,59	19,13	0
2/3	456,15	12,73	2,79	35,64	7,81	29,47	6,46	82,51	18,09	0
5/7	216,94	7,68	3,54	21,50	9,91	16,92	7,80	47,36	21,83	1 Cochran
6/8	513,78	21,47	4,18	60,10	11,70	32,60	6,35	91,29	17,77	1 Grubbs
Mean	—	16,50	**3,63**	46,19	10,17	29,98	**6,86**	83,94	19,20	—

Table B.3 — Statistical evaluation of the pepsin content, expressed in milligrams per litre, from 10 laboratories

Sample	**Mean** mg/l	s_r	$C_{V,r}$ %	r	r_{rel} %	s_R	$C_{V,R}$ %	R	R_{rel} %	**Outliers**
1/4	193,65	16,22	8,38	45,43	23,46	19,40	10,02	54,33	28,06	0
2/3	1 169,90	43,98	3,76	123,15	10,53	107,72	9,21	301,63	25,78	0
5/7	2 665,90	366,60	13,75	1 026,47	38,50	468,92	17,59	1 312,98	49,25	0
6/8	612,35	47,53	7,76	133,09	21,73	68,49	11,18	191,77	31,32	0
Mean	—	118,58	**8,41**	332,03	23,56	166,13	**12,00**	465,18	33,60	—

Bibliography

[1] ISO 707 | IDF 50:2008, *Milk and milk products — Guidance of sampling*

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

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

[4] 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*

[5] ANDRÉN, A., Rennets and coagulants. In: FUQUAY, J.W., FOX, P.F., MCSWEENEY, P. editors. *Encyclopedia of dairy sciences*, 2nd edition, pp. 574–578. Elsevier, 2011

[6] HARBOE, M., BROE, M.L., QVIST, K.B. The production, action and application of rennet and coagulants. In: LAW, B.A., TAMINE, A.Y., editors. *Technology of cheesemaking*, second edition, pp. 98–129. Oxford: Wiley–Blackwell, 2010

[7] ANDRÉN, A. Milk-clotting activity of various rennets and coagulants: Background and information regarding IDF Standards. In: *The use of enzymes in dairying,* Document No. 332, pp. 9–14. Brussels: International Dairy Federation, 1998

[8] GARNOT, P., THAPON, J.L., MATHIEU, C.M., MAUBOIS, J.L., RIBADEAU-DUMAS, B. Determination of rennin and bovine pepsins in commercial rennets and abomasal juices. *J. Dairy Sci.* 1972, **55**, pp. 1641–1650

[9] COLLIN, J.C., MARTIN, P., GARNOT, P., RIBADEAU-DUMAS, B., MOCQUOT, G. Determination of chymosin and bovine pepsin A in commercial bovine rennets and pepsins. *Milchwissenschaft* 1981, **36**, pp. 32–35

[10] COLLIN, J.C., MUSET DE RETTA, G., MARTIN, P. Immunological identification of milk-clotting enzymes. *J. Dairy Res*. 1982, **49**, pp. 221–230

[11] International Collaborative Study on Calf Rennet and Adult Bovine Rennet — Determination of Chymosin and Bovine Pepsin Contents — *Bull. IDF* (in press)

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