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Assessment of heat class - Heat-number

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EAST AFRICAN STANDARD

Milk powders — Methods for the analysis — Part 7: Assessment of heat class — Heat-number reference method

EAST AFRICAN COMMUNITY

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Foreword

Development of the East African Standards has been necessitated by the need for harmonizing requirements governing quality of products and services in East Africa. It is envisaged that through harmonized standardization, trade barriers which are encountered when goods and services are exchanged within the Community will be removed.

In order to achieve this objective, the Partner States in the Community through their National Bureaux of Standards, have established an East African Standards Committee.

The Committee is composed of representatives of the National Standards Bodies in Partner States, together with the representatives from the private sectors and consumer organizations. Draft East African Standards are circulated to stakeholders through the National Standards Bodies in the Partner States. The comments received are discussed and incorporated before finalization of standards, in accordance with the procedures of the Community.

East African Standards are subject to review, to keep pace with technological advances. Users of the East African Standards are therefore expected to ensure that they always have the latest versions of the standards they are implementing.

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International Standard 6735

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION•МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ•ORGANISATION INTERNATIONALE DE NORMALISATION

Dried milk — Assessment of heat class — Heat-number reference method

Lait sec — Évaluation de la classe de traitement thermique — Méthode de référence de l'indice de traitement thermique

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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.

Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council. They are approved in accordance with ISO procedures requiring at least 75 % approval by the member bodies voting.

International Standard ISO 6735 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*.

NOTE — The method specified in this International Standard has been developed jointly with the International Dairy Federation (IDF) and the Association of Official Analytical Chemists (AOAC) and will also be published by these organizations.

Users should note that all International Standards undergo revision from time to time and that any reference made herein to any other International Standard implies its latest edition, unless otherwise stated.

Printed in Switzerland

Dried milk — Assessment of heat class — Heat-number reference method

0 Introduction

The heat treatment applied in the manufacture of spray-dried milk, predominantly in the preheating of the liquid milk, can be varied to produce a product suitable for a particular use. It is therefore necessary, both for the manufacturer and for the buyer or user, to be able to assess the degree of overall heat treatment so that a dried milk can be classified as, for example, "low heat", "medium heat", etc. The basis of classification is that an increasing proportion of the milk-serum protein (whey protein) in milk is denatured (and becomes associated with the casein) as the degree of heat treatment increases. The methods involve either the direct measurement of residual undenatured milk-serum protein or the indirect measurement of denatured milk-serum protein, and the concentration found in the dried milk in relation to the range of possible concentrations indicates the heat class. Ideally, because of the small seasonal variations in the concentration and relative proportions of the proteins in raw bulk cows' milk, a sample of the original liquid milk should be available for comparison when assessing the heat class of a dried milk, but this is only possible for the manufacturer. In practice, heat class is usually assessed solely by examining the dried milk and this inevitably imposes some limitation on the accuracy of all protein-based heat-classification methods.

The most widely used heat-classification method is probably that published by the American Dry Milk Institute (ADMI) in 1971, in which the concentration in the dried milk of undenatured milk-serum protein nitrogen (UMSPN) is determined turbidimetrically so that the dried milk can be placed in one of the following heat classes :

- low heat (not less than 6,0 mg of UMSPN per gram);
- medium heat (1,51 to 5,99 mg of UMSPN per gram);
- high heat (not more than 1,5 mg of UMSPN per gram).

The procedure for determining the so-called "ADMI whey-protein index" of dried milk has several drawbacks, however, and improved or alternative procedures utilizing dye-binding have been developed [for example Sanderson (1970), McGann, O'Connell and McFeely (1975)].

The heat-classification of dried milk is possible by the indirect measurement of denatured milk-serum protein (DMSP), for example by isolating the complex of casein and DMSP and determining its content of cystine plus cysteine (predominantly derived from the DMSP) by the method of de Koning, van Rooijen and Draaisma (1976) and Mrowetz and Klostermeyer (1977). However, it has been recognized for some time that another method of this general type would be to utilize the procedure of Rowland (1938a) for determining the casein number of raw liquid milk (Rowland, 1938b), which is obtained by multiplying the ratio of casein nitrogen content to total nitrogen content by 100 and is useful in diagnosing subclinical mastitis [Rowland and Zein-El-Dine (1938)]. When this procedure is applied to heated milk, or to reconstituted dried milk, the "casein number" obtained is, in fact, 100 times the ratio of casein nitrogen content plus DMSP nitrogen content [or, more accurately, acid-insoluble (pH 4,8) protein nitrogen content] to total nitrogen content, and thus increases with the degree of heat treatment sustained by the milk or dried milk.

