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**Cheese and processed cheese —  
Determination of the nitrogenous  
fractions**

*Fromages et fromages fondus — Détermination des fractions azotées*



Reference numbers  
ISO 27871:2011(E)  
IDF 224:2011(E)



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## Foreword

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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 27871|IDF 224 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF). It is being published jointly by ISO and IDF.

## Foreword

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

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

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ISO 27871|IDF 224 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 ISO-IDF Project Group on *Nitrogen fractions* of the Standing Committee on *Analytical Methods for Composition* under the aegis of its project leader, Mr. P. Trossat (FR).

# Cheese and processed cheese — Determination of the nitrogenous fractions

## 1 Scope

This International Standard specifies a method for determining the nitrogenous fractions in cheeses and processed cheese from cow milk.

## 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 8968-1 | IDF 20-1, *Milk — Determination of nitrogen content — Part 1: Kjeldahl method*

ISO 8968-2 | IDF 20-2, *Milk — Determination of nitrogen content — Part 2: Block-digestion method (Macro method)*

## 3 Abbreviated terms

TN	total nitrogen mass fraction	g(N)/100 g
SN	soluble nitrogen mass fraction	g(N)/100 g
TCA-SN	soluble nitrogen content in trichloroacetic acid	g(N)/100 g
PTA-SN	soluble nitrogen content in phosphotungstic acid	g(N)/100 g

## 4 Principle

The fractions are obtained after separation by precipitation of the insolubles in a medium having a pH of 4,4 (SN) or in 12 % trichloroacetic acid (TCA-SN) or in 5 % phosphotungstic acid (PTA-SN). The nitrogen content in each fraction is determined in the filtrates thus obtained in accordance with the procedures specified in ISO 8968-1 | IDF 20-1 or ISO 8968-2 | IDF 20-2.

## 5 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

**5.1 Water**, ISO 3696<sup>[4]</sup>, grade 3 or equivalent purity.

**5.2 Trisodium citrate dihydrate solution**,  $c(\text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \cdot 2\text{H}_2\text{O}) = 0,5 \text{ mol/l}$ .

Weigh 147,05 g of trisodium citrate dihydrate in a 1 000 ml one-mark volumetric flask (6.8). Shake to dissolve. Make up to the mark with water and mix again.

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

**5.4 Trichloroacetic ( $\text{C}_2\text{HCl}_3\text{O}_2$ ) acid solution (TCA)**, with a mass-volume fraction of 24 % of TCA.

**5.5 Phosphotungstic acid ( $\text{H}_3\text{PW}_{12}\text{O}_{40} \cdot x\text{H}_2\text{O}$ ) solution (PTA)**, with a mass-volume fraction of 25 % of PTA.

**5.6 Sulfuric acid ( $\text{H}_2\text{SO}_4$ ) solution**, with a mass fraction of 25 % of  $\text{H}_2\text{SO}_4$  ( $\rho_{20} \approx 1,84 \text{ g/ml}$ ).

Prepare the sulfuric acid solution by very carefully adding and mixing 260 g of 98 %  $\text{H}_2\text{SO}_4$  into 740 g of water. Use the solution obtained in the nitrogen determination.

## 6 Apparatus

Usual laboratory equipment and in particular the following.

**6.1 Grinding device or grating device**, easy to clean, appropriate for preparing the test sample.

**6.2 Analytical balance**, capable of weighing to 1 mg, with a readability of 0,1 mg.

**6.3 Homogenizer and mixer** [e.g. Ultraturrax type T25<sup>1)</sup>], equipped with a rotor capable forming a 10  $\mu\text{m}$  to 50  $\mu\text{m}$  suspension.

**6.4 Beakers**, capacity 250 ml.

**6.5 Measuring cylinders**, capacities 50 ml, 100 ml, and 200 ml respectively, ISO 4788<sup>[5]</sup>, class A.

**6.6 Water bath**, capable of maintaining a temperature of  $45 \text{ }^\circ\text{C} \pm 5 \text{ }^\circ\text{C}$ .

**6.7 Magnetic stirrers**, equipped with bar magnets (optional).

**6.8 One-mark volumetric flasks**, capacities 100 ml and 200 ml, ISO 1042<sup>[3]</sup>, class A.

**6.9 Graduated pipettes**, capacities 15 ml, 20 ml and 50 ml, ISO 835<sup>[2]</sup>, class A.

**6.10 pH meter**, accurate to 0,01 unit of pH.

**6.11 Glass funnels**.

**6.12 Filters**, of porosity 8  $\mu\text{m}$  [e.g. Whatman 40<sup>2)</sup>].

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1) Ultraturrax type T25 is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO or IDF of this product.

