Metrohm

Application Bulletin 87/3 e

Analysis of dairy products

Branch

General analytical chemistry; food, stimulants, beverages, flavours

Keywords

Dairy products; titration; potentiometric titration; oxidation stability; ion chromatography; IC; titratable acidity; chloride; sodium; lactose; branch 1; branch 7; Porotrode; Ag Titrode; Thermoprobe; Metrosep Carb 2 – 150/4.0; DIN 10316; ISO/TS 11869; IDF/RM 150; EN ISO 5943; IDF 88; ISO 15648; IDF 179; AOCS Cd 12b-92; ISO 6886

Summary

This Bulletin describes potentiometric titration methods for the determination of the acidity in milk and yoghurt according to DIN 10316, ISO/TS 11869, IDF/RM 150, ISO 6091 and IDF 86, the chloride content in milk, butter and cheese according to EN ISO 5943, IDF 88, ISO 15648, IDF 179, ISO 21422, and IDF 242. Additionally the determination of the sodium content in milk using the thermometric titration is described. The determination of the oxidation stability of butter in accordance to AOCS Cd 12b-92, ISO 6886 and GB/T 21121 as well as the determination of lactose in lactose free milk by ion chromatography is also described.

For the determination of the pH value in dairy products see Application Bulletin No. 86 and for the determination of calcium and magnesium see Application Bulletin No. 235.

Determination of acidity

Summary

Milk, dried milk or yoghurt is titrated to a pH value of 8.3 with NaOH. The result is given as titratable acidity, respectively as titratable acidity according to Soxhlet-Henkel.

It is important to titrate quickly.

Instruments

- Titrator with SET mode
- 10 mL burette
- Stirrer

Electrode

Porotrode	6.0235.200

Reagents

- Sodium hydroxide, NaOH
- Metrohm buffer pH = 4.0 (6.2307.100)
- Metrohm buffer pH = 7.0 (6.2307.110)
- Metrohm buffer pH = 9.0 (6.2307.120)
- Potassium hydrogen phthalate, KHP

Solutions

Titrant	c(NaOH) = 0.1 mol/L If possible this solution should be bought from a supplier.
Electrolyte	Porolyte (6.2318.000)

Standard

KHP	KHP is dried over night in a drying oven at 105 °C and allowed to cool down in a desiccator for at least 1 h.

Sample preparation

- For milk, no sample preparation is required.
- For dried milk, an appropriate amount of sample is weighed into a conical flask. 50 mL deion. water (approx. 20 °C) is added and the sample is vigorously agitated before allowing it to stand for about 20 min.
- For yoghurt, 10.0 g is weighed into a titration beaker and diluted with 10 mL dist. water.

Analysis

Electrode calibration

The electrode is calibrated using the buffer solutions pH = 4.0, pH = 7.0 and pH = 9.0.

Titer

90 to 120 mg dried KHP is weighed into a titration beaker and dissolved in approximately 50 mL deion. H_2O. The solution is then titrated with c(NaOH) = 0.1 mol/L until after the equivalence point.

Sample

50 mL milk or the prepared dried milk respectively yoghurt sample is titrated by a SET titration to pH = 8.3 using c(NaOH) = 0.1 mol/L.

Parameters

Titer

Mode	DET U
Stirring rate	8
Signal drift	50 mV/min
Max. waiting time	26 s
Meas. point density	4
Min. increment	10 µL
Stop volume	10 mL
EP criterion	5
EP recognition	greatest

Sample

Mode	SET pH
Stirring rate	8
EP at pH	8.30
Dynamics	1
Max. rate	10 mL/min
Min. rate	25 µL/min
Stop criterion	Drift
Stop drift	20 µL/min

Calculation

Titer

$$f = \frac{m_s}{V_{EP1} \times c_{NaOH} \times M_{Std}}$$

f: Titer of the sodium hydroxide solution; c(NaOH) = 0.1 mol/L

m_s: Mass of standard (KHP) in mg

V_{EP1}: Titrant consumption until the equivalence point in mL

c_{NaOH}: Concentration of the sodium hydroxide solution in mol/L; here c(NaOH) = 0.1 mol/L

