COMPENDIUM OF INDIAN STANDARDS ON TEST METHODS FOR MILK POWDERS

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1. Introduction

1.1 The milk production in our country is characterized by seasonal variations and drying of milk, an important method of preservation, facilitates later consumption during the lean season. The dried milk products, thus, have become an essential part of the chain between the producer and the consumer. Milk Powders/powdered milk/dried milk/dry milk are milk products manufactured using the technology of evaporation of water from milk by multiple methods to make it dry. Milk powders have very less moisture content resulting in longer shelf-life under ambient storage conditions. Hence, drying of milk has been the most popular method of preservation for a highly perishable commodity like milk. Additional advantage of milk powders is the reduction in the bulk which results in economy of transportation.

1.2 BIS through its Dairy Products Sectional Committee, FAD 19 has developed the following 4 Indian Standards on milk powder:

i) IS 1165 : 2022 Whole milk powder – Specification (*sixth revision*)
ii) IS 13334 (Part 1) : 2014 Skimmed milk powder — Specification Part 1 Standard grade (*second revision*)
iii) IS 13334 (Part 2) : 2014 Skimmed milk powder — Specification Part 2 Extra grade (*first revision*)
iv) IS 14542 : 1998 Partly skimmed milk powder — Specification

1.3 Physicochemical characteristics of milk powders (such as moisture content, milk protein in milk solids not fat, milk fat, insolubility index, total ash, titratable acidity, lactate content and scorched particles), microbiological requirements and limits of heavy metals and contaminants (Lead, Arsenic, Aflatoxin M_1 and melamine) have been specified in these standards which are required to be tested as per relevant Indian Standards on test methods.

1.4 This compendium aims at providing an overview of Indian Standards on methods of test for physicochemical parameters and contaminants in milk powders. This document may be used as ready reference by the industry, laboratory and other stakeholders involved in manufacture and testing of milk powders.

2. Testing of Physicochemical Characteristics

2.1 Moisture content

- **IS 11623 : 2008/ISO 5537 : 2004 'Dried milk — Determination of moisture content (Reference method) (*first revision*)'**
- **IS 16072 : 2012 'Determination of moisture content in milk powder and similar products (Routine method)'**

Moisture content is one of the most vital indicators of shelf stability of milk powders. For the determination of moisture content, IS 11623 has been prescribed for reference purpose and IS 16072 for routine purpose. IS 11623 is identical adoption of ISO 5537 : 2004. It is based on the principle that a test portion is dried in a drying oven set at 87 °C for 5 h while dry air is passed through the test portion. The loss of mass of the test portion (which is related to the content of "non-chemically bound" water) is determined. IS 16072 : 2012 provides the method for moisture content determination for routine analysis. As per IS 16072, the sample is dried using flat-bottom moisture dishes (with covers made of stainless steel, nickel or aluminium having approximately 50 mm diameter and 25 mm depth) in a forced draft hot air oven to constant weight at 102 ± 2 °C and the loss in weight reported as moisture.

2.2 Fat content

- **IS 1224 (Part 2) : 1977 'Determination of fat by the Gerber method: Part 2 Milk products (*first revision*)'**
- **IS 11721 : 2013/ISO 1736 : 2008 'Dried milk and dried milk products — Determination of fat content — Gravimetric method (Reference method) (*second revision*)'**

The fat content of milk powder plays a crucial role in product classification and consumer information. For the analysis of fat content, Gerber method given in IS 1224 (Part 2) has been prescribed for routine purpose and IS 11721 has been prescribed for reference purpose. The Gerber method for determination of fat in milk and milk products depends on the liberation of the fat by the action of sulphuric acid on milk or milk products in specially shaped, calibrated glass containers, called butyrometers which are then

centrifuged to aid the separation of fat, the volume of which is finally read off against the percentage scale etched on the butyrometer.

