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Liquid milk — Determination of acidsoluble β -lactoglobulin content — Reverse-phase HPLC method

Lait liquide — Détermination de la teneur en β -lactoglobuline soluble dans l'acide — Méthode par chromatographie liquide haute performance en phase inverse





Reference numbers ISO 13875:2005(E) IDF 178:2005(E)

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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 13875 IDF 178 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF), in collaboration with AOAC International. It is being published jointly by ISO and IDF and separately by AOAC International.

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 and AOAC International 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 National Committees casting a vote.

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All work was carried out by the Joint ISO/IDF/AOAC Action Team, *Characterization of heat treatment*, of the Standing Committee on *Minor components and characterization of physical properties*, under the aegis of its project leader, Prof. L. Pellegrino (IT).

Liquid milk — Determination of acid-soluble β -lactoglobulin content — Reverse-phase HPLC method

1 Scope

This International Standard specifies a method for the quantitative determination of the β -lactoglobulin content, soluble at pH 4,6, in liquid milk. The method has been tested over a range between 0 mg and 3 500 mg of β -lactoglobulin per litre of milk. It is suitable for distinguishing different categories of heat-treated liquid 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 Terms and definitions

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

3.1 β-lactoglobulin content

 β -LG content

mass fraction of substance determined by the procedure specified in this International Standard

NOTE It is expressed in milligrams per litre of test sample.

4 Principle

Casein and denatured whey protein are precipitated isoelectrically from milk at pH 4,6. The acid whey is separated by centrifuging and filtering. The acid-soluble β -LG content in the acid whey is determined by reverse-phase HPLC. The soluble β -LG content in the test sample is quantified by single-point or a multi-point calibration using a reference sample.

5 Reagents

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

5.1 Standard sample, pure β -lactoglobulin (A+B genetic variants).

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Test the chromatographic purity of the β -LG standard sample by the HPLC procedure described in Clause 10. Determine its precise concentration as described in 10.2.

5.2 Reference sample

The reference sample is reconstituted from a freeze-dried raw bulk milk sample which was originally prepared from a skimmed raw bulk milk sample. It contains a known amount of soluble β -LG (A+B), which is determined by the HPLC procedure described in Clause 10.

The freeze-dried reference sample may be stored at 4 °C for 6 months, preventing hydration.

5.3 Reagents for sample preparation

- **5.3.1** Hydrochloric acid, dilute, c(HCI) = 2 mol/l.
- **5.3.2** Phosphate buffer solution, of pH 6,7 (final concentration 0,1 mol/l).

Add 57 ml of 0,2 mol/l sodium dihydrogen orthophosphate (NaH₂PO₄) solution to a 200 ml volumetric flask (6.10). Add 43 ml of 0,2 mol/l disodium hydrogen orthophosphate (Na₂HPO₄) solution and mix the phosphate solutions. Dilute to the mark with water and mix again.

5.4 HPLC elution solvents

Use elution solvents prepared from reagents of recognized HPLC-grade.

SAFETY PRECAUTIONS — Take appropriate safety precautions when handling the elution solvents as the chemicals may be carcinogenic.

5.4.1 Water, of HPLC-grade.

Laboratory-prepared water may be not sufficiently pure. Impure water produces column contamination and loss of resolution. If impure or improperly stored trifluoracetic acid is used, peaks can be unresolved or absent from the chromatogram.

- **5.4.2** Acetonitrile (CH₃CN).
- **5.4.3** Trifluoracetic acid (CF₃COOH), of the highest purity.

6 Apparatus

Usual laboratory equipment and, in particular, the following.

- **6.1 pH-meter**, calibrated over the pH range 4,0 to 7,0, and accurate to 0,1 pH units.
- 6.2 Centrifuge, capable of operating at 2 000 g.
- 6.3 Centrifuge glass tubes, of capacity about 30 ml.
- 6.4 Glass vials, of capacity about 5 ml.
- 6.5 Glass funnels, of diameter about 7 cm.
- 6.6 Filter paper, fast grade, of diameter about 11 cm.
- 6.7 Glass test tubes, of capacity about 30 ml.
- 6.8 **One-mark pipettes**, capable of delivering 1 ml, 2 ml and 5 ml.

