
International Standard



5542

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

Milk — Determination of protein content — Amido black dye-binding method (Routine method)

Lait — Détermination de la teneur en protéines — Méthode au noir amido (Méthode pratique)

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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 developing International Standards is carried out through ISO technical committees. Every member body interested in a subject for which a technical committee has been authorized 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.

International Standard ISO 5542 was developed by Technical Committee ISO/TC 34, *Agricultural food products*, and was circulated to the member bodies in April 1983.

It has been approved by the member bodies of the following countries :

Australia	Iran	South Africa, Rep. of
Austria	Iraq	Spain
Belgium	Korea, Dem. P. Rep. of	Sri Lanka
Canada	Malaysia	Tanzania
Cuba	Netherlands	Thailand
Czechoslovakia	New Zealand	Turkey
Egypt, Arab Rep. of	Philippines	United Kingdom
France	Poland	USSR
Germany, F. R.	Portugal	Yugoslavia
Hungary	Romania	

No member body expressed disapproval of the document.

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.

Milk — Determination of protein content — Amido black dye-binding method (Routine method)

1 Scope and field of application

1.1 This International Standard describes the amido black dye-binding method, used as a routine method, for the determination of the protein content of milk.

As the composition of the amido black dyestuff is variable, the method described is empirical and depends upon constant reference to the protein content derived from determination of the nitrogen content of milk by the Kjeldahl reference method (for example, as described in IDF Standard 20).

1.2 The method is applicable to raw or thermally or mechanically processed (for example pasteurized, sterilized, homogenized, reconstituted) whole milk, partially skimmed milk and skimmed milk, provided that the samples are in good condition. The method is also applicable, in some cases, to preserved samples (see 10.1).

The method allows a rapid and simple determination of the protein content of milk to be made and is suitable for a single determination or for determinations on small numbers of samples and for series of multiple determinations. For determinations in series, special apparatus (i.e. multiple pipetting apparatus, centrifuges for racks) is required (see 6.4 and 6.7), and frequent checking of control samples for correction of "drift" is required (see 8.6.2). In view of the time-consuming method of calibration, laboratories which make only a few determinations on certain types of samples often have recourse to central laboratories for dye solutions and control samples.

NOTE — According to the origin of the sample and the reference method used, the method described in this International Standard may be used not only for the usual determination of the protein content of milk (i.e. total nitrogen $\times 6,38$), but also for the determination of the "true protein" content, or with modifications, the casein or whey protein content of cow's milk and milk from other species (goat, sheep, etc.).

2 References

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

IDF Standard 20 : *Determination of the nitrogen content of milk by the Kjeldahl method*.

3 Definition

protein content : A conventional value obtained by multiplying the nitrogen content, expressed as a percentage by mass, determined in accordance with the Kjeldahl reference method (for example, as described in IDF Standard 20), by an appropriate factor.

NOTE — Care should be taken to distinguish the "protein content" of milk as defined above from the "true protein content" which excludes the non-protein nitrogen (NPN) fraction of milk.

4 Principle

Addition of amido black solution, buffered at pH 2,4, to a test portion, resulting in the formation of an insoluble dye-protein complex. Removal of the insoluble complex by centrifuging (or filtration), and determination of the protein content from the absorbance of the resulting solution containing an excess of dye.

5 Reagents

All reagents shall be of recognized analytical grade unless otherwise stated. The water used shall be distilled water or water of at least equivalent purity.

NOTE — Calcium ions interfere with the determination.

5.1 Amido black 10B dyestuff (acid black 1, CI 20470)¹⁾ for milk testing, having a moisture content of less than 5 % (m/m), or a further purified product.

NOTE — The dyestuff is hygroscopic and should be protected from moisture uptake.

¹⁾ Suitable material is available commercially. Details may be obtained from the Secretariat of ISO/TC 34 (MSZH, Hungary) or from ISO Central Secretariat.