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EAS 81-6 (2006) (English): Milk powders
– Determination of insolubility index

ISO INSIDE



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EAST AFRICAN STANDARD

Milk powders — Methods for analysis — Part 6: Determination of insolubility index

EAST AFRICAN COMMUNITY

EAS 81-8:2006

Foreword

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**Dried milk and dried milk products —
Determination of insolubility index**

*Lait sec et produits laitiers en poudre — Détermination de l'indice
d'insolubilité*



Reference numbers
ISO 8156:2005(E)
IDF 129:2005(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 8156|IDF 129 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.

This edition of ISO 8156|IDF 129 cancels and replaces ISO 8156:1987, of which it constitutes a minor revision.

Foreword

IDF (the International Dairy Federation) is a worldwide federation of the dairy sector with a National Committee in every member country. Every National Committee has the right to be represented on the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO in the development of standard methods of analysis and sampling for milk and milk products.

Draft International Standards adopted by the Action Teams and Standing Committees are circulated to the National Committees for voting. Publication as an International Standard requires approval by at least 50 % of the IDF National Committees casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. IDF shall not be held responsible for identifying any or all such patent rights.

ISO 8156|IDF 129 was prepared by the International Dairy Federation (IDF) and Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*. It is being published jointly by IDF and ISO.

All work was carried out by the Joint ISO/IDF/AOAC Group of Experts on *Physical properties of dried milk products*, under the aegis of its chairman, Mr J. de Vilder (BE).

This edition of ISO 8156|IDF 129 cancels and replaces IDF 129A:1988, of which it constitutes a minor revision.

Introduction

0.1 There are several rather elaborate gravimetric methods for determining the solubility of dried milk (for example, References [1] and [2]), but for routine purposes, including grading, the most widely used procedure is the so-called solubility index method of the American Dry Milk Institute (see Reference [3]), in which a test portion is mixed with water and the reconstituted product is centrifuged. The volume, in millilitres, of the sediment finally obtained (i.e. insoluble residue) is the solubility index. Since the solubility index is thus inversely related to solubility, it seems more direct and more rational to use the term “insolubility index” to describe what is determined by a “solubility” method such as that of the ADMI. Accordingly, “insolubility index” was adopted to designate what is determined in the sediment-volume solubility method described in this International Standard. Use of this new expression also serves to differentiate the method described in this International Standard from the solubility index method of the ADMI.

Although the ADMI solubility index method has been in use in many countries for a considerable period, it became evident some time ago that its precision (repeatability, reproducibility), which is not stated by ADMI, is unsatisfactory with some types of spray-dried whole milk and with roller-dried milk and milk products. This led to the conclusion that the apparatus and technique of the ADMI method are inadequately defined, and are unsuitable for some dried milks, and consequently either the ADMI method should be more closely specified, and possibly modified in some respects, or an alternative method developed. The latter approach was at first favoured because of difficulty in obtaining the special mixer (and spare parts) manufactured in the USA for the ADMI method. However, when improved models of this mixer came to be manufactured in several countries and hence were readily available, it was decided to concentrate on improving the precision of the ADMI method while retaining its principal features so that most of the existing ADMI solubility index specifications for grading would still be applicable.

0.2 In any sediment-volume solubility method applied to a dried milk or a dried milk product, the temperature at which the test portion is reconstituted is the main factor influencing what the result will be. In the ADMI solubility index method, a reconstituting temperature of 75 °F (23,9 °C) is used with spray-dried or roller-dried whole milk, skimmed milk and buttermilk, instant or non-instant as appropriate. But for the insolubility index method, it was decided to adopt the principle that the reconstituting temperature should be either 24 °C or 50 °C depending on whether the product, in normal usage from its quality specification, is expected to be reconstitutable in “cold” water or “warm” water respectively. **This means that the reconstituting temperature to be used in the insolubility index method will, in general, be 24 °C for spray-dried products and 50 °C for roller-dried products.** Exceptions to this general rule are spray-dried milk-based baby food and, in some instances, spray-dried whole milk or partly skimmed milk, intended to be reconstituted in warm water. However, it is important to note that if the insolubility index of spray-dried fat-containing milks is determined at 50 °C, the values obtained will all tend to be very small because the method will no longer detect products that have been subjected to excessive dry heat through faulty manufacture or storage. This is because milk protein denatured by dry heat is insoluble at 24 °C and, along with entrapped or combined fat, is precipitated as sediment when centrifuging is performed. At 50 °C, the dry-heat-denatured protein is soluble and this, with the release of the associated fat, can cause a marked reduction in the volume of sediment (see References [4] to [6]).