IS 11721 : 2013 is identical adoption of ISO 1736 : 2008. As per the method given in IS 11721, an ammoniacal ethanolic solution of a test portion is extracted with diethyl ether and light petroleum. The solvents are removed by distillation or evaporation. The mass of the substances extracted is determined. This is usually known as the Röse-Gottlieb principle. The method is also applicable to dried milk with a fat content of 40 % mass fraction or more, dried whole, dried partially skimmed, and dried skimmed milk, dried whey, dried buttermilk and dried butter serum. The method is not applicable when the powder contains hard lumps which do not dissolve in ammonia solution or free fatty acids in significant quantities.

2.3 Protein – IS 11917: 2018/ISO 8968-1 : 2014 'Milk and milk products — Determination of nitrogen content — Kjeldahl principle and crude protein calculation (*first revision*)'

Protein levels reflect the quality of the milk used and help detect any adulteration with non-dairy solids such as starch or whey substitutes. IS 11917: 2018 is identical adoption of ISO 8968-1 : 2014. It specifies a method for the determination of the nitrogen content and crude protein calculation of milk and milk products by the Kjeldahl principle, using traditional and block digestion methods.

The Kjeldahl method is used as the standard reference method for determining the protein content of foods. It involves digesting (wet ashing) the organic matter in the sample (lipids, proteins, carbohydrates, etc.) with concentrated H_2SO_4 at 370 to 400 °C. K_2SO_4 is added to increase the boiling point of the H_2SO_4 and a catalyst [Cu ($CuSO_4$), is added. During digestion, Carbon in the sample is converted to CO_2, oxygen to CO_2 and H_2O, Hydrogen to H_2O and Nitrogen to $(NH_4)_2SO_4$. Some H_2SO_4 is degraded to the irritating and toxic gas, SO_2. When digestion is complete, as indicated by clearing of the sample (light blue colour), on distillation the solution is made strongly to green; the NH_3 trapped raises the pH abruptly (boric acid has little buffering at acidic pH) and causes a change in the colour of the indicator; at the end of distillation, the H_3BO_3 is back-titrated with standard

HCl to restore the original colour of the indicator. Only one standard solution, HCl, is required. The protein content of the milk sample can be determined by multiplying % N by 100/15.7 [i.e., %N × 6.38].

2.4 Ash – Annex C of IS 14433 : 2022 'Infant milk substitutes — Specification (*second revision*)'

Excessive ash levels may indicate either adulteration (e.g., with sodium carbonate) or the result of over-processing and thermal stress. Method given in Annex C of IS 14433 : 2022 has been referred in the Indian Standards for determination of ash content in milk powders. Dry ashing refers to the use of a muffle furnace capable of maintaining temperatures of 500–600 °C. Water and volatiles are vaporized, and organic substances are burned in the presence of oxygen in air to CO_2 and oxides of N_2. Most minerals are converted to oxides, sulfates, phosphates, chlorides, and silicates. Ash content represents the total mineral content in foods.

2.5 Titratable acidity – IS 11765 : 2017/ISO 6091 : 2010 'Dried milk – Determination of titratable acidity (Reference Method) (*first revision*)'

Titratable acidity is often used to monitor the acidity of dairy products since most dairy fermentations predominately produce lactic acid. Titratable acidity serves as an indicator of freshness and microbial stability. IS 11765 specifies a reference method for the determination of the titratable acidity of all types of dried milk. IS 11765 : 2017 is identical adoption of ISO 6091 : 2010. As per the method given in IS 11765, sodium hydroxide (0.1 N) solution is gradually added to milk in the presence of phenolphthalein indicator. The end point of the neutralization reaction between NaOH and lactic acid is indicated by the color of the samples changing to a light pink. For dried milk the titratable acidity is expressed as the number of millilitres of 0.l mol/l sodium hydroxide solution required to neutralize, in the presence of phenolphthalein, quantity of the reconstituted milk corresponding to 10 g of solids-not-fat, until the appearance of a pink coloration.

2.6 Lactic acid and lactate – IS 11202 : 2012/ISO 8069 : 2005 'Dried milk — Determination of content of lactic acid and lactates (*second revision*)'

Measuring lactate content in milk powder is crucial for ensuring food safety, quality, and compliance with standards, as high levels can indicate bacterial contamination or improper processing, and it helps assess the product's history and preservation status. IS 11202 : 2012 which is identical adoption of ISO 8069 : 2005 specifies an enzymatic method for the determination of the lactic acid and lactates content of all types of dried milk.

