Citric acid

UV-method

for the determination of citric acid in foodstuffs and other materials

Cat. No. 10 139 076 035

Test-Combination for 3 × 12 determinations

BOEHRINGER MANNHEIM / R-BIOPHARM
Enzymatic BioAnalysis / Food Analysis

For *in vitro* use only

Store at 2-8°C

For recommendations for methods and standardized procedures see references (2)

Principle (Ref. 1)

Citric acid (citrate) is converted to oxaloacetate and acetate in the reaction catalyzed by the enzyme citrate lyase (CL) (1).

(1) Citrate $\xrightarrow{CL}$ oxaloacetate + acetate

In the presence of the enzymes L-malate dehydrogenase (L-MDH) and L-lactate dehydrogenase (L-LDH), oxaloacetate and its decarboxylation product pyruvate are reduced to L-malate and L-lactate, respectively, by reduced nicotinamide-adenine dinucleotide (NADH) (2, 3).

(2) Oxaloacetate + NADH + H^+ $\xrightarrow{L\text{-}MDH}$ L-malate + NAD^+

(3) Pyruvate + NADH + H^+ $\xrightarrow{L\text{-}LDH}$ L-lactate + NAD^+

The amount of NADH oxidized in reactions (2) and (3) is stoichiometric to the amount of citrate. NADH is determined by means of its light absorbance at 334, 340 or 365 nm.

(Note: Free pyruvate in the sample is not measured because of the order of pipetting the reagents.)

The Test-Combination contains

1. Three bottles 1, each with approx. 1.4 g lyophilizate, consisting of: glycylglycine buffer, pH approx. 7.8; L-malate dehydrogenase, approx. 136 U; L-lactate dehydrogenase, approx. 280 U; NADH, approx. 5 mg
2. Three bottles 2, each with approx. 50 mg lyophilizate citrate lyase, approx. 12 U
3. Bottle 3 with citric acid assay control solution for assay control purposes (measurement of the assay control solution is not necessary for calculating the results.) Use the assay control solution undiluted. (Expiry date: see pack label)

Preparation of solutions for 10 determinations

1. Dissolve contents of one bottle 1 in 12 ml redist. water.
2. Dissolve contents of one bottle 2 in 0.3 ml redist. water.

Stability of reagents

The contents of bottle 1 and 2 are stable at 2-8°C (see pack label).

Solution 1 is stable for 2 weeks at 2-8°C or for 4 weeks at −20 to −25°C. Bring solution 1 to 20-25°C before use.
Solution 2 is stable for 1 week at 2-8°C or for 4 weeks at −20 to −25°C.

Procedure

Wavelength[1]: 340 nm, Hg 365 nm or Hg 334 nm
Glass cuvette[2]: 1.00 cm light path
Temperature: 20-25°C
Final volume: 3.020 ml
Read against air (without a cuvette in the light path) or against water
Sample solution: 1-80 µg of citric acid/assay[3] (in 0.200-2.000 ml sample volume)

Pipette into cuvettes	Blank	Sample
solution 1	1.000 ml	1.000 ml
sample solution*	-	0.200 ml
redist. water	2.000 ml	1.800 ml
Mix**, read absorbances of the solutions (A_1) after approx. 5 min, and start reaction by addition of:		
solution 2	0.020 ml	0.020 ml
Mix**, on completion of the reaction (approx. 5 min), read absorbances of the solutions (A_2).		

* Rinse the enzyme pipette or the pipette tip of the piston pipette with sample solution before dispensing the sample solution.

** For example, with a plastic spatula or by gentle swirling after closing the cuvette with Parafilm (trademark of the American Can Company, Greenwich, Ct., USA)

Determine the absorbance differences (A_1-A_2) for both, blank and sample. Subtract the absorbance difference of the blank from the absorbance difference of the sample.

$$\Delta A = (A_1\text{-}A_2)_{sample} - (A_1\text{-}A_2)_{blank}$$

Occasionally a negative value with $(A_1\text{-}A_2)_{blank}$ is obtained. This value is then to be added to $(A_1\text{-}A_2)_{sample}$ according to the calculation formula.

