
**Dried milk, dried ice-mixes and processed
cheese — Determination of lactose
content —**

**Part 2:
Enzymatic method utilizing the galactose
moiety of the lactose**

*Lait sec, mélanges secs pour crèmes glacées et fromages fondus —
Détermination de la teneur en lactose —*

Partie 2: Méthode enzymatique par la voie galactose



Reference numbers
ISO 5765-2:2002(E)
IDF 79-2:2002(E)

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Printed in Switzerland

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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 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 part of ISO 5765|IDF 79 may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 5765-2|IDF 79-2 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.

ISO 5765|IDF 79 consists of the following parts, under the general title *Dried milk, dried ice-mixes and processed cheese — Determination of lactose content*:

- Part 1: *Enzymatic method utilizing the glucose moiety of the lactose*
- Part 2: *Enzymatic method utilizing the galactose moiety of the lactose*

Annex A forms a normative part of this part of ISO 5765|IDF 79.

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 *Lactose and lactate determination*, of the Standing Committee on *Main components of milk*, under the aegis of its project leader, Mr J. Labrijn (NL).

Introduction

This part of ISO 5765|IDF 79 describes the enzymatic method for the determination of lactose utilizing the galactose moiety of the lactose. It is complementary to ISO 5765-1|IDF 79-1 which utilizes the glucose moiety of the lactose.

The choice of whether to use the method described in part 1 or part 2 of ISO 5765|IDF 79 depends from the amount of glucose or galactose present in the sample to be analysed. If the glucose content of a sample is considerably higher than its lactose content, it is recommended to use the method described in this part of ISO 5765|IDF 79. Conversely, for a sample with a considerably higher galactose content than its lactose content, it is recommended to use the method described in ISO 5765-1|IDF 79-1.

For samples with a low content of both glucose and galactose, either method may be used without preference. For samples with a high content of both glucose and galactose, the accuracy of the lactose determination is considerably reduced for both methods.

In heat-treated milk and milk products, a proportion of lactose may have been converted to lactulose. Lactulose cannot be determined by applying the method described in ISO 5765-1|IDF 79-1. If, however, the method in this part of ISO 5765|IDF 79 is applied, the lactulose will partially be determined as lactose. Moreover, in intensively heat-treated milk (e.g. sterilized milk) or milk products, a proportion of the lactose may be bound to protein because of a Maillard reaction. In such cases the bound lactose cannot be determined by the method described either in part of ISO 5765|IDF 79.

Only when the good laboratory practice (GLP) rules for enzymatic analyses have been applied strictly, will reliable results be obtained. The GLP rules are stated in annex A.

Dried milk, dried ice-mixes and processed cheese — Determination of lactose content —

Part 2:

Enzymatic method utilizing the galactose moiety of the lactose

1 Scope

This part of ISO 5765|IDF 79 specifies an enzymatic method for the determination of the lactose content of all types of dried milk, of ice-mixes in dry form in the presence of other carbohydrates and reducing substances, and of processed cheese.

2 Terms and definitions

For the purposes of this part of ISO 5765|IDF 79, the following term and definition applies.

2.1

lactose content

mass fraction of substances determined by the procedure in this part of ISO 5765|IDF 79

NOTE The lactose content is expressed as a percentage by mass.

3 Principle

3.1 A solution or suspension of the test portion is deproteinated to obtain a pure extract.

3.2 The purified extract of the test portion is reacted with the following enzymes and biochemical substances:

- a) β -galactosidase, to split the lactose into glucose and galactose;
- b) β -galactosidase dehydrogenase in the presence of nicotinamide adenine dinucleotide phosphate (NAD^+) to catalyse the oxidation of galactose into galactonic acid, the NAD^+ being reduced to NADH.

3.3 The amount of NADH is determined from the absorbance of the test solution at 340 nm.

3.4 The lactose content is calculated, which is proportional to the amount of NADH if a correction is made for the galactose present in the test sample at the start of the analysis.

4 Reagents

Use only reagents of recognized analytical grade unless otherwise specified. The water used for the preparation of the enzyme solutions shall be of at least doubly glass-distilled purity. The water used for other purposes shall be glass-distilled or of at least equivalent purity.