0.3 The insolubility index method described in this International Standard is thus basically the same as the ADMI solubility index method, but with all the apparatus and experimental conditions as closely defined as practicable and the reconstituting temperature either 24 °C or 50 °C, as appropriate (see 0.2). The latter innovation means that an insolubility index value will require to be accompanied by the reconstituting temperature used, for example 0,25 ml (24 °C), 0,10 ml (50 °C). The precision of the insolubility index method has been determined in an interlaboratory collaborative study and is considered to be satisfactory.

Dried milk and dried milk products — Determination of insolubility index

1 Scope

This International Standard specifies a method of determining the insolubility index, as a means of assessing the solubility, of dried whole milk, dried partly skimmed milk and dried skimmed milk, whether non-instant or instant.

NOTE These milks are defined in Reference [7] as “whole milk powder”, “partly skimmed milk powder” and “skimmed milk powder”, respectively.

The method is also applicable to dried whey, dried buttermilk and dried milk-based baby food, as well as to any of the dried products listed in which milk fat has been replaced by another fat, or which has been roller-dried instead of spray-dried.

2 Terms and definitions

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

2.1

insolubility index

volume, in millilitres, of sediment (insoluble residue) obtained when a dried milk or dried milk product is reconstituted and the reconstituted milk or milk product is centrifuged, under the conditions specified in this International Standard

3 Principle

Water at 24 °C (or at 50 °C if appropriate, see 0.2) is added to a test portion, which is reconstituted using a special mixer. After a specified standing period, a specified volume of the reconstituted milk or milk product is centrifuged in a graduated tube. The supernatant liquid is removed and the sediment is redispersed after the addition of water at the same temperature as used for the reconstitution. The mixture is centrifuged and the volume of sediment (insoluble residue) obtained is recorded.

4 Reagents

Use only distilled water or demineralized water, or water of at least equivalent purity.

4.1 Silicone antifoaming agent, for example an aqueous emulsion containing 30 % (mass fraction) of silicone.

Test the suitability of the silicone antifoaming agent by carrying out the procedure described in Clause 7 without a test portion. No more than a trace of silicone fluid ($\leq 0,01$ ml) should be visible at the bottom of the tube at the end of the procedure.

5 Apparatus

Usual laboratory apparatus and, in particular, the following.

5.1 Thermometer(s), capable of measuring a temperature of 24 °C and/or 50 °C with an error not exceeding $\pm 0,2$ °C.

Since the temperature of reconstitution is the most important experimental factor governing values obtained for the insolubility index, it is essential that a thermometer of the specified accuracy is used for the procedures specified in 7.1 and 7.3 (and also 7.4.8).

5.2 Water bath(s), capable of being maintained at $24,0$ °C $\pm 0,2$ °C and/or $50,0$ °C $\pm 0,2$ °C, in which one or more mixing jars (5.3) can be placed (see 9.3).

5.3 Mixing jar, made of glass, of capacity 500 ml, as supplied for use with the mixer (5.8).

The mixing jar (clover-leaf pattern) is illustrated in Figure 1; the dimensions are approximate.

5.4 Scoop, with a smooth surface, or **sampling paper**, black, glazed (of dimensions 140 mm \times 140 mm), for weighing the test portion (7.3).

5.5 Balance, accurate to 0,01 g.

5.6 Measuring cylinder, made of plastic material, of capacity 100 ml $\pm 0,5$ ml (at 20 °C).

NOTE The lower heat capacity of a plastic measuring cylinder, as compared to a glass one, minimizes possible changes in the temperature of the water placed in the cylinder (see 7.4.1).