The measured absorbance differences should, as a rule, be at least 0.100 absorbance units to achieve sufficiently precise results (see "Instructions for performance of assay" and "Sensitivity and detection limit", pt. 4).

If the absorbance difference of the sample (ΔA_{sample}) is higher than 1.000 (measured at 340 nm, Hg 334 nm) or 0.500 (measured at Hg 365 nm) respectively, the concentration of citric acid in the sample solution is too high. The sample solution is to be diluted according to the dilution table in that case.

Calculation

According to the general equation for calculating the concentration:

$$c = \frac{V \times MW}{\varepsilon \times d \times v \times 1000} \times \Delta A \text{ [g/l]}$$

V = final volume [ml]
v = sample volume [ml]
MW = molecular weight of the substance to be assayed [g/mol]
d = light path [cm]
ε = extinction coefficient of NADH at:
340 nm = 6.3 [l × $mmol^{-1}$ × cm^{-1}]
Hg 365 nm = 3.4 [l × $mmol^{-1}$ × cm^{-1}]
Hg 334 nm = 6.18 [l × $mmol^{-1}$ × cm^{-1}]

It follows for citric acid (calculated as the anhydrous acid):

$$c = \frac{3.020 \times 192.1}{\varepsilon \times 1.00 \times 0.200 \times 1000} \times \Delta A = \frac{2.900}{\varepsilon} \times \Delta A \text{ [g citric acid/l sample solution]}$$

for citric acid (calculated as monohydrate):

$$c = \frac{3.020 \times 210.1}{\varepsilon \times 1.00 \times 0.200 \times 1000} \times \Delta A = \frac{3.173}{\varepsilon} \times \Delta A \text{ [g citric acid monohydrate/l sample solution]}$$

If the sample has been diluted during preparation, the result must be multiplied by the dilution factor F.

When analyzing solid and semi-solid samples which are weighed out for sample preparation, the result is to be calculated from the amount weighed:

$$\text{Content}_{citric\ acid} = \frac{c_{citric\ acid}\ [g/l\ sample\ solution]}{\text{weight}_{sample}\ in\ g/l\ sample\ solution} \times 100 \text{ [g/100 g]}$$

1. Instructions for performance of assay

The amount of citric acid present in the assay has to be between 1 µg and 80 µg. In order to get a sufficient absorbance difference, the sample solution is diluted to yield a citric acid concentration between 0.04 and 0.4 g/l.

1 The absorption maximum of NADH is at 340 nm. On spectrophotometers, measurements are taken at the absorption maximum; if spectralline photometers equipped with a mercury vapor lamp are used, measurements are taken at a wavelength of 365 nm **or** 334 nm.
2 If desired, disposable cuvettes may be used instead of glass cuvettes.
3 See instructions for performance of assay
4 Reduced nicotinamide-adenine dinucleotide, NADH-Na_2, Cat. No. 127 345, available from Roche Applied Science

0511.**10248819001**④

Dilution table

Estimated amount of citric acid per liter	Dilution with water	Dilution factor F
< 0.4 g	-	1
0.4-4.0 g	1 + 9	10
4.0-40 g	1 + 99	100
> 40 g	1 + 999	1000

If the measured absorbance difference (ΔA) is too low (e.g. < 0.100), the sample solution should be prepared again (weigh out more sample or dilute less strongly) *or* the sample volume to be pipetted into the cuvette can be increased up to 2.000 ml. The volume of water added must then be reduced so as to obtain the same final volume in the assays for sample and blank. The new sample volume v must be taken into account in the calculation.

2. Technical information

2.1 In carrying out the calculation, a clear indication should be given as to whether the results are to be given as citric acid (molar mass 192.1 g/mol), as citric acid monohydrate (molar mass 210.1), or as citrate (molar mass 189.1 g/mol). (In enzymatic determinations, the citrate ion is measured.)

