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Cheese and processed cheese products — Determination of citric acid content — Enzymatic method

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

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.

In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of normative document:

— an ISO Publicly Available Specification (ISO/PAS) represents an agreement between technical experts in an ISO working group and is accepted for publication if it is approved by more than 50 % of the members of the parent committee casting a vote;

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An ISO/PAS or ISO/TS is reviewed after three years in order to decide whether it will be confirmed for a further three years, revised to become an International Standard, or withdrawn. If the ISO/PAS or ISO/TS is confirmed, it is reviewed again after a further three years, at which time it must either be transformed into an International Standard or be withdrawn.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO/TS 2963|IDF/RM 34 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF). It is being published jointly by ISO and IDF.

This edition of ISO/TS 2963|IDF/RM 34 cancels and replaces ISO 2963:1997, which has been technically revised.

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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 in the development of standard methods of analysis and sampling for milk and milk products.

The main task of technical committees is to prepare International Standards. 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 the IDF National Committees casting a vote.

In other circumstances, particularly when there is an urgent market requirement for such documents, a Standing Committee may decide to publish an other type of normative document which is called by IDF: *Reviewed method*. Such a method represents an agreement between the members of a Standing Committee and is accepted for publication if it is approved by at least 50 % of the committee members casting a vote. A *Reviewed method* is equal to an ISO/PAS or ISO/TS and will, therefore, also be published jointly under ISO conditions.

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All work was carried out by the Joint ISO-IDF Action Team on *Lactose and lactate determination*, of the Standing Committee on *Main components of milk*, under the aegis of its project leader, Mr C. Hughes (NZ).

This edition of ISO/TS 2963|IDF/RM 34 cancels and replaces IDF 34C:1992, which has been technically revised.

Introduction

The method described in ISO 2963:1997 and IDF 34C:1992 did not fulfil the requirements for a fully validated International Standard. No new interlaboratory tests could be organized with the method according to ISO 5725-1 and ISO 5725-2 due to a lack of participants, hence the publication of this revision as a Technical Specification/Reviewed method.

Reliable results with enzymatic methods will only be obtained if the good laboratory practice (GLP) rules for such analyses are applied strictly. These GLP rules are given in Annex A.

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Cheese and processed cheese products — Determination of citric acid content — Enzymatic method

1 Scope

This Technical Specification specifies an enzymatic method for the determination of the citric acid content of cheese and processed cheese products.

2 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

2.1
citric acid content
mass fraction of substances determined by the procedure described in this Technical Specification

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NOTE The citric acid content is expressed as mass fraction in percent.

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3 Principle

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An extract of the sample is treated with the following enzymes and biochemical substances:

a) citrate lyase (CL) to convert citric acid to oxalacetate and acetate;

b) malate dehydrogenase (MDH) and lactate dehydrogenase (LDH), in the presence of reduced nicotinamide adenine dinucleotide (NADH), to catalyse the reduction of oxalacetate and its decarboxylation product pyruvate to L-malate and L-lactate, respectively, with the subsequent conversion of NADH to its oxidized form (NAD^+).

The decrease in concentration of NADH is determined by measurement of the absorbance of the test solution at 340 nm. The citric acid content is proportional to the decrease in NADH concentration.

4 Reagents

Use only reagents of recognized analytical grade and distilled water or water of at least equivalent purity, unless otherwise specified. Take note of the production and expiry dates given by the manufacturer of the reagents.

4.1 Enzymes

If an enzyme suspension is applied with other than the prescribed activity, the volume of the suspension stated in the pipetting scheme (8.5.1) shall be increased or decreased proportionally.

The reagents described in 4.7 to 4.10 inclusive may be obtained commercially as a test combination.

4.2 Trichloroacetic acid solution (CCl_3COOH).

Dissolve 200,0 g of trichloroacetic acid in water. Dilute to 1 000 ml with water and mix.

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4.3 Sodium hydroxide solution I, $c(NaOH) = 5,0$ mol/l.

Dissolve 200,0 g of sodium hydroxide in water. Dilute to 1 000 ml with water and mix.

4.4 Sodium hydroxide solution II, $c(NaOH) = 1,0$ mol/l.

Dissolve 40,0 g of sodium hydroxide in water. Dilute to 1 000 ml with water and mix.

4.5 Sodium hydroxide solution III, $c(NaOH) = 0,1$ mol/l.

Dissolve 4,0 g of sodium hydroxide in water. Dilute to 1 000 ml with water and mix.

4.6 Zinc chloride solution, $c(ZnCl_2) = 800$ mg/l.

Dissolve 800 mg of zinc chloride in water. Dilute to 1 000 ml with water and mix.

