
INTERNATIONAL STANDARD 3594

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

Milk fat — Detection of vegetable fat by gas-liquid chromatography of sterols (Reference method)

Matières grasses du lait — Détection des matières grasses végétales par chromatographie en phase gazeuse des stérols (Méthode de référence)

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FOREWORD

ISO (the International Organization for Standardization) is a worldwide federation of national standards institutes (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 set up 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 3594 was drawn up by Technical Committee ISO/TC 34, *Agricultural food products*, and was circulated to the Member Bodies in August 1974.

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

Australia	Hungary	Poland
Austria	India	South Africa, Rep. of
Belgium	Iran	Spain
Chile	Ireland	Sweden
Czechoslovakia	Israel	Turkey
France	Netherlands	United Kingdom
Germany	New Zealand	U.S.S.R.

No Member Body expressed disapproval of the document.

NOTE — This International Standard has been developed jointly with the IDF (International Dairy Federation) and the AOAC (Association of Official Analytical Chemists, U.S.A.) on the basis of an IDF Standard for the purpose of being included in the FAO/WHO Code of Principles concerning Milk and Milk Products and Associated Standards.

The text as approved by the above organizations has also been published by the IDF (IDF Standard No. 54A) and by the AOAC (Official Methods of Analysis).

Milk fat — Detection of vegetable fat by gas-liquid chromatography of sterols (Reference method)

1 SCOPE AND FIELD OF APPLICATION

This International Standard specifies a reference method for the detection, by gas-liquid chromatography (GLC), of the presence in milk fat of vegetable fat containing β -sitosterol. The limit of detection depends upon the β -sitosterol content of the added vegetable fat.

2 REFERENCES

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

ISO 3595, *Milk fat — Detection of vegetable fat by the phytosterol acetate test*.

3 PRINCIPLE

Preparation of sterol digitonides as described in ISO 3595 and dissolution in a mixture of formamide and dimethylformamide. Extraction of the liberated sterols with pentane. Separation of the sterols by gas-liquid chromatography.

If, on the chromatogram, a peak with the retention time of β -sitosterol is obtained, the presence of vegetable fat in the fat sample under investigation is demonstrated. Peaks of other phytosterols may support this conclusion.

4 REAGENTS AND MATERIALS

All reagents shall be of analytical quality.

4.1 Formamide and dimethylformamide, mixture, in equal volumes.

4.2 *n*-Pentane.

4.3 Column packing: 2 to 4 % loading of a methyl silicone gum rubber, stable up to at least 300 °C, on a flux-calcined diatomaceous earth, acid washed and silanized, mesh size 80/100 (175 to 150 μm) or 100/120 (150 to 125 μm).

4.4 Sensitivity test solution: 1 mg of milk fat sterols in 1 ml of *n*-pentane, freshly prepared from milk fat (see 7.2).

4.5 Peak resolution test solution: 0,9 mg of rape seed oil sterols and 0,1 mg of milk fat sterols in 1 ml of *n*-pentane, each sterol being freshly prepared from rape seed oil and milk fat respectively (see 7.2).

4.6 Reference test solution: 1 mg of soyabean oil sterols in 1 ml of *n*-pentane, freshly prepared from soyabean oil (see 7.2).

4.7 Carrier gas: nitrogen.

4.8 Hydrogen.

4.9 Oxygen or air.

5 APPARATUS

Usual laboratory equipment and

5.1 Gas chromatograph, fitted with hydrogen flame ionization detector, silver or glass injection system, or direct-on-column injection device, and recorder.

5.2 Gas chromatographic column, glass, U-shaped or coiled, length 100 to 200 cm, inside diameter 2 to 4 mm.

NOTE — Stainless steel should not be used as some types cause false results by deterioration of sterols.

5.3 Micro-syringe, capable of delivering a volume of up to 5 or 10 μl .

6 SAMPLING

See ISO/R 707.

7 PROCEDURE

7.1 Preparation of test sample

See ISO 3595.

7.2 Preparation of sterols

Dissolve about 10 mg of sterol digitonide, prepared as described in ISO 3595, in 0,5 ml of the formamide and dimethylformamide mixture (4.1) in a small test tube, if necessary with gentle heating. Add 2,5 ml of *n*-pentane (4.2) to the cooled solution, stopper the tube and shake. Let the layers separate and use the clear upper pentane layer, containing the liberated sterols, for gas chromatographic analysis. This solution contains about 1 mg of sterol per millilitre.