The test can be run on milk powder after reconstitution in water. A test portion of dried milk is dissolved in warm water. The fat and proteins are precipitated then filtered. The filtrate is treated with the following enzymes and biochemical substances, added simultaneously, but acting in sequence:

a) L-lactate dehydrogenase (L-LDH) and D-lactate dehydrogenase (D-LDH), in the presence of nicotinamide adenine dinucleotide (NAD), to oxidize lactate to pyruvate and to convert NAD to its reduced form NADH;
b) glutamate pyruvate transaminase (GPT), in the presence of L-glutamate, to transform pyruvate into L-alanine and to convert L-glutamate to α-ketoglutarate.

The amount of NADH produced is determined by spectrophotometric measurement at a wavelength of 340 nm, and is proportional to the lactic acid and lactates content.

2.7 Solubility/insolubility index – IS 12759 : 2019 'Dried milk and dried milk products — Determination of insolubility index (*first revision*)'

Insolubility Index is volume in ml, 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 standard. IS 12759 specifies a method of determining the insolubility index, as a means of assessing the solubility, of whole milk powder, partly skimmed milk powder and skimmed milk powder, whether non instant or instant. 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.

Water at 24°C (or at 50°C if appropriate) 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.

2.8 Scorched particles – IS 13500 : 1992 'Spray dried milk powders — Scorched particles — Determination'

The presence of scorched particles, visible as small brown or black flakes, is indicative of poor heat control during the drying stage. These particles degrade the sensory profile and consumer perception of quality.

IS 13500 specifies method for determination of scorched particles in spray dried milk powders including infant foods. In this method, 25 g of non-fat dry milk or dry butter milk or 32.5 g dry whole milk is reconstituted in 250 ml sediment free water along with 0.5 mL of diglycollaurate S in a blender for 60 secs and filter the entire solution through a standard cotton disc, using an aspirator or pressure type tester and dried. The resultant disc is compared with the scorched particle disc test cards.

3. Testing for Heavy Metals and Contaminants

3.1 Lead – IS 12074 : 1987 'Method for determination of lead by atomic absorption spectrophotometer'

Lead (Pb) is a type of heavy metal with toxic properties that easily accumulate in organs, making it very dangerous for human life. Milk can be contaminated with heavy metals from the environment, such as Pb. IS 12074 prescribes the method for determination of lead by atomic absorption spectrophotometer. The sample is brought into the solution by

suitable treatment with acids or acid combinations, diluted with distilled water, filtered and suitable dilutions are made for aspiration into the air acetylene flame. The standard solution is made in the same way for calibration. The most sensitive lead line is 217.0 nm, however, other lines suitable for higher concentration can also be used.

3.2 Arsenic – IS 11124 : 1984 'Method for atomic absorption spectrophotometric determination of arsenic'

Milk has been reported to be contaminated with arsenic (As) in some parts of the world. The hydride generation technique (HG) has been widely used in the determining of chemical forms of As in biological matrices. IS 11124 prescribes the atomic absorption spectrophotometric method for the determination of arsenic by hydride generation technique. Samples are usually digested using HNO_3–$HClO_4$–H_2SO_4 and the analytical solution is prepared with HCl. Warming of the analytical solution is needed to pre-reduce from As(V) to As(III), usually with potassium iodide prior to generation of their hydrides with sodium borohydride. The hydrides are transported to a quartz tube or graphite furnace and heated to produce atomization for atomic absorption.