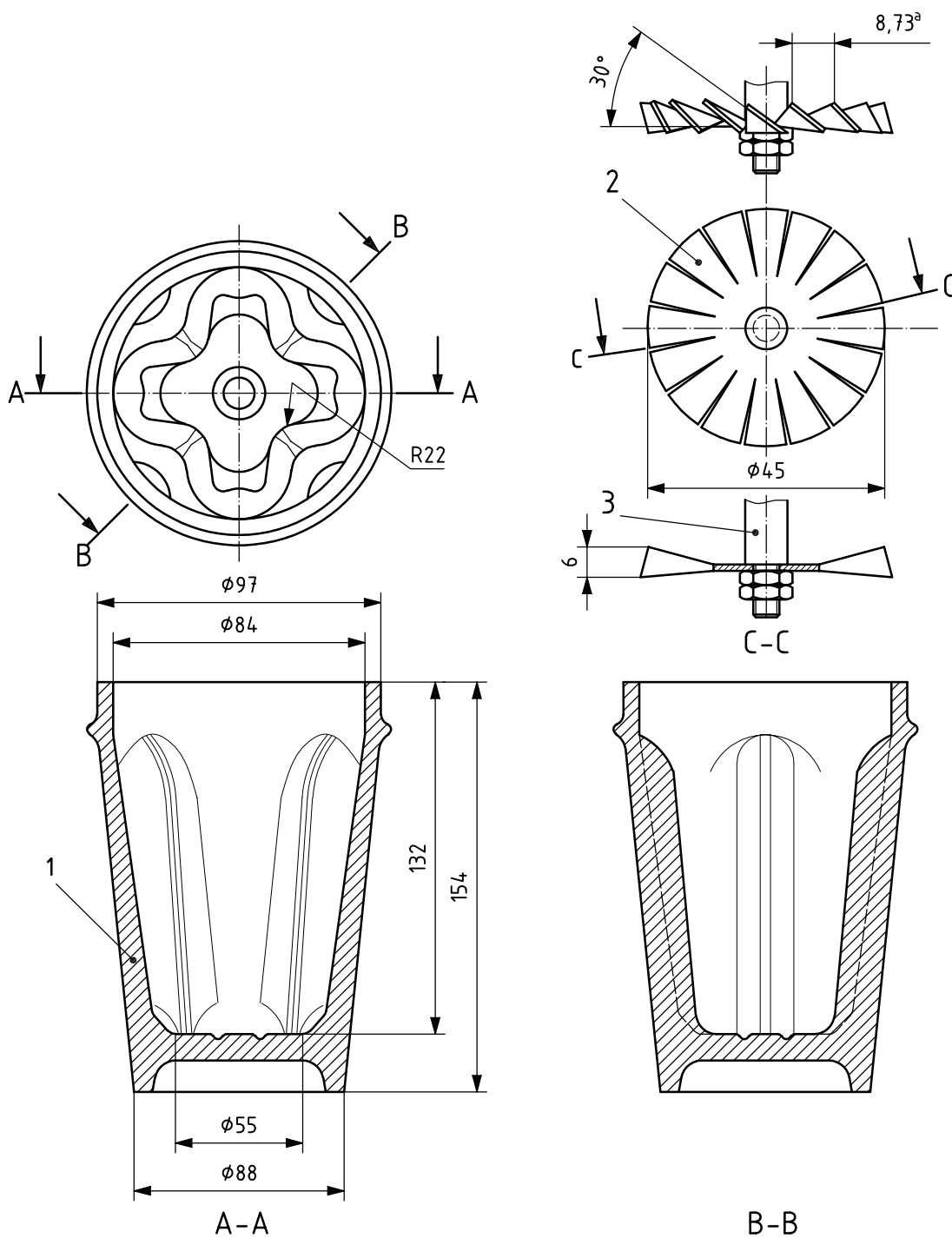
5.7 Brush, suitable for removing any residual test portion from the scoop or sampling paper (5.4).

5.8 Electric mixer, equivalent to that manufactured for the solubility index method of the American Dry Milk Institute^[3] with the following characteristics.