Consideration of all the available heat-classification methods for dried milk and extensive trials of most of these, including examination by gel electrophoresis of the casein / DMSP complex and the UMSP isolated from dried milks, led to the conclusion that a method based on the determination of "casein number" — renamed "heat number" to avoid confusion — would be the best overall general-purpose method and that this should be regarded as the reference method. During these trials, it became evident that for the accurate and precise determination of the heat number of dried milk, the methods described in IDF Standard 20 and IDF Standard 29, for the determination, respectively, of the total nitrogen content and of the casein content of milk, were unsuitable and, accordingly, alternative methods were developed. An advantage of the heat-number method of heat classification is that it enables the total nitrogen content of the dried milk to be determined.

No single heat-classification method is likely to be suitable for all purposes and, therefore, it is recognized that additional methods may be required for detecting extra-low heat and extra-high heat dried milks; in addition, a rapid routine method (for example employing dye binding) would be useful for plant control purposes. It is also recognized that even when the heat class of a dried milk has been accurately assessed and is a relevant criterion of suitability for a particular use, complementary information about the dried milk may be necessary, for example its rennetability [Voss and Schmanke (1973)].

The macro-Kjeldahl method employed in the determination of heat number is described in annex A and the proposed heat-classification scheme is described in annex B. These annexes form integral parts of this International Standard.

1 Scope and field of application

This International Standard specifies the reference method, based on the determination of heat number, for assessing the heat class of dried whole milk, dried partly skimmed milk and dried skimmed milk (defined in FAO/WHO Standard A-5[1] as "whole milk powder", "partly skimmed milk powder" and "skimmed milk powder", respectively) manufactured by the spray process. The method is also applicable to all types of instant dried milk.

2 Reference

ISO 707, *Milk and milk products — Methods of sampling.*

3 Definition

For the purpose of this International Standard, the following definition applies.

3.1 heat number (of dried milk) : Ratio of acid-insoluble (pH 4,8) protein-nitrogen content to total nitrogen content, multiplied by 100. (The acid-insoluble protein is casein plus heat-denatured milk-serum protein.)

4 Principle

Precipitation of the casein plus heat-denatured milk-serum protein in a specified volume of reconstituted dried milk, at a pH of approximately 4,8, by adding acetic acid solution and then sodium acetate solution. Washing of the precipitate and determination of its nitrogen content (in terms of the equivalent volume of a standard volumetric solution) by the Kjeldahl method.

Determination of the total nitrogen content of the same volume of the reconstituted dried milk in the same manner.

Calculation of the heat number of the dried milk directly from the two volumes of standard volumetric solution used, each being corrected by an appropriate blank Kjeldahl determination.

Derivation of the heat class of the dried milk from the heat number according to a proposed heat-classification scheme consisting of four heat classes, i.e. :

- low heat;
- medium heat;
- medium-high heat;
- high heat.

NOTE — The acetic acid/sodium acetate procedure used is that developed by Rowland (1938a) for the precipitation of casein or casein plus heat-denatured milk-serum protein, in determining the nitrogen distribution in cows' milk. The buffered reaction mixture, whether containing milk or reconstituted dried milk, has a pH (glass electrode) at 20 °C of approximately 4,8 and not approximately 4,6 as is commonly assumed.

5 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical quality and only distilled water or water of equivalent purity.

5.1 Antifoaming agent : octan-2-ol (capryl alcohol), containing at least 97 % (*m/m*) of $CH_3(CH_2)_5CH(OH)CH_3$, or a silicone preparation, for example an aqueous emulsion containing 30 % (*m/m*) of silicone.