## 7 Sampling

A representative sample should be 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<sup>[1]</sup>.

## 8 Procedure

### 8.1 Preparation of test sample

Prior to analysis, remove the rind, the smear or the mouldy surface layer of the cheese in such a way as to obtain a test sample representative of the cheese as it is usually consumed. Grind or grate the test sample by using an appropriate grinding or grating device (6.1). Mix the ground mass quickly.

If necessary, e.g. for semi-hard and hard cheese, grind the mixture a second time and mix thoroughly. Preferably, cut the hard and semi-hard cheese into cubes of side ~15 mm. Grind or grate the test sample as previously described. Clean the device after preparing each sample. If the test sample cannot be ground or grated, mix it thoroughly by intensive kneading, e.g. with a pestle in a mortar. Avoid moisture loss. Store the prepared test sample in an airtight container until commencing the analysis. Preferably, however, analyse the samples as soon as possible after grinding.

If delay is unavoidable, take all precautions to ensure proper preservation of the test sample.

If refrigerated, bring the test sample to room temperature. Thoroughly mix the sample (to obviate a well-documented transfer of moisture within the cheese that occurs during cooling and warming). Ensure that any condensation of moisture on the inside surface of the container is thoroughly and uniformly re-incorporated into the test sample. Do not examine ground cheese showing unwanted mould growth or signs of deterioration.

### 8.2 Determination of the different fractions

#### 8.2.1 Total nitrogen (TN)

Take a test portion of crushed cheese (8.1), depending on the assumed nitrogen content of the cheese and in order to obtain 0,025 g of nitrogen per digestion tube. Determine the total nitrogen content in accordance with the procedure specified in ISO 8968-1 | IDF 20-1 or ISO 8968-2 | IDF 20-2.

#### 8.2.2 Preparation of the citrate solution of cheese

Weigh, to the nearest 1 mg, approximately 20 g of crushed cheese (8.1) in a 250 ml beaker. Using a measuring cylinder, add 100 ml of citrate solution (5.2) and mix. Place the mixture in the water bath (6.6) maintained at 45 °C for 15 min while shaking regularly (manually or by using a magnetic stirrer). If using a bar magnet (6.7), take care to rinse the latter in order to avoid losses.

Using the homogenizer and mixer (6.3), crush until obtaining a homogeneous suspension. Rinse the rotor of the crusher and homogenizer with water (5.1) while collecting the rinsings in the beaker.

Place the obtained mixture in the water bath (6.6) maintained at 45 °C until completely dissolved for 1 h maximum. If small, undissolved pieces still persist, further homogenize the mixture using the homogenizer (6.3). Then rinse the rotor as previously specified while collecting the rinsings again.

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2) Example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO or IDF of this product. Alternative products may be used if they can be shown to lead to comparable results.

Quantitatively transfer the contents of the beaker into a 200 ml one-mark volumetric flask (6.8). Rinse the beaker while collecting the rinsings in the flask. Mix and let the mixture cool to room temperature. Make up to the mark with water (5.1).

The dissolution of processed cheeses can also be conducted in water (5.1).

### **8.2.3 Preparation of the SN fraction**

Using a graduated pipette (6.9), introduce 50 ml of the citrate solution of cheese obtained in 8.2.2 into a beaker. Add approximately 11 ml of hydrochloric acid solution (5.3). Check the pH and adjust, if necessary, to  $4,4 \pm 0,05$ .

Quantitatively transfer into a 100 ml one-mark volumetric flask (6.8). Make up to the mark with water (5.1) and mix. Filter (6.12) the solution. Using a graduated pipette (6.9), transfer 15 ml of filtrate into the digestion tube. Carry out the digestion and determine the SN content in accordance with the procedure specified in ISO 8968-1 | IDF 20-1 or ISO 8968-2 | IDF 20-2.

### **8.2.4 Preparation of the TCA-SN fraction**

Using a graduated pipette (6.9), introduce 50 ml of the citrate solution of cheese obtained in 8.2.2 into a 100 ml one-mark volumetric flask (6.8). Make up to the mark with 24 % TCA solution (5.4) and mix.