2.2 In evaluating the analytical results, it should be taken into account that in the acidimetric determination of "total acid calculated as citric acid" protons are measured and in enzymatic determinations the citrate ion is measured. It is thus not possible to compare such results directly.

3. Specificity (Ref. 1)

The method is specific for citric acid.

In the analysis of commercial citric acid monohydrate, results of >100% are obtained if the crystal water is lost during storage and the results are calculated with the molecular weight of citric acid monohydrate (210.1).

4. Sensitivity and detection limit (Ref. 1.4)

The smallest differentiating absorbance for the procedure is 0.005 absorbance units. This corresponds to a maximum sample volume v = 2.000 ml and measurement at 340 nm of a citric acid concentration of 0.25 mg/l sample solution (if v = 0.200 ml, this corresponds to 2.5 mg/l sample solution).

The detection limit of 0.5 mg/l is derived from the absorbance difference of 0.010 (as measured at 340 nm) and a maximum sample volume v = 2.000 ml.

5. Linearity

Linearity of the determination exists from 1 μg citric acid/assay (0.5 mg citric acid/l sample solution; sample volume v = 2.000 ml) to 80 μg citric acid/assay (0.4 g citric acid/l sample solution; sample volume v = 0.200 ml).

6. Precision

In a double determination using one sample solution, a difference of 0.005 to 0.010 absorbance units may occur. With a sample volume of v = 0.200 ml and measurement at 340 nm, this corresponds to a citric acid concentration of approx. 3-5 mg/l. (If the sample is diluted during sample preparation, the result has to be multiplied by the dilution factor F. If the sample is weighed in for sample preparation, e.g. using 1 g sample/100 ml = 10 g/l, a difference of 0.03-0.05 g/100 g can be expected.)

The following data have been published in the literature:

CV = 4.4 % n = 10 extract from rabbit liver
CV = 1.3 % n = 10 wine (Ref. 1.3)

Fruit juice:
r = 0.095 + 0.025 × ($c_{citric\ acid}$ in g/l) g/l
R = 0.130 + 0.054 × ($c_{citric\ acid}$ in g/l) g/l

For further data see references (Ref. 2.3)

Wine:

citric acid < 400 mg/l:		citric acid > 400 mg/l:		
r = 14 mg/l	R = 39 mg/l	r = 28 mg/l	R = 65 mg/l	(Ref. 2.13, 2.14)

7. Interference/sources of error

If the sample solution contains free pyruvic acid, NADH is already consumed before the measuring of A_1. In this case it is recommended to add NADH to the sample additionally (e.g. 0.100 ml NADH solution, 5 mg/ml)[4], and to use less redist. water, appropriately.

8. Recognizing interference during the assay procedure

8.1 If the conversion of citric acid has been completed according to the time given under "Procedure", it can be concluded in general that no interference has occurred.

8.2 On completion of the reaction, the determination can be restarted by adding citric acid or sodium citrate (qualitative or quantitative): if the absorbance is altered subsequent to the addition of the standard material, this is also an indication that no interference has occurred.

8.3 Operator error or interference of the determination through the presence of substances contained in the sample can be recognized by carrying out a double determination using two different sample volumes (e.g. 0.100 ml and 0.200 ml): the measured differences in absorbance should be proportional to the sample volumes used.

When analyzing solid samples, it is recommended that different quantities (e.g. 1 g and 2 g) be weighed into 100 ml volumetric flasks. The absorbance differences measured and the weights of sample used should be proportional for identical sample volumes.

8.4 Possible interference caused by substances contained in the sample can be recognized by using an internal standard as a control: in addition to the sample, blank and standard determinations, a further determination should be carried out with sample **and** assay control solution in the same assay. The recovery can then be calculated from the absorbance differences measured.

8.5 Possible losses during the determination can be recognized by carrying out recovery tests: the sample should be prepared and analyzed with and without added standard material. The additive should be recovered quantitatively within the error range of the method.