4.7 Buffer solution, pH 7,8.

Dissolve 71,3 g of glycylglycine in about 700 ml of water. Adjust to pH 7,8 with sodium hydroxide solution I (4.3). Add 100 ml of zinc chloride solution (4.6). Dilute to 1 000 ml with water and mix.

The buffer solution may be kept for 4 weeks if stored in a refrigerator at between 0 °C and +5 °C.

4.8 Reduced nicotinamide adenine dinucleotide solution

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Dissolve 50 mg of reduced nicotinamide adenine dinucleotide disodium salt ($C_{21}H_{27}N_7O_{14}P_2Na_2$) and 100 mg of sodium hydrogen carbonate ($NaHCO_3$) in 10 ml of water.

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The reduced nicotinamide adenine dinucleotide solution may be kept for 4 weeks if stored in a refrigerator at between 0 °C and +5 °C.

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4.9 Malate dehydrogenase/lactate dehydrogenase suspension

Mix suitable amounts of malate dehydrogenase (MDH from pig heart; suspension in ammonium sulfate solution, 3,2 mol/l, at pH 6,0 ± 0,2; EC 1.1.1.37)[1)] and lactate dehydrogenase (LDH from rabbit muscle; suspension in ammonium sulfate solution, 3,2 mol/l, at pH 7 ± 0,2; EC 1.1.1.27). Dilute with ammonium sulfate solution (3,2 mol/l) so as to obtain a final suspension containing about 600 units[2)] of MDH/ml and 1 400 units[2)] of LDH/ml.

The malate dehydrogenase/lactate dehydrogenase suspension may be kept for 1 year if stored in a refrigerator at between 0 °C and +5 °C.

4.10 Citrate lyase solution

Dissolve a suitable amount of citrate lyase [lyophilisate (CL) from *Aerobacter aerogenes*; EC 4.1.3.6] in ice-cold water so as to obtain a solution containing 40 units/ml [2)].

The citrate lyase solution may be kept for 1 week if stored at between 0 °C and +5 °C, and for 4 weeks if stored at −20 °C.

4.11 Citric acid standard solution.

Dissolve 1,600 g of citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$) in water. Dilute to 1 000 ml with water and mix.

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1) The EC number refers to the Enzyme Classification number as given in Reference [4].

2) This unit (often called International or Standard Unit) is defined as the amount of enzyme which will catalyse the transformation of 1 µmol of substrate per minute under standard conditions.

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5 Apparatus

Usual laboratory equipment and, in particular, the following:

5.1 Analytical balance, capable of weighing to the nearest 1 mg, with a readability of 0,1 mg.

5.2 pH meter.

5.3 Glass beakers, of capacity 50 ml.

5.4 Macerator, equipped with a suitable beaker.

5.5 One-mark volumetric flasks, of capacity 100 ml.

5.6 Pipettes, capable of delivering 0,02 ml, 1 ml, 2 ml, 5 ml, 25 ml and 40 ml, respectively.

5.7 Graduated pipettes, capable of delivering 10 ml, graduated in 0,1 ml divisions.

5.8 Measuring cylinder, of capacity 50 ml.

5.9 Filter funnel, of diameter approximately 7 cm.

5.10 Filter paper, medium grade, of diameter approximately 15 cm.

5.11 Spectrometer, suitable for measuring at a wavelength of 340 nm, equipped with cells of optical path length 1 cm.

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5.12 Plastic paddles, suitable for mixing the sample-enzyme mixture in the spectrometer cell.

5.13 Water bath, capable of maintaining a temperature of 20 °C to 25 °C, with rack suitable for holding the spectrometer cell (5.11) during the incubation period (optional; see 8.5.1).

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Incubation of the cells in the water bath is only necessary if the room temperature is below 20 °C.

6 Sampling

A representative sample should have been sent to the laboratory. It should not have been damaged or changed during transport or storage.

Sampling is not part of the method specified in this Technical Specification. A recommended sampling method is given in ISO 707|IDF 50.

7 Preparation of test sample

Prepare a homogeneous sample taking care to avoid loss of moisture, using the following procedure.

a) In the case of cheese, remove the rind or mouldy surface layer of the cheese in such a way as to provide a sample representative of the cheese as it is usually consumed.

   Grind or grate the sample by using an appropriate device, mix the ground or grated mass quickly and, if possible, grind or grate a second time and again mix thoroughly by intensive stirring and kneading.

b) In the case of processed cheese, remove a sample representative of the product. Mix the sample mass quickly and grind if necessary. Mix thoroughly by intensive stirring and kneading.

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c) In the case of processed cheese containing pieces of other foods (e.g. ham, fruit, nuts, herbs), determine whether the objective of the analysis is to determine the citric acid content of the processed cheese proper or of the entire product. In the former case, separate the pieces of other food and then proceed as for processed cheese.