M_{Std}: Molecular weight of the standard (KHP); 204.22 g/mol

Sample

Milk

$$TA = \frac{V_{EP1} \times f \times 1000}{V_S}$$

$$°SN = \frac{V_{EP1} \times f \times 1000}{V_S \times 25}$$

TA: Titratable acidity as mL c(NaOH) = 0.1 mol/L for 1 L milk

°SN: Titratable acidity according to Soxhlet-Henkel as mL c(NaOH) = 0.25 mol/L for 100 mL milk

V_{EP1}: Titrant consumption until the equivalence point in mL

f: Titer of the sodium hydroxide solution; c(NaOH) = 0.1 mol/L

V_S: Sample size in mL

1000: Conversion factor for 1 L

25: Conversion factor for the acid number according to Soxhlet-Henkel

Dried milk

$$TA = V_{EP1} \times f \times 2$$

TA: Titratable acidity as mL c(NaOH) = 0.1 mol/L for 10 g of solid-non fat content

V_{EP1}: Titrant consumption until the equivalence point in mL

f: Titer of the sodium hydroxide solution; c(NaOH) = 0.1 mol/L

2: Conversion factor 5 g to 10 g

Yoghurt

$$TA = \frac{V_{EP1} \times c_{NaOH} \times f \times 100}{m_S}$$

TA: Titratable acidty as mmol/100 g yoghurt

V_{EP1}: Titrant consumption until the equivalence point in mL

c_{NaOH}: Concentration of the sodium hydroxide solution in mol/L; here c(NaOH) = 0.1 mol/L

f: Titer of the sodium hydroxide solution; c(NaOH) = 0.1 mol/L

m_S: Sample size in g

100: Conversion factor

Example determination

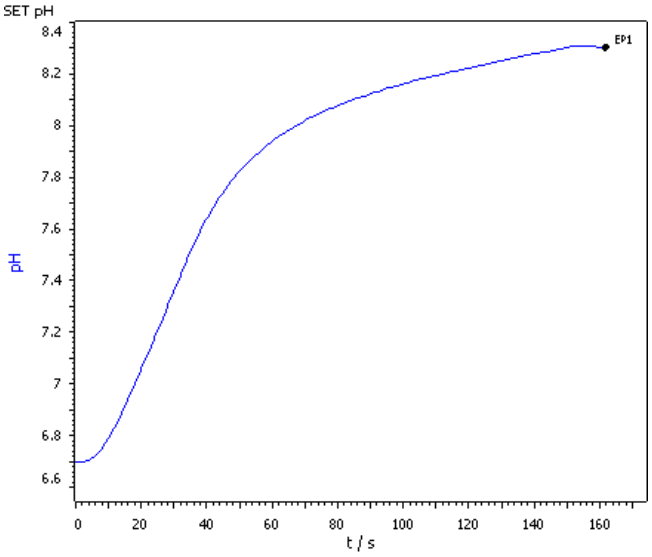


Fig. 1: Example titration curve for the determination of the titratable acidity in milk.

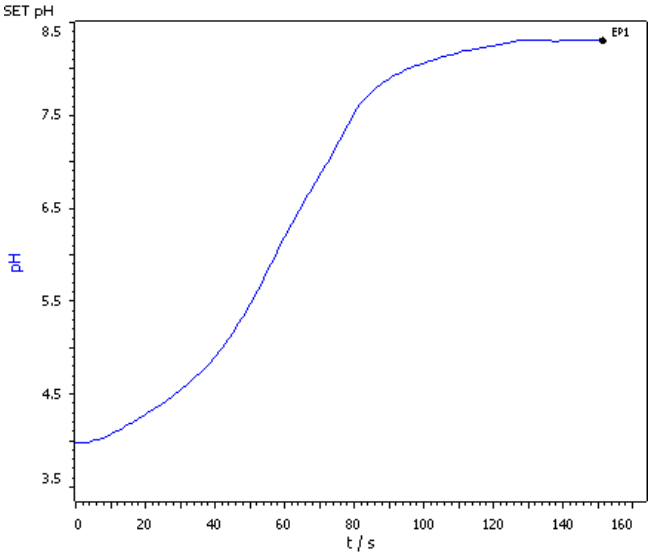


Fig. 2: Example titration curve for the determination of the titratable acidity in yoghurt.