5.2 Acetic acid solution.

Add 25,00 g of at least 99,7 % (*m/m*) acetic acid (CH_3COOH) to 200 ml of water, transfer quantitatively to a 250 ml one-mark volumetric flask and dilute to the mark at 20 °C.

5.3 Sodium acetate solution.

Dissolve 34,02 g of at least 99,0 % (*m/m*) sodium acetate trihydrate ($CH_3COONa \cdot 3H_2O$) in water, transfer quantitatively to a 250 ml one-mark volumetric flask and dilute to the mark at 20 °C.

To delay the growth of mould, 0,5 ml of chloroform ($CHCl_3$) may be added.

5.4 Washing solution.

Add 6,0 ml of the acetic acid solution (5.2) and 14,0 ml of the sodium acetate solution (5.3) to 1 000 ml of water at 20 °C and mix.

Check that the pH of the washing solution, measured with a pH meter, is 4,80 ± 0,05 at 20 °C and, if necessary, adjust by adding more acetic acid solution or sodium acetate solution, as appropriate.

5.5 Sucrose ($C_{12}H_{22}O_{11}$), having a nitrogen (N) content of not more than 0,002 % (*m/m*).

6 Apparatus

Usual laboratory equipment, and in particular

6.1 Balance, accurate to 0,01 g.

6.2 Conical flask, of capacity 150 or 200 ml, with a rubber stopper.

1) FAO/WHO Standard A-5 for whole milk powder, partly skimmed milk powder and skimmed milk powder, elaborated under the *Code of principles concerning milk and milk products,* 8th edition (1984), Rome : Food and Agriculture Organization of the United Nations/World Health Organization.

6.3 Thermometer, general purpose.

6.4 Graduated measuring cylinder, of capacity 100 ml.

6.5 Stirring rods, made of glass, one plain and the other rubber-tipped.

6.6 One-mark pipettes, of capacities 1 and 10 ml.

6.7 Beaker, of capacity 150 ml, short-form, with spout.

6.8 Filter funnel, internal diameter at the top of 75 mm.

6.9 Filter papers, ashless, medium speed and retention, of diameter 125 mm.

6.10 Wash bottles, of capacity 500 ml, for use with water and the washing solution (5.4).

7 Sampling

Take the laboratory sample in accordance with ISO 707. Store it in a clean, dry, securely closed, airtight container, which may be the intact, unopened retail container.

Note and report any deviations from these requirements.

8 Preparation of the test sample

Thoroughly mix the laboratory sample, which should be at laboratory temperature, 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, the mixing shall be carried out gently to avoid reducing the particle size of the sample.

9 Procedure (see also clause 11)

9.1 Test portion

Weigh into the conical flask (6.2) a test portion of 14,00 g of dried whole milk or dried partly skimmed milk, or of 10,00 g of dried skimmed milk, from the test sample (clause 8).

9.2 Determination

9.2.1 Transfer 100 ml of water at 40 °C to the graduated measuring cylinder (6.4) and add 10 ml of the water to the test portion so as to wash any of the test portion adhering to the walls of the flask into the bottom of the flask. Stir the mixture with the plain stirring rod (6.5) to disperse any lumps and to form a paste. Add the remainder of the water in successive small portions, with stirring, rinsing the stirring rod and neck of the flask with the final portions of the water. Close the flask with a rubber stopper, swirl the contents of the flask to mix thoroughly and allow the flask to stand for 30 min with periodic gentle swirling and inversion.

9.2.2 Remove the stopper from the conical flask, add 1 drop of the antifoaming agent (5.1) to the reconstituted milk, re-stopper the flask, and mix its contents by gentle swirling and inversion. Transfer 10 ml of the reconstituted milk, by means of a 10 ml pipette (6.6), filled so that the top level of the milk is coincident with the mark, to the beaker (6.7). Using the same pipette in exactly the same way, transfer 10 ml of the reconstituted milk to one of the four prepared Kjeldahl flasks (see annex A, A.2.1), i.e. each containing 15 g of potassium sulphate (see annex A, A.1.1) and three glass balls (see annex A, A.2.2). Wash the reconstituted milk from the neck into the Kjeldahl flask with a little water and set the flask aside.