- a) The 16-bladed impeller (stainless steel) shall have the shape and diameter show in Figure 1, and shall be attached to the shaft of the mixer so that the "flat" side of the impeller is underneath, as also shown in Figure 1. The slope of the blades is upward from right to left; this is for clockwise rotation (see the Note).
- b) The pitch of the impeller blades shall be 30° and the horizontal distance between the blades (around the circumference of the impeller) shall be 8,73 mm, as shown in Figure 1. With usage of the impeller, these dimensions can change and hence periodic inspection and maintenance are essential.
- c) When the mixing jar (5.3) is fitted to the mixer, the length of the mixer shaft shall be such that the distance from the lowest part of the impeller to the bottom of the jar is 10 mm ± 2 mm; this means that for a jar of depth 132 mm the distance from the top of the jar to the lowest part of the impeller is 122 mm ± 2 mm, and to the plane of the lowest part of the impeller blades is 115 mm ± 2 mm. The impeller shall also be located centrally in the jar.
- d) When the mixing jar, containing 100 ml of water at 24 °C with or without the addition of an appropriate test portion (7.3), is fitted to the mixer and the mixer switched on, the impeller shall come to its operational fixed rotational frequency of $(3\ 600 \pm 100)$ min⁻¹ in less than 5 s. The direction of rotation of the impeller shall be clockwise (viewed from above). The rotational frequency of the impeller under load (as described above) shall be checked periodically with an electronic tachometer; this is especially necessary with older models of the mixer, with a non-synchronous motor, whose rotational frequency has to be adjusted to $(3\ 600 \pm 100)$ min⁻¹ by means of a speed control and a speed indicator (which may not remain accurate).

NOTE Some mixers for the ADMI method cause the impeller to rotate in an anticlockwise direction (viewed from above). These mixers require an impeller whose blades slope upward from left to right so that liquid in the mixing jar moves in the same way as with a clockwise rotating impeller. In all respects, including its manner of attachment to the shaft and its distance from the bottom of the mixing jar, an anticlockwise-rotating impeller is equivalent to a clockwise-rotating impeller.

Dimensions in millimetres



Key

1 mixer jar

2 impeller

3 mixer shaft

^a Horizontal distance between blades (around circumference)