Comments

- The determined titratable acidity in milk is given mainly through the absorption of hydroxyl ions via milk proteins and milk salts at a pH value of 8.3. The total acidity increases with bacterial acidification and with enzymatic lipolysis.
- For dried milk the appropriate sample amount can be calculated as follows:
  $m_s = 500 / a$
  where a is the solids-not-fat content of the sample. The solids-not-fat content can be calculated by subtracting the fat and moisture content from 100.
- For the analysis according to DIN 10316 c(NaOH) = 0.25 mol/L is used as titrant.
- For the analysis according to ISO 6091 / IDF 86 the end point of the titration is at pH 8.4.
- The following table summarizes characteristic values in assessing milk samples:

	Titratable acidity	Titratable acidity according to Soxhlet-Henkel
Normal, fresh milk	160 – 190	6.5 – 7.5
Milk from cows with diseased udders	< 150	< 6.0
Sour and rancid milk	> 200	> 8.0

References

- ISO/TS 11869 / IDF/RM 150
  Fermented milks – Determination of titratable acidity – Potentiometric method
- ISO 6091 / IDF 86
  Dried milk — Determination of titratable acidity (Reference method)
- DIN 10316
  Acidity test of milk and liquid milk products according to Soxhlet-Henkel
- Schweizerisches Lebensmittelbuch
  Chapter 1 – Milk

Determination of chloride

Summary

The chloride content is determined with silver nitrate. From this the total sodium chloride content could be calculated, however not all chloride may come from sodium chloride. It is therefore recommended to also determine the sodium content. For this, please refer to the section *Determination of sodium*.

Instruments

- Titrator with DET mode
- 20 mL burette
- Stirrer
- Polytron with aggregate 6.9012.010 (Cheese)

Electrode

Ag Titrode with sulfide coating	6.0430.100S

Reagents

- Silver nitrate, $AgNO_3$
- Nitric acid, c(HNO_3) = 4 mol/L
- Sodium chloride, NaCl, p.a.
- 2-propanol, isopropanol
- Potassium ferrocyanide trihydrate, $K_4[Fe(CN)_6] \cdot 3\ H_2O$, p.a.
- Zinc acetate dihydrate, $Zn(CH_3COO)_2 \cdot 2\ H_2O$, p.a.
- Glacial acetic acid, CH_3COOH, p.a.

Solutions

Titrant	c($AgNO_3$) = 0.1 mol/L If possible this solution should be bought from a supplier.
Carrez solution 1	Used for milk or milk products with high protein content 106 g PFC3H2O is weight into a 1000 mL volumetric flask and dissolved in deion. H_2O. The flask is then filled up to the mark with deion. H_2O.
Carrez solution 2	Used for milk or milk products with high protein content 220 g ZnAcetate is weight into a 1000 mL volumetric flask and dissolved in deion. H_2O. 30 mL glacial acetic acid is added and the flask is then filled up to the mark with deion. H_2O.

Standard

NaCl	NaCl is dried overnight in a drying oven at 140 °C and allowed to cool down in a desiccator for at least 1 h.

Sample preparation

The sample is homogenized in order to ensure a representative sample.

For cheese, the rind is removed previously to obtain a representative sample as it is usually consumed.

For milk and milk products it might be necessary to remove proteins by precipitation to achieve acceptable performance. In this case an appropriate amount (e.g., 25 g) sample is weighed into a 50 mL centrifuge tube. 2.5 mL Carrez solution 1 and 2.5 mL Carrez solution 2 is added to the tube. The sample is then diluted to 50 mL before centrifugation at 12'000 *g* for 5 min at 4 °C.

Analysis

Titer

50 – 100 mg NaCl is weighed into a titration vessel and dissolved in ca. 50 mL deion H_2O. 2 mL c(HNO_3) = 4 mol/L is added and the solution then titrated with c($AgNO_3$) = 0.1 mol/L until after the equivalence point.

Blank

Milk

Into a clean titration vessel 50 mL hot (55 °C), deion. H_2O is added. After the addition of 2 mL c(HNO_3) = 4 mol/L the solution is titrated with c($AgNO_3$) = 0.1 mol/L until after the equivalence point.

Butter

Into a clean titration vessel 100 mL deion. H_2O is added, the water is then heated to boiling. After cooling down below 55 °C, 2 mL c(HNO_3) = 4 mol/L is added and the solution is titrated with c($AgNO_3$) = 0.1 mol/L until after the equivalence point.