- 6.9 Beakers, of capacities 50 ml and 100 ml.
- 6.10 One-mark volumetric flasks, of capacities 10 ml, 20 ml, 25 ml, 50 ml and 200 ml.
- 6.11 Microfiltration tools.
- 6.11.1 Glass syringe, of capacity 5 ml.
- 6.11.2 Disposable syringe filter units, of pore size 0,22 µm, used with aqueous solutions.
- 6.12 Analytical balance, capable of weighing to the nearest 1 mg, with a readability of 0,1 mg.
- 6.13 Magnetic stirrer.
- 6.14 HPLC equipment.
- 6.14.1 Elution gradient pumping system, capable of operating at 1,0 ml/min at 200 bar.
- 6.14.2 Manual or automatic injector, capable of injecting 20 µl.
- **6.14.3** Column heater, capable of maintaining the column at 40 $^{\circ}C \pm 1^{\circ}C$.

6.14.4 UV detector, capable of operating at 205 nm or at 280 nm wavelength and 0,1 AUFS.

6.14.5 Integrator or data-reprocessing software, capable of measuring peak areas.

6.14.6 PLRP-S column¹⁾, of length 150 mm and internal diameter 4,6 mm, of particle size 5 μ m or 8 μ m, and pore size 30 nm; or an equivalent column packed with underivatized polystyrene divinyl benzene, giving an equivalent chromatographic pattern.

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. A recommended sampling method is given in ISO 707.

8 Procedure

8.1 Preparation of test portion

8.1.1 Check that the reported expiry date of the test sample has not been passed. Bring the closed package of the test sample to 20 °C \pm 2 °C. Just before opening, shake the package and its contents carefully by inversion.

Open the package and transfer about 50 ml of test sample to a 100 ml beaker (6.9).

The test sample package should not be opened until just before starting the preparation.

¹⁾ PLRP-S column is the trade name of a product supplied by Polymer Laboratories Ltd, Church Stretton, United Kingdom.

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. Equivalent products may be used if they can be shown to lead to the same results.

8.1.2 Adjust the pH of the test portion to 4,6 by dropwise addition of the dilute hydrochloric acid (5.3.1) while stirring continuously. Allow the test portion to stand for 20 min at room temperature.

Transfer the prepared test portion to a centrifuge tube (6.3) and centrifuge at 2 000 *g* for 20 min. Filter the supernatant through filter paper (6.6), collecting the casein-free acid whey in a test tube (6.7).

The undiluted acid whey test portion may be stored for 24 h at 4 $^{\circ}$ C or for 2 weeks at –18 $^{\circ}$ C. Once defrosted, the acid whey test portion shall not be refrozen.

8.2 Preparation of test solution

After defrosting, carefully mix the acid whey test portion. Using a pipette (6.8), transfer suitable amounts of the acid whey test portion, depending on the type of test sample, to a 10 ml volumetric flask (6.10):

- a) 1 ml, if from a test sample of raw or pasteurized or high-temperature pasteurized milk (final dilution 1:10);
- b) 2 ml, if prepared from a test sample of UHT milk (final dilution 1:5);
- c) 5 ml, if prepared from a test sample of bottle-sterilized milk (final dilution 1:2).

Dilute the test portion to the mark with the phosphate buffer solution (5.3.2). Mix carefully by inversion and allow to stand for 1 h. Mix again and filter using the microfiltration tools (6.11). Discard the first few millilitres of filtrate. Collect the rest of the filtrate in a glass vial (6.4). The diluted acid whey solution may be stored at 4 $^{\circ}$ C but shall be analysed within 24 h.

8.3 Preparation of reference portion

Weigh, to the nearest 0,01 g, 2,50 g of reference sample (5.2) into a 30 ml beaker (6.9). Add 10 ml of distilled water at 40 °C. Stir using a stirring rod (6.13) in order to dissolve any lumps. Quantitatively transfer the reconstituted reference sample to a 25 ml volumetric flask (6.10). Dilute to the mark with distilled water and mix thoroughly.

Quantitatively transfer the 25 ml of reference solution to a 50 ml beaker (6.9). Prepare the acid whey reference portion as described in 8.1.2 for the test portion.

Standardize the reference sample periodically as described in Clause 10.

8.4 Preparation of reference solutions for the multi-point calibration

After defrosting, carefully mix the acid whey reference portion. Pipette 2 ml of the reference portion into a 20 ml volumetric flask (6.10) (marked D). Dilute to the mark with phosphate buffer solution (5.3.2) and mix.

Immediately pipette 1 ml, 2 ml and 5 ml of this solution into three different 10 ml volumetric flasks (6.10) (marked A, B, C). Dilute to the mark with the phosphate buffer solution (5.3.2). Stopper the flasks and mix carefully.

Filter each reference solution (A, B, C and D) using the microfiltration tools (6.11). Discard the first few millilitres of each filtrate and collect the rest.

Using this procedure, obtain the following four diluted reference solutions:

- reference solution A with dilution ratio 1:100;
- reference solution B with dilution ratio 1:50;
- reference solution C with dilution ratio 1:20;
- reference solution D with dilution ratio 1:10.