Filter (6.12) the solution. Using a graduated pipette (6.9), transfer 20 ml of filtrate into the digestion tube. Carry out the digestion and determine the TCA-SN content in accordance with the procedure specified in ISO 8968-1 | IDF 20-1 or ISO 8968-2 | IDF 20-2.

### **8.2.5 Preparation of the PTA-SN fraction**

Using a graduated pipette (6.9), introduce 50 ml of the citrate solution of cheese obtained in 8.2.2 into a 100 ml one-mark volumetric flask (6.8). Add 20 ml of the 25 % PTA solution (5.5) and mix. Make up to the mark with 25 % sulfuric acid solution (5.6).

Filter (6.12) the solution. Using a graduated pipette (6.9), transfer 20 ml of filtrate into the digestion tube. Carry out the digestion and determine the PTA-SN content in accordance with the procedure specified in ISO 8968-1 | IDF 20-1 or ISO 8968-2 | IDF 20-2.

**NOTE** The weighing of the test portions of citrate solution and filtrate (for digestion) is allowed in 8.2.3, 8.2.4 and 8.2.5 under the conditions which the final dilutions are realized as a mass fraction (instead of a volume fraction).

## **8.3 Blank test**

Simultaneously with the determination of the test sample, carry out a blank test using the same procedure as specified in 8.2.3 to 8.2.5, but omitting the test portion (citrate solution without cheese).

## **9 Calculation and expression of results**

### **9.1 Calculation**

Calculate the soluble nitrogen of the different nitrogenous fractions of the test sample,  $w_{\text{SN}}$ , expressed in grams of nitrogen per 100 g of test sample, using the following equation:

$$w_{\text{SN}} = \frac{1,400\,7\,(V_{\text{s}} - V_{\text{b}})\,c_{\text{r}}}{f_{\text{w}}}$$



where

$w_{\text{SN}}$  is the soluble nitrogen content, in grams of nitrogen per 100 g of test sample, of the different nitrogenous fractions ( $w_{\text{SN}4,4}$ ,  $w_{\text{TCA-SN}}$ ,  $w_{\text{PTA-SN}}$ ) of the sample;

$V_{\text{S}}$  is the volume, in millilitres, of the hydrochloric acid used in the determination of the sample;

$V_{\text{b}}$  is the volume, in millilitres, of the hydrochloric acid used in the determination for the blank test;

$c_{\text{r}}$  is the numerical value of the concentration, in moles per litre, of the hydrochloric acid standard volumetric solution used;

$f_{\text{w}}$  is the “working factor” taking into account, the quantity of the test portion, the different dilutions and the volume of filtrate for the digestion step ( $f_{\text{w}} = 0,75$  for SN fractions;  $f_{\text{w}} = 1,00$  for TCA-SN and PTA-SN fractions).

## 9.2 Expression of test results

Express the test results to two decimal places.

## 10 Precision

### 10.1 Interlaboratory test

The values for repeatability and reproducibility limits are expressed for the 95 % probability level and may not be applicable to concentration ranges and matrices other than those given.

The values obtained were derived from the results of interlaboratory tests carried out in accordance with ISO 5725-1<sup>[6]</sup> and ISO 5725-2<sup>[7]</sup>.

### 10.2 Repeatability

The absolute difference between two individual single test results, obtained with 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 soluble nitrogen:

At pH 4,4: 0,053 g of nitrogen per 100 g of test sample;

In TCA: 0,039 g of nitrogen per 100 g of test sample;

In PTA: 0,028 g of nitrogen per 100 g of test sample.

### 10.3 Reproducibility

The absolute difference between two individual single test results, obtained with 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 soluble nitrogen:

At pH 4,4: 0,089 g of nitrogen per 100 g of test sample;

In TCA: 0,047 g of nitrogen per 100 g of test sample;

In PTA: 0,091 g of nitrogen per 100 g of test sample.

