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Dried milk protein products — Determination of nitrogen solubility index

Poudres de protéines lactiques — Détermination de l'indice de solubilité de l'azote

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

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 International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 15323 | IDF 173 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.

Annex A of this International Standard is for information only.

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 National Committees casting a vote.

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All work was carried out by the Joint ISO/IDF/AOAC Action Team, *Physical properties of dried milk products*, under the aegis of its project leader, Dr P. Paquin (CA).

Dried milk protein products — Determination of nitrogen solubility index

1 Scope

This International Standard specifies a method for the determination of the nitrogen solubility index (NSI) of dried milk protein products. This determination is a means of assessing the solubility of nitrogen-containing compounds.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

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 Term and definition

For the purposes of this International Standard, the following term and definition applies.

3.1
nitrogen solubility index
ratio of the nitrogen content of the supernatant liquid after centrifuging an aqueous dispersion of the test sample to the nitrogen content of the aqueous dispersion before centrifuging, determined by the method specified in this International Standard

NOTE The nitrogen solubility index (NSI) is expressed as a percent.

4 Principle

A test portion of a dried milk protein product is dispersed in water at a fixed pH value and the nitrogen content of the dispersion is determined. Part of the dispersion is centrifuged and the nitrogen content of the filtered supernatant liquid is determined. The ratio between the two nitrogen contents obtained is calculated.

5 Reagents

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

5.1 Sodium hydroxide, c(NaOH) = 0,1 mol/l approximately.

5.2 Hydrochloric acid, c(HCl) = 0,1 mol/l approximately.

5.3 Reagents for the determination of nitrogen, according to ISO 8968-1|IDF 20-1 or ISO 8968-2|IDF 20-2.

6 Apparatus

Usual laboratory equipment and, in particular, the following.

6.1 Analytical balance, capable of weighing to the nearest 0,001 g.

6.2 Beaker, of capacity 250 ml.

6.3 One-mark volumetric flask, of capacity 100 ml.

6.4 Measuring cylinder, of capacity 100 ml.

6.5 Small glass funnels.

6.6 Wash bottle.

6.7 Magnetic stirrer.

6.8 Fixed-angle centrifuge, temperature controlled to maintain a temperature of 22 °C ± 2 °C and capable of producing a radial acceleration of 3 000 g at the bottom of the centrifuge tubes.

6.9 Centrifuge tubes, of capacity 50 ml.

6.10 pH-meter, capable of reading to 0,05 pH units.

6.11 Filter paper, medium grade (Whatman No. 1[1], or equivalent is suitable).

7 Sampling

It is important that the laboratory receive a sample which is truly representative and has not 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).

Store the test sample in such a way that deterioration and change in its composition are prevented.

8 Preparation of test sample

Thoroughly mix the test sample by repeatedly rotating and inverting the test sample container. Keep the test sample in the container to prevent water uptake.

If necessary, transfer the sample to a tightly closed container of sufficient size to do this effectively.

9 Procedure

9.1 Test portion

Weigh, to the nearest 0,001 g, an amount of the prepared test sample (clause 8) which is equivalent to about 1 g of protein (0,16 g of nitrogen) into the beaker (6.2).

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1) Whatman No. 1 is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO or IDF of this product.

9.2 Determination

9.2.1 Gradually add water at a temperature of 22 °C ± 2 °C to the test portion to a total volume of about 75 ml. Stir the mixture, while adding the water, and break up any lumps.

Adjust the pH (6.10) of the dispersion to pH 7,00 ± 0,55 with sodium hydroxide (5.2) or hydrochloric acid (5.3), if necessary. Rinse the pH electrode with some water. Stir the dispersion with a magnetic stirrer (6.7) at a temperature of 22 °C ± 2 °C for 2 h. Readjust the pH to 7,0 after 1 h of stirring, if necessary.

9.2.2 Transfer the dispersion to the 100 ml volumetric flask (6.3). Wash out the contents of the beaker carefully into the volumetric flask. Make up to the mark with water and mix thoroughly.

9.2.3 Take a suitable size aliquot portion of the dispersion (9.2.2). Immediately determine its nitrogen content by using the Kjeldahl method according to ISO 8968-1 | IDF 20-1 or ISO 8968-2 | IDF 20-2.

Low-solubility products (e.g. dried lactalbumin) tend to produce poor repeatability values. In such cases, the nitrogen content should be determined in the powder sample from those products as such. The obtained nitrogen content should be used in the calculation instead. The moisture content of the test sample can influence the result.

9.2.4 Allow the remaining dispersion to stand for 2 min. Decant, using the measuring cylinder (6.4), 35 ml of the dispersion into a centrifuge tube (6.9).

Centrifuge (6.8) at the required rotational frequency to produce a radial acceleration of 3 000 g at 22 °C for 10 min. Decant the supernatant liquid through the filter paper (6.11). Take care not to transfer any sediment to the filter.

9.2.5 Take a suitable aliquot of the filtered supernatant liquid (9.2.4). Determine the nitrogen content of the supernatant liquid in it by using the Kjeldahl method according to ISO 8968-1 | IDF 20-1 or ISO 8968-2 | IDF 20-2.

10 Calculation and expression of results

10.1 Calculation

Calculate the nitrogen solubility index, NSI, using the following equation.

$$\mathrm{NSI} = \frac{w_\mathrm{S}}{w_\mathrm{d}} \times 100\ \%$$

where

NSI is the nitrogen solubility index of the sample, expressed as a percent;

w_S is the nitrogen content of the supernatant liquid obtained in 9.2.5, expressed as grams per 100 ml;

w_d is the nitrogen content of the dispersion obtained in 9.2.3, expressed as grams per 100 ml.

10.2 Expression of results

Express the results to one decimal place.

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.

NOTE IDF 135 provides specific guidance for interlaboratory tests on methods of analysis and milk products. It is based on ISO 5725.

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 the repeatability limit, r, for each type of protein powder as given in Table A.1.

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 the reproducibility limit, R, for each type of protein powder as given in Table A.1.

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 or, if the repeatability has been checked, the final result obtained.

Annex A
(informative)

Results of an interlaboratory trial

An international collaborative test was carried out on three types of protein powder as given in Table A.1. The test was organized by joint ISO/IDF/AOAC action team (JAT), *Measurement of functional properties of dried milk products*. The results obtained were subjected to statistical analysis in accordance with ISO 5725[2)] to give the precision data shown in Table A.1 and have been published (see reference [6]).

Table A.1 — Precision data for different types of protein powder

Product	Number of laboratories	Mean NSI value	Repeatability			Reproducibility		
			r	s_r	RSD	R	s_R	RSD
Whey protein concentrate	10	92,16 %	3,00	1,06	1,15	8,61	3,04	3,30
Co-precipitate	11	85,31 %	1,87	0,66	0,77	2,58	0,91	1,07
Caseinate	13	100,37 %	1,87	0,66	0,66	2,71	0,96	0,96

2) The precision data were obtained using ISO 5725:1986 (now withdrawn).