The diluted reference solutions may be stored at 4 °C but shall be analysed within 24 h.

8.5 Preparation of the reference solution for the "single-point" calibration procedure

Prepare a reference solution which produces a β -LG peak area close (i.e. \pm 20 %) to that of the test solution. Do not use reference solutions containing a β -LG content lower than 50 mg/l.

Usually reference solution B (8.4) is used for the quantification of β -LG in bottle-sterilized milk and in UHT milk. Use reference sample solution D (8.4) for the quantification of raw milk, pasteurized milk and high-temperature pasteurized milk.

8.6 HPLC determination

8.6.1 Elution solvents

Use the following elution solvents:

- a) elution solvent X1: a volume fraction of 0,1 % trifluoracetic acid (5.4.3) in water (5.4.1);
- b) elution solvent Y: a volume fraction of 0,1 % trifluoracetic acid (5.4.3) in acetonitrile (5.4.2);
- c) elution solvent X2: mix volumes of water (5.4.1), acetonitrile (5.4.2) and trifluoracetic acid (5.4.3) in the following ratio: 65:35:0,1.

A low-pressure gradient system may produce a baseline noise due to poor mixing of the elution solvents X1 and Y. In that case, change the elution solvent X1 to the solution X2. Elution solvent Y may remain the same. Calculate the new elution gradient on the basis of that reported in Table 1, taking into account that solvent X2 already contains 35 % acetonitrile.

8.6.2 Elution gradient

Time min	Elution solvent X1 %	Elution solvent Y %			
Initial	65	35			
1,0	65	35			
8,0	62	38			
16,0	58	42			
22,0	54	46			
22,5	0	100			
23,0	0	100			
23,5	65	35			
NOTE The elution gradient might require slight modification in order to achieve the resolution shown in Figure 1.					

Table 1 — Suggested elution gradient

Set the flowrate of the elution gradient pumping system (6.14.1) of the HPLC equipment (6.14) at 1,00 ml/min. Set the temperature of the column heater (6.14.3) at 40 °C.

Determine the equilibration time by monitoring the column elution. The detector response at the end of the run (baseline) should be equal to its initial value. An isocratic flushing of 15 min is usually sufficient.

8.6.3 Injection volume

Use a manual or automatic injector (6.14.2) to inject 20 µl of the solutions into the column.

8.6.4 Column equilibration

When starting up the system each day, flush the column with 100 % elution solvent Y (8.6.1) for between 5 min and 10 min. Then set the initial conditions (8.6.2) and equilibrate for 15 min. Perform a blank run by injecting the phosphate buffer solution (5.3.2).

In the case of long-term storage of the column, flush it with a mixture of acetonitrile (5.4.2) and water (5.4.1) having a volume ratio of 70:30.

8.6.5 Determination of the β -LG content in the test sample

Perform the sequence of chromatographic analyses while keeping constant the run-to-run time in order to obtain a baseline without drift and also constant retention times.

Always perform a multi-point or a single-point calibration procedure in every series of analyses. Inject a reference solution every 10 to 15 test samples in order to evaluate the response factor. If necessary, recalibrate the system. Either a multi-point or a single-point calibration procedure may be used. Periodically, a multi-point calibration is needed in order to check the response linearity of the HPLC system.

If a test sample contains a very low content of β -LG (e.g. sterilized milk), always run a blank sample (8.6.4) before injecting such samples in order to minimize a possible "carry-over effect" of the system. Clean the column by flushing with 100 % elution solvent Y (8.6.1) every 20 to 25 runs for at least 30 min.

8.7 Integration mode

Under the described conditions, β -LG elutes in three partially unresolved peaks. Integrate these peaks as a cluster (see Figure 1). Determine the whole area of the β -LG peaks as follows.

Set the baseline from the starting point of the first β -LG peak to the endpoint of the last β -LG peak. Integrate the area of the peak group in this interval (see Figures 1 and 2).

When the starting and/or the endpoints cannot be clearly identified because of a baseline drift or a poor shape of the peaks, adopt the integration interval of the reference sample (see Figure 2).