Transfer the test sample into a container provided with an airtight lid, for storage prior to analysis. Close the container immediately. Analysis should be carried out as soon as possible after preparation of the test sample.

8 Procedure

8.1 Check tests

8.1.1 Carry out the test in 8.1.2 to 8.1.4 to check the recovery of citric acid whenever

— a new batch of reagents (4.7 to 4.10 inclusive) is brought into use,

— such reagents have been kept in a refrigerator without being used for more than 2 weeks,

— restarting analytical work after a period of analytical inactivity, or

— when conditions justify such a test.

8.1.2 Pipette 5,0 ml and 10,0 ml of citric acid standard solution (4.11) into each of two 100 ml one-mark volumetric flasks (5.5).

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Add 10 ml of trichloroacetic acid solution (4.2) to each flask. Dilute the contents of each flask to the 100 ml mark with water and mix.

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Determine the citric acid content of both solutions as described in 8.4.3 to 8.5.3 inclusive.

8.1.3 Calculate the citric acid monohydrate content of the citric acid standard solution (4.11) according to Equation (2) in 9.1, but using the following values:

— V_5 is the volume, in millilitres, of the citric acid standard solution (4.11) (V_5 = 1 000 ml);

— V_6 is the volume, in millilitres, of the citric acid standard solution (8.1.2) (V_6 = 5 ml and 10 ml respectively);

— V_7 is the total volume, in millilitres, of the diluted citric acid standard solution (8.1.2) (V_7 = 100 ml).

8.1.4 Taking into account the purity of the citric acid monohydrate, the recovery obtained for both dilutions (8.1.2) shall be within the range 100 % ± 5 %. If the recoveries are not within this range, the reagents, the operating technique, the accuracy of the pipettes and the condition of the spectrometer shall be checked and the required action shall be taken to obtain the appropriate results. The test shall be repeated until satisfactory results are obtained.

8.2 Test portion

Weigh, to the nearest 0,1 mg, approximately 1 g of the prepared test sample (Clause 7) in a beaker (5.3). Suspend the test portion in about 50 ml of water preheated to between 40 °C and 50 °C using the macerator (5.4). Transfer the contents of the beaker quantitatively to a 100 ml one-mark volumetric flask (5.5). Cool the contents of the flask to about 20 °C.

8.3 Reagent blank test

Carry out a blank test in duplicate. Proceed as specified in 8.4 and 8.5, using all reagents but omitting the test portion.

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8.4 Deproteination

8.4.1 Add 10 ml of trichloroacetic acid (4.2) to the suspension (8.2) in the 100 ml one-mark volumetric flask. Dilute to the mark with water and mix thoroughly.

8.4.2 Let the mixture stand for 30 min. Do not remix the contents of the volumetric flask prior to filtration.

8.4.3 Filter the supernatant liquid through a filter paper (5.10), discarding the first fraction of filtrate.

8.4.4 Pipette 25 ml of the filtrate into a glass beaker (5.3). Adjust the pH to approximately 4 by adding sodium hydroxide solution II (4.4) and, subsequently, to approximately pH 8 by adding sodium hydroxide solution III (4.5) while checking using the pH meter (5.2).

Transfer the contents of the beaker quantitatively to a 100 ml one-mark volumetric flask (5.5). Dilute to the mark with water and mix.

8.4.5 Filter through a filter paper (5.10), discarding the first fraction of filtrate.

8.5 Determination

8.5.1 Scheme for procedure

Carry out the determination according to the scheme in Table 1, taking care to bring the buffer solution (4.7) and the water to be used to room temperature just before use.

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Table 1 — Determination scheme

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Pipette into the spectrometer cells	Test portion or check test	Blank test
Buffer solution (4.7)	1,00 ml	1,00 ml
NADH solution (4.8)	0,10 ml	0,10 ml
MDH/LDH suspension (4.9)	0,02 ml	0,02 ml
Test or check test filtrate	2,00 ml	—
Blank test filtrate	—	2,00 ml
Mix the contents of the cells, using the plastic paddles (5.12) and incubate at a temperature above 20 °C for 5 min (see 5.13). Measure the absorbance, A_0, of the solution in each cell against air at a wavelength of 340 nm. Then add to the spectrometer cells:		
Citrate lyase solution (4.10)	0,02 ml	0,02 ml
Mix the contents of the cells and incubate at a temperature above 20 °C for 10 min (see also 5.13). Measure the absorbance A_{10} of the solution in each cell, against air.		

8.5.2 Calculation of absorbance

Calculate the absorbance, A, of each cell content to be used for the calculation of citric acid content (9.1) using the following equation:

$$A = A_0 - A_{10} \quad (1)$$

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