9.2.3 Using the measuring cylinder (6.4), add 75 ml of water at 40 °C to the reconstituted milk in the beaker and then add, by means of a pipette (6.6), 1,0 ml of the acetic acid solution (5.2). Stir the mixture gently but thoroughly with the rubber-tipped stirring rod (6.5). Leave the stirring rod in the beaker.

9.2.4 After 10 min, add, by means of a pipette (6.6), 1,0 ml of the sodium acetate solution (5.3) to the contents of the beaker and stir the mixture gently but thoroughly. Leave the stirring rod in the beaker.

9.2.5 Allow the contents of the beaker to cool for 45 min, add 13 ml of water (at laboratory temperature) using the measuring cylinder, stir gently, and allow to stand for 15 min with the stirring rod in the beaker.

9.2.6 Filter the supernatant liquid in the beaker through a folded (not pleated) filter paper (6.9), previously wetted with the washing solution (5.4) (see the note). Using the stirring rod and the washing solution, transfer all the precipitate to the filter paper, taking care to include the precipitate adhering to the beaker. Finally, wash the precipitate and filter paper three times with the washing solution, allowing time for drainage after each washing.

NOTE — The adequacy of the precipitation and filtration procedure is indicated by the filtered supernatant liquid being perfectly clear and having a pH of 4,80 ± 0,05 at 20 °C.

9.2.7 Carefully remove the filter paper from the filter funnel (6.8), fold the paper to enclose the precipitate, and transfer the paper and precipitate to another of the prepared Kjeldahl flasks (see annex A, A.2.1).

9.2.8 Place a filter paper (6.9) in one of the two remaining prepared Kjeldahl flasks; this flask will be used to obtain the blank value V_1 (see 10.1). Place 0,1 g of the sucrose (5.5) in the remaining prepared Kjeldahl flask; this flask will be used to obtain the blank value V_3 (see 10.1).

9.2.9 Proceed as described in annex A, clause A.3.

10 Expression of results

10.1 Calculation of heat number

Calculate the heat number H of the laboratory sample by means of the formula

$$H = \frac{V_0 - V_1}{V_2 - V_3} \times 100$$

where

V_0 is the volume, in millilitres, of the standard volumetric solution used in the Kjeldahl determination with the precipitate (and filter paper) from 10 ml of the reconstituted milk (see annex A, A.3.5);

V_1 is the volume, in millilitres, of the standard volumetric acid solution used in the blank Kjeldahl determination with a filter paper (see annex A, A.3.5);

V_2 is the volume, in millilitres, of the standard volumetric acid solution used in the Kjeldahl determination with 10 ml of the reconstituted milk (see annex A, A.3.5);

V_3 is the volume, in millilitres, of the standard volumetric acid solution used in the blank Kjeldahl determination with 0,1 g of sucrose (see annex A, A.3.5).

Round the heat number to the nearest 0,1.

10.2 Precision

NOTE — The following values for repeatability and reproducibility, which are expressed for the 95 % probability level, were determined in an inter-laboratory collaborative study evaluated as described in ISO 5725.[1)]

10.2.1 Repeatability

The difference between two single values for heat number, obtained concurrently or within a short time-interval by one analyst on the same test sample using the same apparatus, should not exceed 1,2.

10.2.2 Reproducibility

The difference between two single values for heat number, obtained by two analysts in different laboratories on identical test samples, should not exceed 1,5.

10.3 Derivation of heat class

Derive the heat class of the laboratory sample from its heat number according to the proposed heat-classification scheme given in annex B.

11 Note on procedure

The total nitrogen content of the test sample can be calculated from the values for V_2 and V_3 (see 10.1) if

a) the mass of the reconstituted milk is obtained (by difference) by weighing the empty stoppered conical flask in 9.1 and the stoppered conical flask containing the reconstituted milk (see 9.2.1);

b) the mass of the reconstituted milk delivered by the 10 ml pipette, when used as described in 9.2.2, is determined; and

c) if the exact value for the concentration of the standard volumetric acid solution (see annex A, A.1.7) is known.