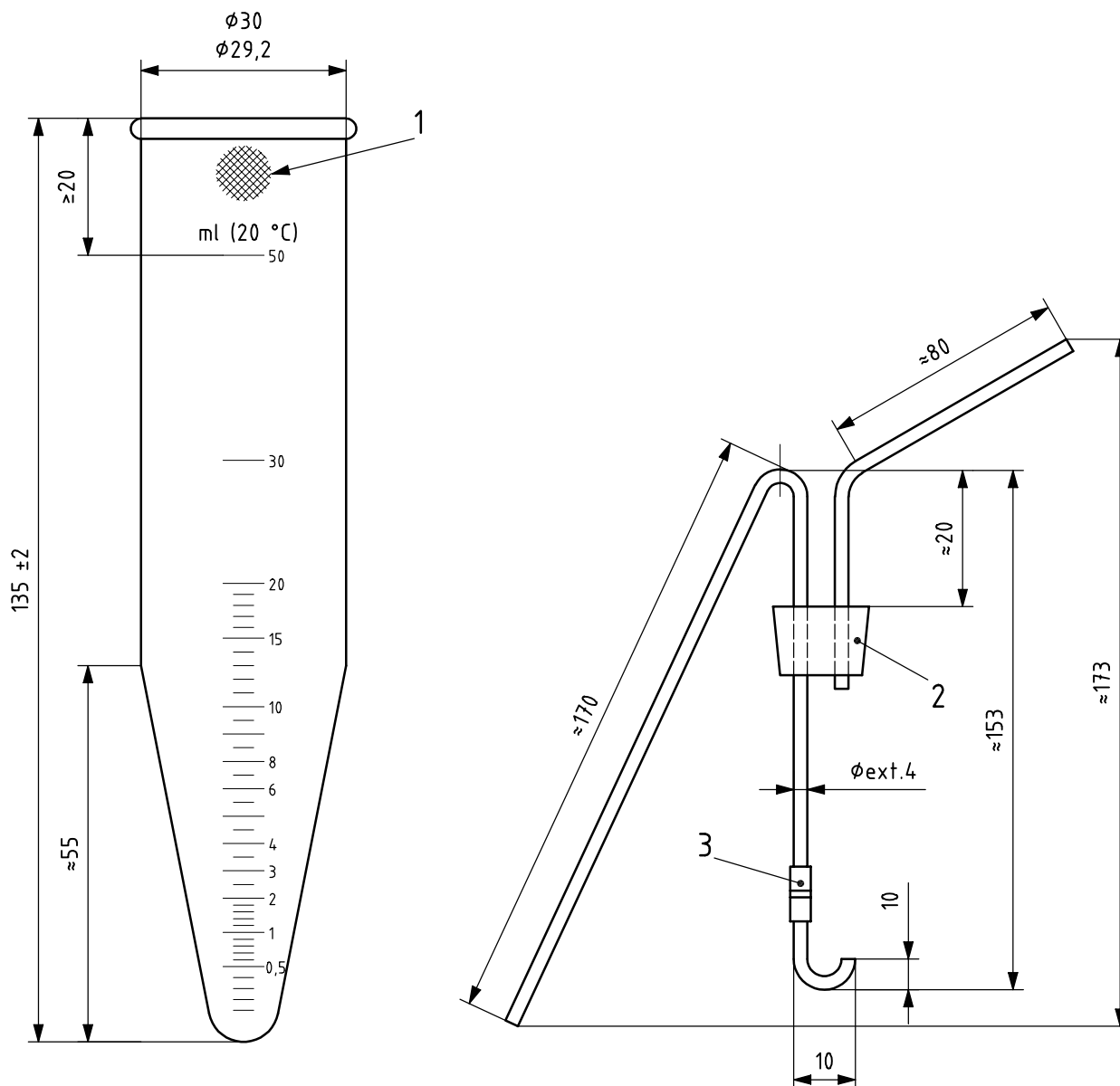
Figure 1 — Mixing jar and impeller

5.9 Interval timer, indicating 0 s to 60 s and 0 min to 60 min.

5.10 Spoon spatula(s), of length approximately 210 mm.

5.11 Centrifuge tubes, of glass, conical, with the shape, dimensions, graduation scheme, inscriptions and patch of matt surface shown in Figure 2, and provided with rubber stoppers.

Dimensions in millimetres



Key

- 1 matt surface
- 2 rubber stopper
- 3 flexible plastic tubing

Figure 2 — Centrifuge tube and siphon fitting

The graduation lines, graduation numbers and the inscription “ml (20 °C)” shall be marked in a permanent manner and the graduation lines shall be fine and clean. The maximum errors (\pm) in capacity at 20 °C shall be as follows:

- at 0,1 ml: $\pm 0,05$ ml;
- from 0,1 ml to 1 ml inclusive: $\pm 0,1$ ml;
- from 1 ml to 2 ml inclusive: $\pm 0,2$ ml;
- from 2 ml to 5 ml inclusive: $\pm 0,3$ ml;
- from 5 ml to 10 ml: $\pm 0,5$ ml;
- at 10 ml: ± 1 ml.

For routine production control purposes, tubes of different dimensions may be used provided that they comply with limits for maximum error in capacity, as listed above. In all cases of dispute or where a definitive results is required, the tubes specified in this subclause should be used.

5.12 Centrifuge, electric, with speed indicator (min^{-1} or r/min), with vertical-loading swing-out cups for accommodating the centrifuge tubes (5.11), and capable of producing an acceleration of $160 g_n$ at the internal bottom of the tubes and maintaining a temperature of 20 °C to 25 °C within the closed centrifuge.

NOTE The acceleration, in terms of g_n , produced in a centrifuge is equal to

$$1,12 r n^2 \times 10^{-6}$$

where

- r is the effective horizontal radius of spinning, in millimetres;
- n is the rotational frequency, per minute.

5.13 Siphon fitting or suction tube attached to water pump, made of glass tubing and with upturned tip, for removing supernatant liquid from a centrifuge tube (5.11). A suitable siphon fitting is shown in Figure 2.

5.14 Stirring rod, made of glass, of length 250 mm and diameter 3,5 mm.

5.15 Magnifying lens, suitable for aiding the reading of the volume of sediment (see 7.4.10).

6 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 | IDF 50.

If necessary, store the laboratory sample in a clean, dry, securely closed, airtight and preferably opaque container, which may be the intact unopened retail container. If the laboratory sample is in a transparent container, keep the container in the dark.

Note and report any deviations from these requirements.