9. Reagent hazard

The reagents used in the determination of citric acid contain hazardous materials in the sense of the Hazardous Substances Regulations, the Chemicals Law or EC Regulations 67/548 and 99/45 and subsequent alteration, supplementation and adaptation guidelines. Please refer to the safety date sheet or the labels of the affected vials for further information.

10. General information on sample preparation

In carrying out the assay:
Use **clear, colorless and practically neutral liquid samples** directly, or after dilution according to the dilution table, and of a volume up to 2.000 ml;
Filter **turbid solutions**;
Degas **samples containing carbon dioxide** (e.g. by filtration);
Adjust **acid samples** to approx. pH 8 by adding sodium or potassium hydroxide solution;
Adjust **acid and weakly colored samples** to approx. pH 8 by adding sodium or potassium hydroxide solution and incubate for approx. 15 min;
Treat **"strongly colored" samples** that are used undiluted or with a higher sample volume with polyvinylpolypyrrolidone (PVPP) or with polyamide (e.g. 1 g/100 ml);
Crush or homogenize **solid or semi-solid samples,** extract with water or dissolve in water and filter if necessary;
Deproteinize **samples containing protein** with perchloric acid;
Extract **samples containing fat** with hot water (extraction temperature should be above the melting point of the fat involved). Cool to allow the fat to separate, make up to the mark, place the volumetric flask in an ice bath for 15 min and filter.

Important note

The Carrez-clarification cannot be used in the sample preparation for citric acid determination due to a too low recovery rate (adsorption of citric acid).

If, in addition to free citric acid, esterified citric acid is to be determined - e.g. citric acid esters of polyphenols or anthocyans - the esters must be converted to the free acid by alkaline hydrolysis. Proceed as stated under "wine".

11. Application examples

Determination of citric acid in fruit juices, refreshment drinks, tea and similar beverages
Remove turbidities by filtration and dilute sample to obtain a citric acid concentration between 0.04 and 0.4 g/l. The diluted solution can be used for the assay even if it is colored. Only *intensely colored* juices must be decolorized when they are used undiluted for the assay because of their low citric acid concentration. In such cases, proceed as follows:

Mix 10 ml juice and 0.1 g polyamide or polyvinylpolypyrrolidone (PVPP), stir for 1 min, and filter. Use the clear, slightly colored solution for the assay, neutralize, if necessary.

Determination of citric acid in wine
Slightly colored wines can be used directly for the assay or after dilution according to the dilution table. Dark colored wines have to be decolorized when they are used directly for the assay, especially with an increased sample volume because of their low citric acid concentration:

Mix 10 ml wine and 0.1 g polyamide or polyvinylpolypyrrolidone (PVPP), stir for 1 min, and filter. Use the clear or slightly colored solution for the assay.

Determination of citric acid esters in wine
Heat 20 ml sample and 6 ml alcoholic potassium hydroxide (approx. 2 M; methanol or ethanol) for 10 min at a reflux condenser while stirring, allow to cool to 20-25°C, and neutralize with sulfuric acid (2 M). Transfer quantitatively to a 50 ml volumetric flask and fill up to the mark with redist. water. Use the sample directly for the assay or after dilution, if necessary (= total citric acid, which is the sum of free and esterified citric acid).

Determination of citric acid in beer
For removal of carbonic acid, stir approx. 5-10 ml beer for 1 min with a glass rod or filter. The largely CO_2-free sample of beer is used for the assay without further dilution.

Determination of citric acid in bread, meat products, cheese, vegetable and fruit products
Grind approx. 20-50 g sample material (using e.g. a mortar, a meat grinder or homogenizer). Accurately weigh approx. 10 g of the well mixed sample into a homogenizer beaker and add 50 ml perchloric acid (1 M); homogenize for 2 min (up to 10 min) with the homogenizer. A resultant warming of up to 35°C is allowed. Centrifuge homogenate.

Adjust 20 ml of the supernatant to pH 8-10 with approx. 4 ml (measure the volume of KOH which is needed) potassium hydroxide solution (5 M). Place the solution for 15 min in the refrigerator for the quantitative precipitation of the formed potassium perchlorate, filter, and discard the first few ml. Use the filtrate directly for the assay or after dilution according to the dilution table.