Cheese

Into a clean titration vessel 80 mL hot (55 °C), deion. H_2O is added. After the addition of 2 mL c(HNO_3) = 4 mol/L the solution is titrated with c($AgNO_3$) = 0.1 mol/L until after the equivalence point.

Sample

Milk

10 mL supernatant is pipetted into a titration beaker or an appropriate aliquot of untreated sample is weighed into a titration beaker and diluted with 50 mL deion. H_2O. After the addition of 5 mL c(HNO_3) = 4 mol/L the solution is titrated with c($AgNO_3$) = 0.1 mol/L until after the equivalence point.

Buret tip and electrode are rinsed with isopropanol after each titration in order to remove organic residue.

Butter

2 to 4 g butter is weighed into a titration vessel. 100 mL deion. H_2O is added and heated to boiling. The hot suspension is cooled below 55 °C, 2 mL c(HNO_3) = 4 mol/L is added and the suspension is then titrated with c($AgNO_3$) = 0.1 mol/L until after the equivalence point

Buret tip and electrode are rinsed with isopropanol after each titration in order to remove organic residue.

Cheese

2 to 5 g cheese is weighed into a titration vessel. 80 mL hot (55 °C) deion. H_2O is added and the cheese is then suspended in the titration vessel using the Polytron. The suspension time needed depends on the type of cheese. 2 mL c(HNO_3) = 4 mol/L is added and the suspension is then titrated with c($AgNO_3$) = 0.1 mol/L until after the equivalence point.

The polytron, buret tip and electrode are rinsed with isopropanol after each titration in order to remove organic residue.

Parameters

Titer

Mode	DET U
Signal drift	50 mV/min
Max. waiting time	26 s
Meas. point. density	4
Min. increment	10 µL
Stop Volume	20 mL
EP criterion	5
EP recognition	greatest

Blank and Sample

Mode	DET U
Pause	10 s
Signal drift	50 mV/min
Max. waiting time	26 s
Meas. point. density	4
Min. increment	10 µL
EP criterion	5
EP recognition	greatest

Calculation

Titer

$$f = \frac{m_s}{V_{EP1} \times c_{AgNO_3} \times M_{Std}}$$

f:	Titer of $AgNO_3$ solution
m_s:	Mass of standard in mg
V_{EP1}:	Titrant consumption until the equivalence point in mL
c_{AgNO_3}:	Concentration of the titrant in mol/L; c($AgNO_3$) = 0.1 mol/L
M_{Std}:	Molecular weight of the standard (NaCl); 58.44 g/mol

Blank

Blank = V_{EP1}

Blank:	Blank of the silver chloride solution in mL
V_{EP1}:	Titrant consumption until the equivalence point in mL

Sample

$$w_{Cl} = \frac{(V_{EP1} - Blank) \times c_{AgNO_3} \times f \times M_A \times 0.1}{m_s}$$

w_{Cl}:	Content of chloride in %
V_{EP1}:	Titrant consumption until the equivalence point in mL
c_{AgNO_3}:	Concentration of the titrant in mol/L; c($AgNO_3$) = 0.1 mol/L
f:	Titer of $AgNO_3$
M_A:	Molecular weight of the analyte (Cl or NaCl); 35.45 respectively 58.44 g/mol
m_S:	Sample size in g
0.1:	Conversion factor