7 Procedure (see also Clause 10, and 10.3 in particular)

7.1 Preparation of test sample

Before proceeding with the determination, ensure that the laboratory sample (Clause 6) has been maintained at laboratory temperature (20 °C to 25 °C) for at least 48 h so that any influence on the insolubility index attributable to the physical state of the fat is constant from sample to sample. Then thoroughly mix the laboratory sample by repeatedly rotating and inverting the container. If the container is too full to allow thorough mixing, transfer all the laboratory sample to a clean, dry, airtight container of adequate capacity and mix as described.

In the case of instant dried milk, mixing shall be performed very gently to avoid reducing the particle size of the sample.

7.2 Preparation of the mixing jar

According to whether the insolubility index is to be measured at 24 °C or at 50 °C (see 0.2), adjust the temperature of the mixing jar (5.3) to 24,0 °C ± 0,2 °C or 50,0 °C ± 0,2 °C, respectively, by standing the jar in the water bath (5.2), with the water level near the top of the jar, for a sufficient period of time.

NOTE In the remainder of the test, the phrase "at 24,0 °C ± 0,2 °C or 50,0 °C ± 0,2 °C as appropriate" means whichever of these temperatures was adopted in this subclause.

7.3 Test portion

Weigh, to the nearest 0,01 g, in the scoop or on the sampling paper (5.4), a test portion of

- a) 13,00 g in the case of dried whole milk, dried partly skimmed milk, and infant food based on either of these,
- b) 10,00 g in the case of dried skimmed milk and dried buttermilk, or
- a) 7,00 g in the case of dried whey.

(See 10.4.)

7.4 Determination

7.4.1 Remove the mixing jar (see 7.2) from the water bath, quickly wipe dry the outside of the jar, and, using the measuring cylinder (5.6), add to the jar 100 ml ± 0,5 ml of water at 24,0 °C ± 0,2 °C or 50,0 °C ± 0,2 °C as appropriate (see the Note to 7.2).

7.4.2 Add 3 drops of the silicone antifoaming agent (4.1) to the water in the mixing jar and then transfer the test portion (7.3) to the jar, using the brush (5.7), if necessary, so that all of the test portion falls onto the surface of the water.

7.4.3 Fit the mixing jar to the mixer (5.8), switch on the mixer, and after the mixer has operated for exactly 90 s, switch it off. If the mixer is of the type with a non-synchronous motor, speed control and speed indicator, bring the rotational frequency of the impeller to $(3\ 600 \pm 100) \text{ min}^{-1}$ within the first 5 s of the 90 s mixing period.

7.4.4 Remove the mixing jar from the mixer (allow a few seconds for liquid on the impeller to drain into the jar), and leave the jar to stand, at laboratory temperature (see 10.2), for not less than 5 min and not more than 15 min (see 10.3).

7.4.5 Add 3 drops of the silicone antifoaming agent to the mixture in the mixing jar (see 10.5), thoroughly mix the contents of the jar by stirring (not too vigorously) for 10 s with a spoon spatula (5.10). Immediately pour sufficient of the mixture into a centrifuge tube (5.11) to fill the tube up to the 50 ml mark, i.e. until the top level is coincident with the 50 ml mark.

7.4.6 Place the centrifuge tube (counterbalanced) in the centrifuge (5.12) set at 20 °C to 25 °C. Bring the centrifuge as quickly as possible to the rotational frequency producing an acceleration of 160 g_n at the internal bottom of the tube, and then spin the tube at this rotational frequency for 5 min.

7.4.7 Remove the centrifuge tube from the centrifuge and, using a spoon spatula (5.10), remove and discard any top layer of fatty material in the tube.

Hold the centrifuge tube in a vertical position and remove the supernatant liquid with a siphon fitting (5.13) or a suction tube (5.13) until the top level is coincident with the 15 ml mark if a roller-dried product is being tested or coincident with the 10 ml mark if a spray-dried product is being tested, taking care not to disturb the sediment. However, if it is evident that the volume of sediment exceeds 15 ml or 10 ml, respectively, discontinue the procedure at this stage and record the insolubility index as “greater than 15 ml” or “greater than 10 ml”, indicating the reconstituting temperature as shown in 8.1. Otherwise, proceed as described in 7.4.8.