For calculating the content (in g/100 g) according to the above-mentioned formula (see calculation) the content of the sample in the sample solution is needed. When applying the above-mentioned sample preparation and considering the water content of the sample the weight of the sample is calculated according to the following furmula:

$$\text{Weight}_{\text{sample}} = \frac{a \times 1000 \times d}{(b + a \times w) \times (d + e)} \text{ [g/l sample solution]}$$

It is:
a: the weighed sample in g
b: volume of perchloric acid in ml
d: volume of supernatant for pH adjustment in ml
e: volume of KOH to adjust pH to 8-10 in ml
w : water content of the sample in (%;w/w)/100
1000: factor for g expressed in mg

(The specific gravity of water from the sample at 20-25°C is approx. 1 g/ml. It can be neglected for the calculation.)

Determination of citric acid in margarine, edible oil and salves
Accurately weigh approx. 5 g homogeneous sample into a beaker, add approx. 70 ml redist. water and while vigorously stirring on a heatable magnetic stirrer, bring to boil. Transfer the aqueous phase with a pipette into a 100 ml volumetric flask. Repeat the extraction with approx. 20 ml redist. water.

Allow the flask to cool to 20-25°C and fill up to the mark with redist. water. Place the flask for 15 min in an ice-bath or in the refrigerator. Filter through a folded filter. According to the expected citric acid concentration, use the filtrate diluted or undiluted for the assay.

Determination of citric acid esters
(e.g. glyceride citric acid esters, emulsifying agents)

Citric acid which is bound in monoglyceride or diglyceride citric acid esters, respectively, can be determined in the presence of free citric acid (citrates) if the sample is extracted with chloroform and the esters are subsequently saponified with potassium hydroxide. In this case proceed as follows:

Boil the well minced and homogenized samples which contain up to approx. 120 mg monoglyceride citric acid ester (e.g. monooleylcitryl glyceride ester, MW approx. 550) or up to 170 mg diglyceride citric acid ester (e.g. dioleylcitryl glyceride ester, MW approx. 810) with approx. 50 ml chloroform under a reflux condenser for approx. 2 h in a 250 ml round bottomed flask. Filter and rinse with chloroform. Evaporate the chloroform in a rotary evaporator. Boil the nearly dry residue with 25 ml methanolic potassium hydroxide (1 M) for 10 min under a reflux condenser. Allow to cool to 20-25°C and neutralize or weakly acidify respectively with approx. 5 ml hydrochloric acid (5 M). Transfer the solution quantitatively into a 100 ml volumetric flask, make up to the mark with water, mix and filter. Use the nearly clear solution for the assay.

For determination of the content the molecular weight of the glyceride must be taken into account.

12. Further applications

The method may also be used in the examination of paper, cosmetics, detergents (Ref. 3.6), pharmaceuticals, as well as in research when analyzing biological materials.

For details of sampling, treatment and stability of the sample see Ref. 1.3 and 1.5.

Determination of citric acid in fermentation samples and cell culture media
Place the sample (after centrifugation, if necessary) in a water-bath at 80°C for 15 min to stop the enzymatic reactions. Centrifuge and use the supernatant (diluted according to the dilution table, if necessary) for the assay. Alternatively, deproteinization can be carried out with perchloric acid. See the above-mentioned examples.

Homogenize gelatinous agar media with water and treat further as described above.

