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**INTERNATIONAL STANDARD**



**2963**

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**Cheese and processed cheese products — Determination of citric acid content (Reference method)**

*Fromages et fromages fondus — Détermination de la teneur en acide citrique (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 2963 was drawn up by Technical Committee ISO/TC 34, *Agricultural food products*, and circulated to the Member Bodies in August 1972.

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

Australia	France	New Zealand
Austria	Germany	Poland
Belgium	Hungary	Romania
Brazil	India	South Africa, Rep. of
Chile	Iran	Spain
Czechoslovakia	Ireland	Thailand
Egypt, Arab Rep. of	Israel	Turkey
Finland	Netherlands	United Kingdom

This International Standard has also been approved by the International Union of Pure and Applied Chemistry (IUPAC).

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 was also published by FAO/WHO (Code of Principles, Standard No. B-13), by the IDF (IDF Standard No. 34B) and by the AOAC (Official Methods of Analysis).

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# Cheese and processed cheese products – Determination of citric acid content (Reference method)

## 1 SCOPE AND FIELD OF APPLICATION

This International Standard specifies a reference method for the determination of the citric acid content of cheese and processed cheese products.

## 2 REFERENCE

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

## 3 DEFINITION

**citric acid content of cheese and processed cheese products:** The percentage by mass of anhydrous citric acid determined by the procedure specified

## 4 PRINCIPLE

Dispersion of the cheese in water, clearing by the addition of trichloroacetic acid and filtration. Treatment with pyridine and acetic anhydride of the clear filtrate obtained and photometric measurement of the yellow colour developed in the presence of citric acid.

## 5 REAGENTS

All reagents used shall be of analytical reagent quality and the water used shall be distilled water or water of at least equivalent purity.

### 5.1 Trichloroacetic acid, 300 g/l solution.

Dissolve 300 g of trichloroacetic acid in water and make up to a volume of 1 000 ml.

### 5.2 Pyridine.

### 5.3 Acetic anhydride.

### 5.4 Citrate standard solution.

Dissolve 0,956 5 g of trisodium citrate ( $C_6H_5O_7Na_3 \cdot 2H_2O$ ) in water and make up to a volume of 1 000 ml.

*This solution corresponds to 625 mg of anhydrous citric acid per litre.*

1) Whatman No. 540, Schleicher and Schuell 589 medium, or equivalent.

## 6 APPARATUS

*Usual laboratory equipment used in particular*

### 6.1 Analytical balance.

6.2 Photoelectric colorimeter or spectrophotometer, suitable for making readings at a wavelength of 428 nm.

### 6.3 Water bath maintained at $32 \pm 1$ °C.

### 6.4 Suitable grinding device.

6.5 Test tubes, with glass or plastics stoppers, 16 mm or 18 mm x 150 mm

6.6 Pestle and mortar, porcelain, approximately 50 ml capacity.

6.7 Volumetric flasks, 50, 100 and 1 000 ml, complying with ISO/R 1042.

6.8 Pipette or burette, to deliver 1 – 1,3 – 4 – 5,7 – 8 – 12 – 16 and 20 ml, complying with ISO/R 648 and ISO/R 385 as appropriate.

6.9 Glass funnels, of convenient size, for example 5 cm diameter.

### 6.10 Hard filter paper.<sup>1)</sup>

## 7 SAMPLING

See ISO/R 707.

## 8 PROCEDURE

### 8.1 Preparation of the test sample

Before analysis, remove the rind or mouldy surface layer of the cheese so as to give a test sample representative of the cheese as it is usually consumed. Grind or treat the sample so obtained in such a way that it will be homogeneous; avoid losses by evaporation. Keep the test sample so prepared in an airtight container until analysis, which shall be carried out on the same day.

## 8.2 Test portion

Weigh, to the nearest 0,001 g, about 0,5 g of the test sample.

## 8.3 Dispersion and clearing

8.3.1 Place the test portion in the porcelain mortar (6.6) and disperse it by crushing with the pestle, adding small portions of warm water (60 to 70 °C).

8.3.2 Transfer the contents of the mortar quantitatively to a 100 ml volumetric flask (6.7). Do not use more than about 50 ml of water.

8.3.3 Cool to room temperature.

8.3.4 Add 40 ml of the trichloroacetic acid solution (5.1), mix by swirling, fill to the mark with distilled water and mix again.

8.3.5 Allow to stand at room temperature for 30 min and filter through a dry filter paper. Discard the first runnings of the filtrate until it runs clear, discarding in any case at least 10 ml.

## 8.4 Determination

8.4.1 Pipette 1 ml of the clear filtrate into a stoppered test tube (6.5).

8.4.2 Add to the test tube 1,3 ml of pyridine (5.2). Mix and immediately add 5,7 ml of acetic anhydride (5.3). Stopper the tube, mix thoroughly and place in the water bath (6.3) without delay.

8.4.3 After 30 min, remove the tube from the water bath, cool to room temperature, dry it and measure the absorbance against the blank (8.6) at a wavelength of 428 nm, within 30 min.

8.4.4 Carry out two determinations on the same test sample.

## 8.5 Preparation of the calibration curve

8.5.1 Place in six 50 ml volumetric flasks, 0, 4, 8, 12, 16 and 20 ml respectively of the citrate standard solution (5.4); add to each flask distilled water to make a volume of about 25 ml.

8.5.2 Add 20 ml of trichloroacetic acid solution (5.1); mix by swirling, fill to the mark with water and mix again.

8.5.3 Pipette 1 ml of each diluted standard solution into a stoppered test tube (6.5) to obtain a range of standards containing 0 (zero value), 50, 100, 150, 200 and 250 µg of anhydrous citric acid and proceed as described in 8.4.2 and 8.4.3.

8.5.4 Prepare the calibration curve by plotting the absorbance against the quantity of anhydrous citric acid in micrograms.

## 8.6 Blank test

Carry out a blank test following the same procedure but without the test portion.

## 9 EXPRESSION OF RESULTS

### 9.1 Method of calculation and formula

9.1.1 Convert the reading obtained as directed in 8.4.3 to micrograms of anhydrous citric acid by reference to the calibration curve.

9.1.2 The anhydrous citric acid content, as a percentage by mass, is equal to

$$\frac{m_1}{100 m_0}$$

where

$m_0$  is the mass, in grams, of the test portion;

$m_1$  is the mass, in micrograms, as obtained in 9.1.1.

### 9.2 Repeatability

The difference between the results of two determinations carried out simultaneously or in rapid succession by the same analyst shall not exceed 0,1 g of anhydrous citric acid per 100 g of the product.

## 10 TEST REPORT

The test report shall show the method used and the result obtained. It shall also mention any operating conditions not specified in this International Standard, or regarded as optional, as well as any circumstances that may have influenced the result.

The report shall include all details required for the complete identification of the sample.

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