12 Test report

The test report shall include the following information :

a) a full identification of the laboratory sample, including sampling date and date received;

b) the heat number (see 10.1) and heat class (see 10.3), and the date they were determined;

c) a reference to this International Standard;

d) any observation that indicates that the results may be unreliable.

1) ISO 5725, *Precision of test methods — Determination of repeatability and reproducibility by inter-laboratory tests.*

Annex A
Kjeldahl method

(This annex forms part of the standard.)

A.0 Introduction

A feature of this new Kjeldahl method is that a small amount (0,05 g) of copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) is used as the catalyst instead of the mercury oxide specified in IDF Standard 20 (1962), a change consistent with current views [Rexroad and Cathey (1976)]. However, so that the new method could cope with the larger amounts of material to be digested for the determination of heat number in accordance with this International Standard (*cf.* IDF Standard 20, 1962, and IDF Standard 29, 1964), the volume of sulfuric acid (A.1.3) has had to be increased to 30 ml (see A.3.1) from the 25 ml appropriate for 5 ml of milk. As a consequence, the volume of water and sodium hydroxide solution (A.1.4) added has had to be reduced from 250 ml to 240 ml and increased from 60 ml to 70 ml, respectively (see A.3.3).

A.1 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical quality and only distilled water or water of equivalent purity.

A.1.1 Potassium sulfate (K_2SO_4).

A.1.2 Copper(II) sulfate solution.

Dissolve in water 5,0 g of copper(II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), transfer quantitatively to a 100 ml one-mark volumetric flask and dilute to the mark at 20 °C.

A.1.3 Sulfuric acid, at least 98,0 % (m/m), ϱ_{20} approximately 1,84 g/ml.

A.1.4 Sodium hydroxide, 47 % (m/m) solution, corresponding to 704 g of NaOH per litre at 20 °C.

NOTES

1 The sodium hydroxide need not be of recognized analytical grade.

2 A less concentrated sodium hydroxide solution may be used, for example 40 % (m/m) (572 g of NaOH per litre at 20 °C) or 30 % (m/m) (399 g of NaOH per litre at 20 °C); see the note to A.3.3.

A.1.5 Boric acid solution.

Dissolve 40,0 g of boric acid (H_3BO_3) in 1 000 ml of hot water, allow to cool, and store in a borosilicate-glass bottle.

A.1.6 Indicator solution.

Mix equal volumes of 2 g/l ethanolic methyl red solution and of 1 g/l ethanolic methylene blue solution [the ethanol should be at least 94 % (V/V)].

A.1.7 Sulfuric acid or **hydrochloric acid,** standard volumetric solution, $c(1/2\ H_2SO_4)$ or $c(HCl) = 0,1$ mol/l.

NOTE — The exact concentration of this solution is not required for the calculation of the heat number (see 10.1), but see clause 11.

A.2 Apparatus

Usual laboratory equipment, and

A.2.1 Kjeldahl flasks, of capacity 500 ml.

A.2.2 Glass balls, of diameter approximately 5 mm.

A.2.3 Burette or **automatic pipette,** for delivering 1,0 ml portions of the copper sulfate solution (A.1.2).

A.2.4 Graduated measuring cylinders, made of glass, of capacities 50, 100 and 250 ml.

A.2.5 Digestion apparatus, to hold the Kjeldahl flasks (A.2.1) in an inclined position (approximately 45°), with electric heaters or gas burners that do not heat the flasks above the level of their contents, together with a fume extraction system.

A.2.6 Distillation apparatus, made of borosilicate glass, to which a Kjeldahl flask (A.2.1) can be fitted, and consisting of an efficient splash-head connected to an efficient condenser (with straight inner tube; jacket at least 300 mm long) with an outlet tube attached to its lower end; the connecting tubing and stopper(s) shall be close-fitting and shall preferably be made of neoprene rubber.

A.2.7 Pipette or **automatic pipette,** for delivering 0,10 ml portions of the indicator solution (A.1.6).

A.2.8 Conical flasks, of capacity 500 ml, graduated at 200 ml.

A.2.9 Burette, of capacity 50 ml, graduated in 0,1 ml, maximum error ± 0,05 ml.