References

1.1 Gruber, W. & Möllering, H. (1966) Citrat-Lyase und Bestimmung von Citrat, Biochemische Zeitschrift **346,** 85-88

1.2 Möllering, H. & Gruber, W. (1966) Determination of citrate with citrate lyase, Anal. Biochem. **17,** 369-376

1.3 Dagley, St. (1974) in Methoden der enzymatischen Analyse (Bergmeyer, H. U., Hrsg.) 3. Aufl., Bd. 2, S. 1607-1611; Verlag Chemie Weinheim and (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.) 2nd ed., vol. 3, pp. 1562-1565, Verlag Chemie, Weinheim/Academic Press, Inc., New York and London

1.4 Möllering, H. (1985) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.) 3rd ed., vol. VII, pp. 2-12; Verlag Chemie, Weinheim, Deerfield Beach/Florida, Basel

1.5 Passonneau, J. V. & Brown, J. G. (1974) in Methoden der enzymatischen Analyse (Bergmeyer, H. U., Hrsg.) 3. Aufl. Bd. 2, S. 1613-1614, Verlag Chemie Weinheim and (1974) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.) 2nd ed., vol. 3, p. 1568, Verlag Chemie, Weinheim/Academic Press, Inc., New York and London

2.1 Bundesverband der Deutschen Feinkostindustrie e.V. Bonn; Analysenmethoden: Bestimmung von Citronensäure in Tomatenmark, IV/41 (Dezember 1979)

2.2 Norme Française Homologuée NF V 76-104 (Octobre 1980) Jus de Fruits et Jus de Légumes, Détermination de la Teneur en Acides Carboxyliques

2.3 Amtliche Sammlung von Untersuchungsverfahren nach §35 LMBG; Untersuchung von Lebensmitteln: Bestimmung von Citronensäure (Citrat) in Fleischerzeugnissen, 07.00-13 (November 1981); Bestimmung von Citronensäure (Citrat) in Wurstwaren, 08.00-15 (November 1981); Bestimmung von Citronensäure in Tomatenmark, 26.11.03-5 (Mai 1983); Bestimmung von Citronensäure in Tomatenketchup und vergleichbaren Erzeugnissen, 52.01.01-5 (November 1983); Bestimmung von Citronensäure (Citrat) in Fruchtsäften, 31.00-14 (November 1984); Enzymatische Bestimmung des Gehaltes an Citronensäure (Citrat) in Frucht- und Gemüsesäften, L 31.00-14 (Januar 1997); Bestimmung des Gehaltes an Citronensäure (Citrat) in Gemüsesäften, spektralphotometrische Bestimmung von NADH, 26.26-12 (Januar 1997)

2.4 Schweizerisches Lebensmittelbuch, Kapitel 61B (Enzymatische Bestimmungen)/3.1 (1981), Kapitel 2A (Milchmischgetränke)/18 (1980), Kapitel 2B (Sauermilchprodukte)/15 (1980), Kapitel 4 (Milchdauerwaren)/10.3 (1993), Kapitel 28A (Frucht- und Gemüsesäfte u.a.)/7.5 (1988), Kapitel 30 (Wein)/36 (1967), Kapitel 30A (Wein aus Trauben)/6.4 (1993), Kapitel 34 (Gärungsessig)/4.5 (1994), Kapitel 34A (Essig und essigähnliche Erzeugnisse)/21 (1970)

2.5 Gombocz, E., Hellwig, E., Vojir, F. & Petuely, F. (1981) Deutsche Lebensmittel-Rundschau **77,** 4-5

2.6 Brautechnische Analysenmethoden, Band III, S. 565-568 (1982), Methodensammlung der Mitteleuropäischen Brautechnischen Analysenkommission (MEBAK)

2.7 International Federation of Fruit Juice Producers (IFU, Methods of Analysis, no. 22-1985); contained in "Code of Practice for Evaluation of Fruit and Vegetable Juices" (1996) edited by Association of the Industry of Juices and Nectars from Fruits and Vegetables of the European Economic Community (A.I.J.N.)

