INTERNATIONAL STANDARD 3356

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

Milk and dried milk, buttermilk and buttermilk powder, whey and whey powder — Determination of phosphatase activity (Reference method)

Lait et lait sec, babeurre et poudre de babeurre, sérum et poudre de sérum — Détermination de l'activité phosphatasique (Méthode de référence)

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FOREWORD

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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 3356 was drawn up by Technical Committee ISO/TC 34, *Agricultural food products*, and circulated to the Member Bodies in April 1974.

It has been approved by the Member Bodies of the following countries :

Austria	Hungary	Poland
Belgium	India	Romania
Bulgaria	Iran	South Africa, Rep. of
Canada	Ireland	Spain
Chile	Israel	Thailand
Czechoslovakia	Italy	Turkey
France	Netherlands	U.S.S.R.
Germany	New Zealand	Yugoslavia

The Member Body of the following country expressed disapproval of the document on technical grounds :

United Kingdom

Printed in Switzerland

Milk and dried milk, buttermilk and buttermilk powder, whey and whey powder — Determination of phosphatase activity (Reference method)

1 SCOPE AND FIELD OF APPLICATION

This International Standard specifies a reference method for the determination of the phosphatase activity in milk and dried milk, buttermilk and buttermilk powder, whey and whey powder.

The method can be applied for the control of proper pasteurization of these products.

2 REFERENCE

ISO/R 707, *Milk and milk products — Sampling.*

3 DEFINITION

For the purposes of this International Standard, the following definition applies :

phosphatase activity : The quantity of active alkaline phosphatase present in the product, expressed as the quantity of phenol, in micrograms, liberated under the specified conditions by 1 ml of the liquid product or, in the case of dried products, by 1 ml of the reconstituted liquid product.

4 PRINCIPLE

Dilution of the liquid product or the reconstituted liquid product with a buffer at pH 10,6 containing disodium phenylphosphate and incubation at 37 °C for 1 h, liberating phenol by reaction with the alkaline phosphatase present in the product. Reaction of the phenol with dibromoquinonechloroimide and photometric measurement of the colour formed.

5 REAGENTS

All reagents shall be of analytical reagent quality and water shall be freshly boiled distilled water, or water of a least equal purity, free from carbon dioxide.

5.1 Barium borate-hydroxide buffer.

5.1.1 Dissolve 50,0 g of barium hydroxide [$Ba(OH)_2 \cdot 8H_2O$], free from carbonate, in water to a volume of 1 000 ml.

5.1.2 Dissolve 22,0 g of boric acid (H_3BO_3) in water to a volume of 1 000 ml.

5.1.3 Warm 500 ml of each solution to 50 °C, mix the solutions, stir, cool rapidly to about 20 °C, adjust the pH if necessary to 10,6 ± 0,1 by addition of solution 5.1.1 or 5.1.2 and filter.

Store the solution in a tightly stoppered container.

Dilute the solution before use with an equal volume of water.

5.2 Colour development buffer.

Dissolve 6,0 g of sodium metaborate ($NaBO_2$) or 12,6 g of $NaBO_2 \cdot 4H_2O$, and 20,0 g of sodium chloride (NaCl) in water to a volume of 1 000 ml.

5.3 Colour dilution buffer.

Dilute 10 ml of the colour development buffer (5.2) to 100 ml with water.

5.4 Buffer substrate.

Dissolve 0,5 g of disodium phenylphosphate ($Na_2C_6H_5PO_4 \cdot 2H_2O$) in 4,5 ml of the colour development buffer (5.2), add two drops of the BQC solution (5.6) and let stand at room temperature for 30 min. Extract the colour so formed with 2,5 ml of butan-1-ol and let stand until the butan-1-ol separates. Remove the butan-1-ol and discard. Repeat this extraction if necessary.

The solution may be stored in a refrigerator for a few days; develop the colour and re-extract before use. Prepare the buffer substrate immediately before use by diluting 1 ml of this solution to 100 ml with the barium borate-hydroxide buffer (5.1).

5.5 Zinc-copper precipitant.

Dissolve 3,0 g of zinc sulphate ($ZnSO_4 \cdot 7H_2O$) and 0,6 g of copper(II) sulphate ($CuSO_4 \cdot 5H_2O$) in water to a volume of 100 ml.

5.6 2,6-dibromoquinonechloroimide solution (Gibb's reagent).

Dissolve 40 ± 1 mg of 2,6-dibromoquinonechloroimide (BQC) (O = $C_6H_2Br_2$ = NCl) in 10 ml of 96 % (*V/V*) ethanol.