Key

Х	time	in	minutes	

Y relative response at 205 nm

A and B are the peaks for the two genetic variants of β -LG

T is a test solution with a dilution ratio of 1:5, obtained from a UHT milk sample containing 500 mg/l of β -LG

R is a reference solution with a dilution ratio of 1:50, obtained from a reconstituted freeze-dried milk sample containing 3 470 mg/l of β -LG

Figure 1 — HPLC patterns from a test solution of UHT milk and a reference solution



Key

- X time, in minutes
- Y relative response at 205 nm
- T is a test solution with a dilution ratio of 1:2, obtained from a sterilized milk sample containing 24 mg/l of β-LG
- R is a reference solution with a dilution ratio of 1:100, obtained from a reconstituted freeze-dried milk sample containing 3 470 mg/l of β -LG

Figure 2 — HPLC patterns from a test solution of sterilized milk and a reference solution

9 Calculation and expression of results

9.1 Multi-point calibration

9.1.1 Calculate the β -LG content, w_x , expressed in milligrams per litre, in each reference solution x, where x = A, B, C or D (8.4), using the following equations:

$$w_{A} = \frac{m}{2500}$$
$$w_{B} = \frac{m}{1250}$$
$$w_{C} = \frac{m}{500}$$

$$w_{\rm D} = \frac{m}{250}$$

where *m* is the mass of β -LG, in milligrams, in 2,5 g of reference sample (5.2).

9.1.2 Using least-squares linear regression analysis for the pairs $A_A - w_A$, $A_B - w_B$, $A_C - w_C$, $A_D - w_D$, with w_A , w_B , w_C , w_D as the independent variables, give the regression coefficients b_0 and b_1 by using the following equation:

$$A_{\mathbf{X}} = (b_0 \cdot w_{\mathbf{X}}) + b_1$$

where

- A_x is the numerical value of the β -LG peak area of solution x (x = A to D);
- b_0 is the numerical value of the first regression coefficient (slope);
- b_1 is the numerical value of the second regression coefficient (intercept).

9.1.3 Calculate the β -LG content, w_t , of the test sample, in milligrams per litre, using the following equation:

$$w_{t} = \frac{A_{t} - b_{1}}{b_{0} \times V_{t}} \times 10$$

where

- A_{t} is the numerical value of the β -LG peak area of the test solution (8.2);
- V_{t} is the numerical value of the volume of the acid whey test portion (8.1) used for the preparation of the test solution (8.2), in millilitres.

9.2 Single-point calibration

Calculate the β -LG content, w_t , of the test sample, in milligrams per litre, using the following equation:

$$w_{t} = \frac{w_{r} \cdot A_{t} \cdot V_{r}}{A_{r} \cdot V_{t}} \times 10$$

where

- w_r is the β -LG content in the reconstituted reference sample (8.3), in milligrams per litre;
- A_r is the numerical value of the β -LG peak area of the reference solution (8.5);
- A_{t} is the numerical value of the β -LG peak area of the test solution (8.2);
- V_r is the volume of acid whey of the reconstituted reference sample (8.3), in millilitres, in 1 ml of reference solution (8.5);
- V_{t} is the volume of acid whey of the test portion (8.1), in millilitres, used for preparing the test solution (8.2).

9.3 Expression of results

Express the results to two decimal places, or as "less than 15 mg/l" (see 11.1).

10 Standardization of the reference sample

10.1 General

Standardize the reference sample periodically in order to check the performance of the laboratory.

10.2 Preparation of the standard sample

Accurately weigh, to the nearest 1 mg, approximately 20 mg of β -LG standard sample (5.1) in a 50 ml volumetric flask (6.10). Dissolve the standard sample in phosphate buffer solution (5.3.2). Dilute to the mark with the phosphate buffer solution (5.3.2) and mix.

Filter the standard solution using the microfiltration tools (6.11). Discard the first few millilitres of filtrate. Collect the rest of the filtrate in a glass vial (6.4).

10.3 Determination of protein content

Determine the nitrogen content, w_N , of the standard sample according to the method described in either ISO 8968-1 | IDF 20-1 or ISO 8968-2 | IDF 20-2.

Calculate the protein content, w_{p} , in milligrams per 100 mg of standard sample, using the following equation:

$$w_{\rm D} = w_{\rm N} \times 6,38$$

where

- $w_{\rm N}$ is the nitrogen content of the sample, as a mass fraction in percent;
- 6,38 is the generally accepted multiplication factor to express the nitrogen content as crude protein content.

10.4 Determination of β -LG content in the reference sample

Run both the standard solution (10.2) and the reference solution D with dilution ratio 1:10 (8.4).

10.5 Calculation of the β -LG content

Calculate the β -LG content, w_r , of the reconstituted reference sample (8.3), in milligrams per litre, using the following formula:

$$w_{\rm r} = \frac{m \times w_{\rm p} \times A_{\rm r}}{A_{\rm s}} \times 2$$

where

- *m* is the mass of the standard sample (10.2), in milligrams;
- $w_{\rm p}$ is the protein content, in milligrams, per 100 mg of standard sample;
- $A_{\rm s}$ is the numerical value of the β -LG peak area of the standard solution (10.2);
- A_r is the numerical value of the β -LG peak area of the reference solution (8.4).