2.8 Henniger, G. & Mascaro, L. (1985) Enzymatic-ultraviolet determination of citric acid in wine, J. Assoc. Off. Anal. Chem. **68,** 1024-1027

2.9 Official Methods of Analysis of the Association of Official Analytical Chemists (1990), 15th ed., vol. 2, p. 746 (985.11)

2.10 Deutsche Norm DIN 10325 (Januar 1986) Bestimmung des Citronensäuregehaltes in Schmelzkäse (Enzymatisches Verfahren)

2.11 Nederlandse Norm NEN 2851 (1e druk, september 1987) Vruchtesappen: Bepaling van het citronenzuurgehalte; Enzymatische methode (Fruits juices - Determination of the citric acid content - Enzymatic method)

2.12 RSK-Values, The Complete Manual, Guide Values and Ranges of Specific Numbers for Fruit Juices and Nectars, Including the Revised Methods of Analysis (1987), 1st ed., Verlag Flüssiges Obst/Liquid Fruit, D-56370 Eschborn, pp. 97-100

2.13 Recueil des méthodes internationales d'analyse des vins et des moûts, Complément n^{o} 1 à l'édition officielle de juin 1990, OFFICE INTERNATIONAL DE LA VIGNE ET DU VIN, S. 187-189

2.14 Amtsblatt der Europäischen Gemeinschaften L 272 (3. Oktober 1990), Rechtsvorvorschriften: Verordnung (EWG) Nr. 2676/90 der Kommission vom 17. September 1990 zur Festlegung gemeinsamer Analysenmethoden fr den Weinsektor (S. 94-96); Official Journal of the European Communities L 272 (3 October 1990), Legislation: Commission Regulation (EEC) No 2676/90 of 17 September 1990 determining Community methods for the analysis of wines (pp. 94-96)

2.15 International Dairy Federation, Provisional Standard 34C (1992) Cheese & Processed Cheese products, Determination of Citric Acid Content (Enzymatic Method)

2.16 Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten, VDLUFA (1993) Enzymatische Bestimmung des Citronensäuregehaltes in Käse und Schmelzkäse, Methodenbuch Band VI, C8.7

2.17 Deutsche Norm DIN EN 1137 (Dez. 1994) Frucht- und Gemüsesäfte; Enzymatische Bestimmung des Gehaltes an Citronensäure (Citrat); Spektralphotometrische Bestimmung von NADH (Fruit and vegetable juices; Enzymatic determination of citric acid (citrate) content; NADH spectrometric method)

2.18 European Standard EN 1137 (Dec . 1994) Fruit and vegetable juices, Enzymatic determination of citric acid (citrate) content by the NADH spectrometric method

2.19 Deutsche Norm DIN 10259 (Juni 1998) Material zur Herstellung von Umhüllungen für Zigarettenfilter, Zigaretten und andere Tabakerzeugnisse, Bestimmung des Citratgehaltes

2.20 International Standard ISO 2963 (März 1997) Cheese and processed cheese products - Determination of citric acid content - Enzymatic method

2.21 Standard der Russischen Föderation / Standard of the Russian Federation / Gosstandart Rossii GOST R 51129-98 (1998) Fruit and vegetable juices. Method for determination of citric acid (citrate)

2.22 Standard der Russischen Föderation / Standard of the Russian Federation / Gosstandart Rossii GOST R 51257-99 (1999) Processed cheese. Method for determination of citric acid content

3.1 Mayer, K. & Pause, G. (1965) Eine enzymatische Citronensäure-Bestimmung, Mitt. Geb. Lebensmittelunters. Hyg. **56,** 454-458

3.2 Mayer, K. & Pause, G. (1969) Enzymatische Zitronensäurebestimmung an gerb- und farbstoffreichen Weinen, Lebensm.-Wiss. Technol. **2,** 143

3.3 Büsching, L. (1968) Methode zur enz. Bestimmung von Citronensäure und Brenztraubensäure in Zuckerfabrikationsprodukten, Zucker **21,** 531-535

3.4 Schiweck, H. & Büsching, L. (1971) Citronensäure- und Raffinosegehalt in Zuckerrübenwurzelkörpern und -blättern während des Wachstums und Füllung der Citronensäure während der Saftreinigung, ZUCKER **24,** 249-253