3.3 Melamine – IS 16195 : 2014/ISO/TS 15495 : 2010 'Milk, milk products and infant formulae — Guidelines for the quantitative determination of melamine and cyanuric acid by LC-MS/MS'

The presence of melamine and cyanuric acid in milk powder raises concerns due to their potential health risks. IS 16195 : 2014 which is identical adoption of ISO/TS 15495 : 2010 gives guidance for the quantitative determination of melamine and cyanuric acid content in milk, powdered milk products, and infant formulae by electrospray ionization liquid chromatography tandem mass spectrometry (LC-MS/MS). The principle of the method is that the sample is made homogenous and optionally reconstituted in the case of powdered samples. A suitable solvent is added to the test sample to precipitate proteins from the matrix and to extract melamine and cyanuric acid. After centrifugation, an aliquot of the supernatant is analysed by LC-MS/MS. LC-MS/MS designates any method combining either high-performance liquid chromatography (HPLC) or ultra-performance liquid chromatography (UPLC), with either triple quadrupole or ion-trap mass

spectrometric detection. Chromatographic separation is based on hydrophilic interaction liquid chromatography (HILIC) to ensure good separation of melamine and cyanuric acid. Ionization of the substance is accomplished by electrospray ionization (ESI) and detection operates in the selected reaction monitoring (SRM) mode. Quantification of both melamine and cyanuric acid is based on isotope dilution using labelled ($^{13}C_3$, $^{15}N_3$)-melamine and labelled ($^{13}C_3$, $^{15}N_3$) - cyanuric acid as internal standards (ISs).

3.4 Aflatoxin M_1

- **IS 18108 : 2023/ISO 14674:2005 'Milk and milk powder — Determination of aflatoxin M_1 content — Clean-up by immune affinity chromatography and determination by thin-layer chromatography'**
- **IS 18110 : 2023/ISO 14501:2021 'Milk and milk powder — Determination of aflatoxin M1 content — Clean-up by immune affinity chromatography and determination by high-performance liquid chromatography'**

Aflatoxins are toxic, carcinogenic, and/or teratogenic to humans and animals. Aflatoxin M_1 and M_2, are potentially important contaminants in dairy products. AFM_1 is relatively stable in raw and processed milk products and cannot be destroyed by heat treatments or pasteurization.

IS 18108 : 2023 which is identical adoption of ISO 14674:2005 specifies a method for the determination of the aflatoxin M_1 (AFM1) content of milk and milk powder by a method including a clean-up step using immunoaffinity chromatography followed by a thin-layer chromatography (IAC-TLC). The method is applicable to raw milk, low fat or skimmed liquid milk and milk powder. The lowest quantity of AFM1 that can commonly be determined is 2 ng, which corresponds to a limit of quantification close to 0.10 µg/l for liquid milk or dissolved milk powder (for a spot of 20 µl). Aflatoxin M_1 (AFM1) is extracted by passing the test portion through an immunoaffinity column. The column contains specific antibodies bound onto a solid support material. As the sample passes through the column, the antibodies selectively bind with any AFM1 (antigen) present and form an antibody-antigen complex. All other components of the sample matrix are washed off the column with water. Then the AFM1 is eluted from the column with methanol and acetonitrile. After concentration of the eluate, the amount of AFM1 is

determined by one-dimensional thin-layer chromatography. In the case of interference, two-dimensional thin layer chromatography is carried out to separate the AFM1 from its impurities.

IS 18110 : 2023 which is identical adoption of ISO 14501:2021 specifies a method for the determination of aflatoxin M_1 content in milk and milk powder. The lowest level of validation is 0.08 µg/kg for whole milk powder, i.e. 0.008 µg/l for reconstituted liquid milk. The limit of detection (LOD) is 0.05 µg/kg for milk powder and 0.005 µg/kg for liquid milk. The limit of quantification (LOQ) is 0.1 µg/kg for milk powder and 0.01 µg/kg for liquid milk. The method is also applicable to low-fat milk, skimmed milk, low-fat milk powder and skimmed milk powder. Aflatoxin M_1 is extracted by passing the test portion through an immunoaffinity column that contains specific antibodies bound onto a solid support material. As the sample passes through the column, the antibodies are selectively bound with any aflatoxin M_1 (antigen) present and form an antibody-antigen complex. All other components of the sample matrix are washed off the column with water. Then aflatoxin M_1 is eluted from the column and the eluate is collected. The amount of aflatoxin M_1 present in this eluate is determined by means of HPLC coupled with fluorimetric detector.