A.2.10 Magnifying lens, for reading the burette (A.2.9).

A.3 Procedure

A.3.1 To each of the four Kjeldahl flasks (see 9.2.2, 9.2.7 and 9.2.8), add 1,0 ml of the copper sulfate solution (A.1.2) and 30 ml of the sulfuric acid (A.1.3), using the acid to wash down any copper sulfate solution on the neck of the flask, and gently mix the contents of each flask.

A.3.2 Heat each Kjeldahl flask on the digestion apparatus (A.2.5), very gently at first so that the black froth stays within the bulb. When the initial frothing has ceased and copious white vapour appears, boil vigorously (acid vapour condensing half-way up the neck of the flask) until no black particles remain and until the digest becomes clear pale yellow-green in colour. Then boil gently for 1,5 h. Note the following requirements :

a) If black particles enter the neck of the flask and these are not all washed down into the bulb by the refluxing acid during the initial stages of the vigorous boiling period, allow the flask to cool sufficiently and wash the particles into the bulb with the minimum of water. Then continue the digestion as described above.

b) The timing of the 1,5 h boiling period shall not be started until a pale green tint becomes visible in the clear digest.

A.3.3 When the Kjeldahl flasks are cool, add 240 ml of water (see the note) to each so as to wash the neck of the flask, and mix the contents thoroughly and until the crystals which separate out are dissolved. Then add to each flask, 70 ml of the sodium hydroxide solution (A.1.4) (see the note) by gently pouring the solution down the inclined neck of the flask to form a bottom layer in the bulb; do not wet the top of the neck with the sodium hydroxide solution.

NOTE — It is necessary that the combined volume of water and sodium hydroxide solution added be 310 ml to enable approximately 150 ml of distillate to be collected just before irregular boiling ("bumping") starts (see A.3.4). Thus, if a larger equivalent volume of a sodium hydroxide solution less concentrated than 47 % (m/m) is to be added, the volume of water added shall be reduced accordingly. For example, if 85 ml of 40 % (m/m), or 125 ml of 30 % (m/m), sodium hydroxide solution are to be added, the volume of water added shall be 225 ml or 185 ml respectively.

A.3.4 Immediately connect each Kjeldahl flask to the distillation apparatus (A.2.6), the tip of the condenser outlet-tube of which shall be immersed in 50 ml of the boric acid solution (A.1.5) plus 0,10 ml of the indicator solution (A.1.6), contained in a conical flask (A.2.8). Swirl the contents of each Kjeldahl flask to mix thoroughly, and boil, gently at first to prevent excessive frothing. When 100 to 125 ml of distillate have been collected, lower each conical flask until the tip of the condenser outlet-tube is approximately 40 mm above the 200 ml mark. Continue distillation of the contents of each flask until irregular boiling ("bumping") starts and then immediately stop heating. Disconnect each Kjeldahl flask and rinse the tip of each condenser outlet-tube with a little water, collecting the rinsings in the conical flask. Note the following requirements :

a) The distillation rate shall be such that approximately 150 ml of distillate are collected in approximately 30 min; when irregular boiling ("bumping") starts, the volume of the contents of each conical flask will be approximately 200 ml, i.e. approximately 150 ml of distillate will have been collected, as required.

b) The efficiency of each condenser shall be such that the temperature of the contents of each conical flask does not exceed 25 °C during the distillation.

A.3.5 Titrate each distillate with the standard volumetric acid solution (A.1.7) until the colour matches that of a freshly prepared solution consisting of 150 ml of water, 50 ml of the boric acid solution (A.1.5) and 0,10 ml of the indicator solution (A.1.6), contained in a conical flask (A.2.8). Take each burette reading to 0,01 ml with the aid of the magnifying lens (A.2.10), avoiding errors of parallax.

NOTE — The titration of each blank distillate will require only a very small volume of the standard volumetric solution.

Annex B

Proposed heat-classification scheme for spray-dried milk

(This annex forms part of the standard.)