7.4.8 Add water at 24 °C or 50 °C as appropriate (see the Note to 7.2) to the centrifuge tube until the top level is coincident with the 30 ml mark. Completely disperse the sediment with the stirring rod (5.14), tap the bottom of the rod against the inside of the tube to collect adhering liquid, and add more water at the same temperature until the top level is coincident with the 50 ml mark.

7.4.9 Close the centrifuge tube with a rubber stopper. Invert the tube quickly five times so as to mix its contents thoroughly, remove the stopper (draw the bottom of the stopper across the rim of the tube to collect adhering liquid), and then spin the tube in the centrifuge for 5 min at the required rotational frequency and temperature, as described in 7.4.6.

It is recommended that the centrifuge tube be placed in the centrifuge cup so that, when the tube is in the spinning position, the scale lines are not facing upwards or downwards but are midway between these two positions. Then, if the top of the sediment is sloping, the volume of sediment will be easier to estimate.

7.4.10 Remove the centrifuge tube from the centrifuge, hold the tube in a vertical position against a suitable background (see the Note) with the top of the sediment at eye level, and, using the magnifying lens (5.15), read the volume of sediment to the nearest 0,05 ml if the volume is less than 0,5 ml and to 0,1 ml if the volume is more than 0,5 ml. Estimate the volume if the top of the sediment is sloping. If the top of the sediment is uneven, leave the tube in a vertical position for a few minutes; the top of the sediment will usually become less uneven and the volume of sediment can be read more easily. Record the temperature of the water used for the reconstitution (see 7.4.8).

NOTE Viewing the tube against either a light or a dark background, as preferred, makes the top of the sediment more distinct.

8 Expression of results

The insolubility index of the laboratory sample is equal to the volume, in millilitres, of sediment recorded in 7.4.10. Report the result with the temperature of the water used for reconstitution, for example as follows:

- 0,10 ml (24 °C);
- 4,1 ml (50 °C).

9 Precision

9.1 Interlaboratory test

The values of repeatability and reproducibility were determined in an interlaboratory test, carried out as described in ISO 5725¹⁾, involving 10 laboratories and 10 samples.

9.2 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 $0,138 M$, where M is the arithmetic mean of the two results.

9.3 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 $0,328 M$, where M is the arithmetic mean of the two results.

10 Notes on procedure

10.1 It is essential that the procedure, once started, be completed without unnecessary delay at any stage and that all requirements relating to temperature and timing be strictly observed.

10.2 Since the determination of the insolubility index may be affected by ambient temperature, it is recommended that the procedure be carried out in a laboratory in which the temperature is maintained in the range 20 °C to 25 °C.

10.3 Tests have shown that the permitted 5 min to 15 min variation in “standing” time (7.4.4) has an insignificant effect on the insolubility index. This range of 10 min allows several samples, preferably requiring the same reconstitution temperature, to be tested as a batch, provided that the temperature of each mixing jar is adjusted simultaneously (see 7.2) and the test portions (7.3) are all weighed together. Under these circumstances, it may be advantageous to modify the procedure described in 7.2 and 7.4.1 by adding 100 ml \pm 0,5 ml of water (at approximately the temperature required) to each of the mixing jars in the water bath and, when the temperature of the water has stabilized to the correct value, to remove one jar from the water bath and proceed as described in 7.4.1 to 7.4.4 inclusive, and so on with each of the other jars in turn. Thereafter, a batch procedure may be adopted, with simultaneous centrifuging of the tubes.

10.4 Each test portion (7.3) is such that, when mixed with 100 ml of water, the total solids content, expressed as a mass fraction of the mixture, approximates to that of the original liquid product. The sizes of the test portions for dried whole milk, dried skimmed milk and dried buttermilk are the same as specified in the solubility index method^[3], which deals only with these three products.

10.5 The addition of 3 drops of the silicone antifoaming agent (4.1) in 7.4.5 may not appear to be necessary with samples whose propensity to foam during mixing is less than others. Nevertheless, the 3 drops should always be added to keep the procedure exactly the same for all samples.

1) ISO 5725:1986, *Precision of test methods — Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests* (now withdrawn).

11 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 operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which have influenced the test result(s);
- e) the test result(s) obtained, or, if the repeatability has been checked, the final quoted result obtained.

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

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