3.5 Piendl, A. (1974) Citrat im Bier, Brauwissenschaft **27,** 250-257 und 305-311

3.6 Taraborelli, J. A. & Upton, R. P. (1975) Enzymatic Determination of Citrate in Detergent Products, J. Am. Oil Chem. Soc. **52,** 248-251

3.7 Gerstenberg, H. (1978) Nachweis von Teigsäuerungsmitteln in Brot aufgrund des Zitronensäuregehalts, Lebensm. Chemie u. gerichtl. Chemie **32,** 125-126

3.8 Seppi, A. & Sperandio, A. (1983) L'acido citrico nei vini, determinazione con metodo enzimatico e con metodo chimico ufficiale, La Rivista della Societa Italiana di Scienza dell' Alimentazione **12,** 479-482

3.9 Lagemann, M., Anders, D., Graef, V. & Bödeker, R.H. (1985) Einfluß von Kakao auf die Ausscheidung von Oxalat, Citrat, Magnesium und Calcium im Urin bei Kindern, Monatsschr. Kinderheilkd. **133,** 754-759

3.10 Klopper, W. J., Angelino, S.A.G.F., Tuning, B. & Vermeire, H.A. (1986) Organic acids and glycerol in beer, J. Inst. Brew. **92,** 225-228

3.11 Talpay, B. (1988) Inhaltsstoffe des Honigs - Citronensäure (Citrat), Deutsche Lebensmittel-Rundschau **84,** 41-44

3.12 Schlimme, E., Lorenzen, P. Chr., Martin, D. & Thormählen, K. (1996) Analytical differentiation of butter types by specific compositional parameters of the aqueous butter phase, Milchwissenschaft **51,** 139-143

3.13 Saalfeld, U. & Freund, W. (1999) Charakterisierung pulverisierter Sauerteige und Möglichkeiten ihrer qualitativen Bestimmung im Brot - Teil 1: Säuregehalt und Abbauvermögen für L-Malat und Citrat, Deutsche Lebensmittel-Rundschau **95**, 209-219

Citric acid assay control solution (Bottle 3)

Concentration*: see bottle label

Citric acid assay control solution is a stabilized aqueous solution of citric acid. It serves as an assay control solution for the enzymatic determination of citric acid in foodstuffs and other materials.

Application:

1. Addition of citric acid assay control solution to the assay mixture:

Instead of sample solution the assay control solution is used for the assay.

2. Restart of the reaction, quantitatively:

After completion of the reaction with sample solution and measuring of A_2 add 0.100 ml assay control solution to the assay mixture. Read absorbance A_3 after the end of the reaction (approx. 10 min). Calculate the concentration from the difference of (A_2-A_3) according to the general equation for calculating the concentration. The altered total volume must be taken into account. Because of the dilution of the assay mixture by addition of the assay control solution, the result differs insignificantly from the data stated on the bottle label.

* Stated as anhydrous citric acid

3. Internal standard:

The assay control solution can be used as an internal standard in order to check the determination for correct performance (gross errors) and to see whether the sample solution is free from interfering substances:

Pipette into cuvettes	Blank	Sample	Standard	Sample + Standard
solution 1	1.000 ml	1.000 ml	1.000 ml	1.000 ml
sample solution	-	0.200 ml	-	0.100 ml
assay control sln.	-	-	0.200 ml	0.100 ml
redist. water	2.000 ml	1.800 ml	1.800 ml	1.800 ml
Mix, and read absorbances of the solutions (A_1) after approx. 5 min. Continue as described in the pipetting scheme under "Procedure". Follow the instructions given under "Instructions for performance of assay" and the footnotes.				

The recovery of the standard is calculated according to the following formula:

$$\text{recovery} = \frac{2 \times \Delta A_{\text{sample + standard}} - \Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \times 100\ [\%]$$

Also available:

Test-Combination D-Isocitric acid, Cat. No. 10 414 433 035

R-BIOPHARM AG
An der neuen Bergstraße 17
D-64297 Darmstadt
Phone + 49 61 51 / 81 02-0
Fax + 49 61 51 / 81 02-20
www.r-biopharm.com

Roche