B.0 Introduction

Fairly recent surveys of the casein number of raw bulk cows' milk have shown that while annual average values are likely to be in the range from 77,0 to 78,0, there is a small, but definite, seasonal variation [Muir, Kelly, Phillips and Wilson (1978)]. This seasonal variation in casein number makes it impracticable to use the heat number of dried milk to identify a dried milk that has received minimal heat treatment during manufacture, i.e. extra-low heat dried milk containing only a very small amount of denatured milk-serum protein. Accordingly, in the proposed heat-classification scheme, the lowest heat class is simply low heat, which includes all dried milks with a heat number of 80,0 or less that will thus have sustained minimal to mild heat treatment during manufacture.

As the degree of heat treatment during manufacture increases, the heat number of dried milk progressively increases, the maximum value obtainable in practice being approximately 91. This maximum is slightly less than might be expected (by approximately 1 or 2) and is attributable to incomplete precipitation of the denatured milk-serum protein with the casein at pH 4,8 and also to slight casein hydrolysis during the more severe preheating treatments. There is, however, no need to set a precise upper limit for heat number in a heat-classification scheme.

The overall range of heat number for spray-dried milks that have received differing degrees of heat treatment during manufacture can be subdivided into four bands corresponding to four heat classes on the basis of the relationship between heat number and the protein pattern obtained by polyacrylamide gel electrophoresis of the undenatured milk-serum protein isolated from the dried milk.

B.1 Proposed heat-classification scheme

The proposed heat-classification scheme for spray-dried milk, based on the heat number of the dried milk determined as described in this International Standard, is shown in the table. The approximate correspondence of each heat class to the polyacrylamide gel (PAG) electrophoresis pattern of the undenatured milk-serum protein (UMSP) and to the concentration of undenatured milk-serum protein nitrogen (UMSPN) obtained by the heat-classification method of the American Dry Milk Institute (the ADMI whey-protein index; see clause 0) is also shown.

Table — Heat-classification scheme

Heat number	Heat class	PAG electrophoresis pattern of UMSP	UMSPN (mg/g of dried milk)
80,0 or less	Low heat	All bands clearly visible or immunoglobulin band faint	$\geqslant$ 6,0
80,1 to 83,0	Medium heat	Immunoglobulin band just visible or absent; blood-serum albumin band faint	4,5 to 5,9
83,1 to 88,0	Medium-high heat	Blood-serum albumin band just visible or absent; β-lactoglobulin bands faint; δ-lactalbumin band reduced	1,5 to 4,4
88,1 or more	High heat	β-lactoglobulin bands just visible or absent; α-lactalbumin band just visible or absent	$\leqslant$ 1,4

Bibliography

American Dry Milk Institute (1971). *Standards for grades of dry milk including methods of analysis,* Bulletin 916 (Revised), Chicago : American Dry Milk Institute, Inc.

de KONING, P. J., van ROOIJEN, P. J. and DRAAISMA, J.T.M. (1976). *Milchwissenschaft,* **31**, 261.

IDF Standard 20 (1962). *Determination of the total nitrogen content of milk by the Kjeldahl method.*

IDF Standard 29 (1964). *Determination of the casein content of milk.*

McGANN, T.C.A., O'CONNELL, J. A. and McFEELY, R. (1975). *Journal of the Society of Dairy Technology,* **28**, 23.

MROWETZ, G. and KLOSTERMEYER, H. (1977). *Milchwissenschaft,* **32**, 9.

MUIR, D. D., KELLY, M. E., PHILLIPS, J. D. and WILSON, A. G. (1978). *Journal of the Society of Dairy Technology,* **31**, 137.

REXROAD, P. R. and CATHEY, R. D. (1976). *Journal of the Association of Official Analytical Chemists,* **59**, 1213.

ROWLAND, S. J. (1938a). *Journal of Dairy Research,* **9**, 30 and 42.

ROWLAND, S. J. (1938b). *Journal of Dairy Research,* **9**, 47.

ROWLAND, S. J. and ZEIN-EI-DINE, M. (1938). *Journal of Dairy Research,* **9**, 174.

SANDERSON, W. B. (1970). *New Zealand Journal of Dairy Science and Technology,* **5**, 46 and 48.

VOSS, E. and SCHMANKE, E. *Milchwissenschaft,* **28** (1973), p. 645.

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