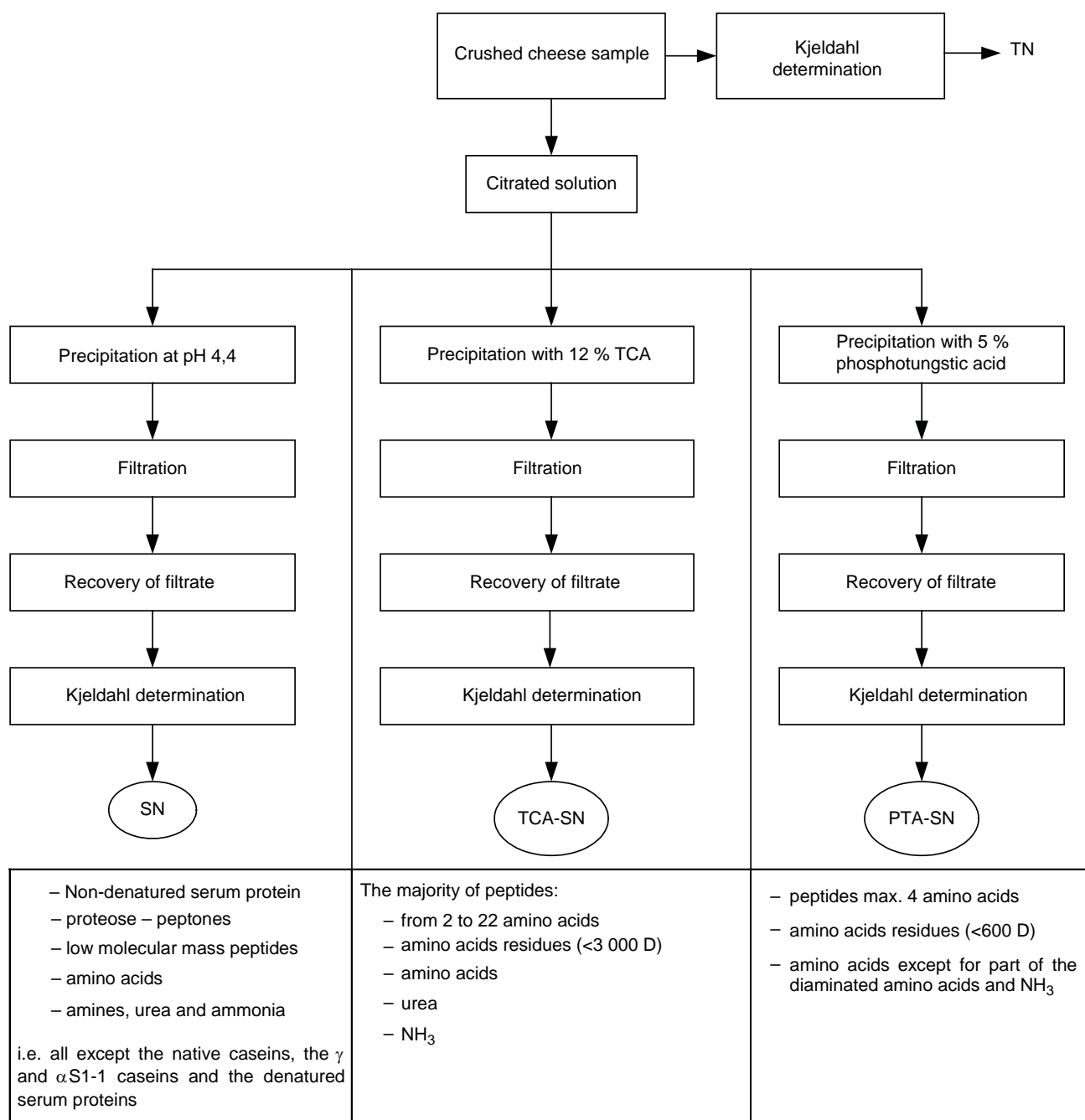
## **11 Test report**

The test report shall contain at least the following information:

- a) all information necessary for the complete identification of the sample;
- b) the sampling method used, if known;
- c) the test method used, with reference to this International Standard (ISO 27871:2011);
- 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 test result(s);
- e) the test result(s) obtained;
- f) if the repeatability has been checked, the final quoted result obtained.

## Annex A (normative)

### Scheme of the method



## Annex B (informative)

### Collaborative trial

#### B.1 General

An interlaboratory collaborative study involving nine laboratories was carried out on six test samples divided into blind duplicates. The obtained results are expressed in grams of nitrogen per 100 g of cheese.

The study was organized with test samples prepared and distributed by Actilait/Cecalait, Poligny (FR), which also performed the statistical analyses in accordance with ISO 5725-1<sup>[6]</sup> and ISO 5725-2<sup>[7]</sup> to give the precision data shown in Tables B.1 to B.3.