In a well-operating system, the difference between the w_r value obtained by this procedure and the declared amount in the reference sample should be within the range described for the reproducibility. Small differences may be due to a β -LG concentration of the standard sample not corresponding to the declared purity.

10.6 Expression of results

Express the results to two decimal places.

11 Precision

11.1 Interlaboratory test

Details of an interlaboratory test on the precision of the method are summarized in Annex A. The values derived from this interlaboratory test may not be applicable to concentration ranges and matrices other than those given.

The lowest limit of the β -LG quantification is set at a content of 15 mg/l. Indicate obtained results below a content of 15 mg/l with the expression "less than 15 mg/l".

11.2 Repeatability

The absolute difference between two independent single test 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 β -LG values between 15 mg/l and 500 mg/l: 16 % of the arithmetic mean,
- for β -LG values between 1 300 mg/l and 3 600 mg/l: 4 % of the arithmetic mean.

The relative standard deviation of repeatability, which expresses the variability of independent analytical results obtained under the former mentioned conditions, will in not more than 5 % of cases be greater than

- for β -LG values between 15 mg/l and 500 mg/l: 6 %,
- for β -LG values between 1 300 mg/l and 3 600 mg/l: 1,4 %.

11.3 Reproducibility

The absolute difference between two single test 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 β -LG values between 15 mg/l and 500 mg/l: 31 % of the arithmetic mean,
- for β -LG values between 1 300 mg/l and 3 600 mg/l: 13 % of the arithmetic mean.

The relative standard deviation of reproducibility, which expresses the variability of independent analytical results, obtained under the former mentioned conditions, will in not more than 5 % of cases be greater than

- for β -LG values between 15 mg/l to 500 mg/l: 11 %,
- for β -LG values between 1 300 mg/l and 3 600 mg/l: 5 %.

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) the test method used, with reference to this International Standard;
- d) all operational 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, and, if the repeatability has been checked, the final quoted result obtained.

Annex A

(informative)

Results of interlaboratory trials

The values for the repeatability and reproducibility limits were derived from the results of two interlaboratory tests organized within the Dairy Expert Group of the EU Commission. The results obtained were subjected to statistical analysis in accordance with ISO 5725-1 and ISO 5725-2 to give the precision data shown in Tables A.1 and A.2.

The first test involved 10 laboratories and was carried out with 9 samples of UHT milk and bottle-sterilized milk containing amounts of β -LG between 0 mg/l and 500 mg/l. The first test results are shown in Table A.1.

Type of milk	Mean value	Repeatability limit	s _r	Reproducibility limit	s _R
	mg/i	mg/l		mg/l	
Bottle-sterilized	19,30	2,64	0,94	4,82	1,72
Bottle-sterilized	24,80	3,56	1,27	9,19	3,28
UHT	54,30	16,01	5,72	12,72	4,54
UHT	64,90	13,89	0,50	20,78	7,42
UHT	80,00	32,50	11,59	36,91	13,18
UHT	86,10	10,07	3,60	18,57	6,63
UHT	133,70	12,04	4,22	67,03	23,94
UHT	240,40	31,53	11,26	57,68	20,60
UHT	424,40	38,92	13,90	89,49	31,96

Table A.1 — Milk samples with β -LG content between 0 mg/l and 500 mg/l

The second test involved 14 laboratories and was carried out with 9 samples of raw milk, pasteurized milk, high-temperature pasteurized milk and UHT milk, containing amounts of β -LG between 1 300 mg/l and 3 600 mg/l. The second test results are shown in Table A.2.

Table A.2 — Milk san	nples with <i>b</i>	B-LG content be	etween 1 300 m	g/l and 3 600 mg/l
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Type of milk Mean value Re		Repeatability limit	s _r	Reproducibility limit	s _R
		mg/l		mg/l	
UHT	1337	95,12	33,97	222,52	79,47
UHT	1429	63,61	22,72	239,90	85,68
HT pasteurized	2021	79,55	28,41	240,79	86,00
Pasteurized	2557	105,15	37,55	335,80	119,93
Pasteurized	2777	123,95	44,27	421,76	150,63
Pasteurized	2920	81,51	29,11	366,35	130,84
Raw	3449	79,17	28,27	474,27	169,38
Raw	3544	87,30	31,18	361,43	129,08
Raw	3565	141,90	50,68	268,85	96,02

Bibliography

- [1] ISO 707, Milk and milk products Guidance on sampling²)
- [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
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²⁾ Equivalent to IDF 50.

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