**Table B.1 — Result of interlaboratory test for soluble nitrogen at pH 4,4  
(grams of nitrogen per 100 g of test sample)**

Parameter	Sample						Mean
	S1	S2	S3	S4	S5	S6	
No. participating laboratories after eliminating outliers <sup>a</sup>	9	9	9	8	7	8	
Mean value	0,420	0,513	0,183	0,633	1,250	0,696	
Repeatability standard deviation, $s_r$	0,017	0,015	0,008	0,012	0,026	0,029	0,019
Repeatability limit, $r$ (2,8 $s_r$ )	0,049	0,041	0,024	0,033	0,072	0,082	0,053
Reproducibility standard deviation, $s_R$	0,038	0,033	0,018	0,041	0,030	0,029	0,032
Reproducibility limit, $R$ (2,8 $s_R$ )	0,108	0,094	0,050	0,117	0,085	0,082	0,089
Coefficient of variation of repeatability, $C_{V, r}$ (%)	4,117	2,853	4,597	1,856	2,043	4,178	
Coefficient of variation of reproducibility, $C_{V, R}$ (%)	9,102	6,458	9,631	6,510	2,398	4,187	

<sup>a</sup> After Grubbs and Cochran 1 % elimination.

**Table B.2 — Result of interlaboratory test for soluble nitrogen in TCA (grams of nitrogen per 100 g of test sample)**

Parameter	Sample						Mean
	S1	S2	S3	S4	S5	S6	
No. participating laboratories after eliminating outliers <sup>a</sup>	8	7	7	8	8	8	
Mean value	0,172	0,250	0,094	0,385	0,876	0,352	
Repeatability standard deviation, $s_r$	0,018	0,007	0,007	0,024	0,013	0,009	0,014
Repeatability limit, $r$ (2,8 $s_r$ )	0,052	0,020	0,020	0,067	0,036	0,025	0,039
Reproducibility standard deviation, $s_R$	0,018	0,008	0,007	0,024	0,024	0,011	0,017
Reproducibility limit, $R$ (2,8 $s_R$ )	0,052	0,022	0,021	0,069	0,067	0,031	0,047
Coefficient of variation of repeatability, $C_{V, r}$ (%)	10,634	2,873	7,522	6,169	1,472	2,556	
Coefficient of variation of reproducibility, $C_{V, R}$ (%)	10,634	3,086	7,890	6,367	2,691	3,086	

<sup>a</sup> After Grubbs and Cochran 1 % elimination.

**Table B.3 — Result of interlaboratory test for soluble nitrogen in PTA  
(g of nitrogen/100 g of test sample)**

Parameter	Sample						Mean
	1	2	3	4	5	6	
No. participating laboratories after eliminating outliers <sup>a</sup>	7	7	7	6	7	6	
Mean value	0,057	0,074	0,045	0,162	0,420	0,163	
Repeatability standard deviation, $s_r$	0,008	0,009	0,006	0,008	0,017	0,007	0,010
Repeatability limit, $r$ (2,8 $s_r$ )	0,022	0,025	0,017	0,023	0,049	0,019	0,028
Reproducibility standard deviation, $s_R$	0,009	0,011	0,007	0,031	0,069	0,024	0,033
Reproducibility limit, $R$ (2,8 $s_R$ )	0,026	0,031	0,020	0,088	0,196	0,067	0,091
Coefficient of variation of repeatability, $C_{V,r}$ (%)	13,880	11,954	13,046	4,936	4,135	4,141	
Coefficient of variation of reproducibility, $C_{V,R}$ (%)	15,978	15,079	15,853	19,178	16,473	14,557	
<sup>a</sup> After Grubbs and Cochran 1 % elimination.							

## Bibliography

- [1] ISO 707|IDF 50, *Milk and milk products — Guidance on sampling*
- [2] ISO 835, *Laboratory glassware — Graduated pipettes*
- [3] ISO 1042, *Laboratory glassware — One-mark volumetric flasks*
- [4] ISO 3696, *Water for analytical laboratory use — Specification and test methods*
- [5] ISO 4788, *Laboratory glassware — Graduated measuring cylinders*
- [6] ISO 5725-1, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*
- [7] 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.*
- [8] Chemical methods for evaluating proteolysis in cheese maturation — List of criteria for evaluation of accelerated ripening of cheese. *Bull. IDF* 1991, (261), pp. 1-36
- [9] INTERNATIONAL DAIRY FEDERATION. Chemical methods for evaluating proteolysis in cheese maturation (Part 2). *Bull. IDF* 1999, (337), pp. 1-32
- [10] INTERNATIONAL DAIRY FEDERATION. Report of the interlaboratory study of ISO 27871|IDF 224. *Bull. IDF* (in press)



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