Example determination

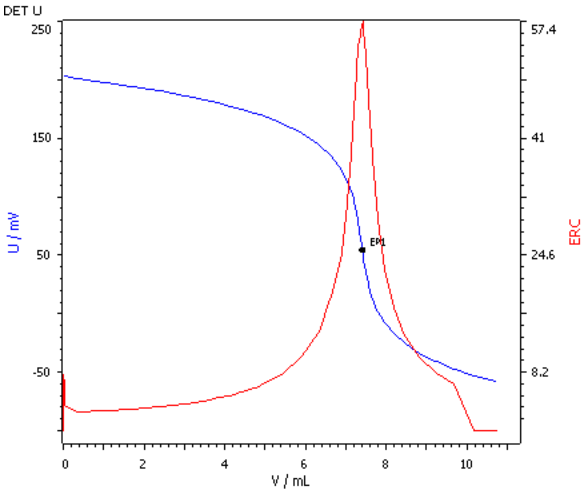


Fig. 3: Titration curve for the determination of chloride in herbal butter

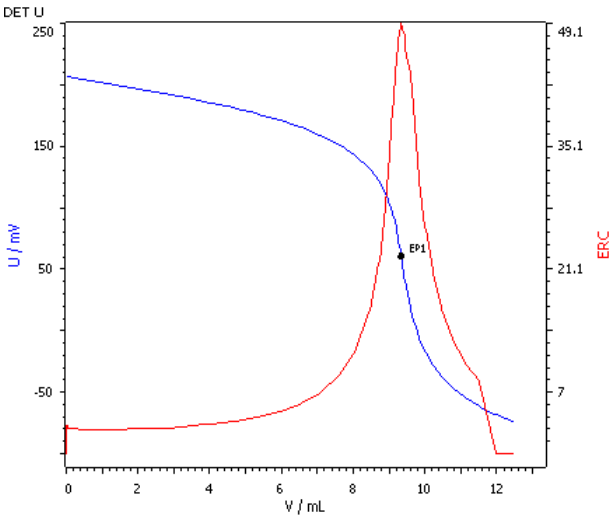


Fig. 4: Titration curve for the determination of chloride in cheese

Comments

- The chloride content in milk is between 90 and 110 mg / 100 mL.
- The described method is only applicable to butter with a salt content higher than 0.1%. The chloride content in unsalted butter is below 0.1%, in salted butter between 0.5 and 2%.
- The pH of the sample solution should be below 1.5 before the start of the titration otherwise a little more c(HNO_3) = 4 mol/L should be added.
- The chloride content in cheese can vary within wide limits.
- Only run the Polytron with the connected aggregate immersed in a liquid. Otherwise the Polytron can be damaged. Running the drive without connected aggregate is possible. For more details on the use of the Polytron please see AB 418.

References

- EN ISO 5943 / IDF 88
  Cheese and processed cheese products – Determination of chloride content – Potentiometric titration method
- ISO 15648 / IDF 179
  Butter – Determination of salt content – Potentiometric method
- ISO 21422 / IDF 242
  Milk, milk products, infant formula and adult nutritionals — Determination of chloride — Potentiometric titration method
- AB 418
  Use of the Polytron PT 1300 D (Metrohm version)

Determination of sodium

Summary

The sodium content of milk can be rapidly and easily titrated thermometrically using a standard solution of Al^{3+} as titrant. Sodium is quantitatively precipitated as NaK_2AlF_6 in the presence of an excess of fluoride and potassium ions.

Instruments

- Thermometric titrator
- 10 mL burette
- Stirrer

Electrode

Thermoprobe HF	6.9011.040

Reagents

- Aluminum nitrate, $Al(NO_3)_3$
- Potassium nitrate, KNO_3
- Sodium chloride, NaCl
- Ammonium fluoride, NH_4F
- Trichloroacetic acid
- Glacial acetic acid

Solutions

Titrant	c($Al(NO_3)_3$) = 0.5 mol/L and c(KNO_3) = 1.1 mol/L 187.57 g $Al(NO_3)_3$ and 111.22 g KNO_3 are weighed into a 1000 mL volumetric flask, dissolved in approx. 800 mL deion. water and filled up to the mark.
Complexing agent	β(NH_4F) = 400 g/L 400 g ammonium fluoride is weighed into a 1 L volumetric flask and dissolved in deion. H_2O. The flask is then filled up to the mark with deion. H_2O.
Coagulant	Trichloroacetic acid 25% (w/v) in water 250 g trichloroacetic acid is weighed into a 1 L volumetric flask containing already approx. 200 mL deion H_2O. The flask is then filled up to the mark with deion. H_2O.

Standard solution

c(NaCl) = 0.25 mol/L	NaCl is dried over night in a drying oven at 140 °C and allowed to cool down in a desiccator for at least 1 h.

Sample preparation

Prepare a quantity of milk serum by transferring 500 mL of milk product into a 1000 mL beaker equipped with a large magnetic stirring bar. Place on a magnetic stirrer, and while stirring, slowly add 50 mL 25% (w/v) trichloroacetic acid. Stir for 20 minutes and wait for another 10 minutes. Either filter the coagulated milk through a Whatman no. 4 filter paper (or similar), or separate the milk serum by centrifugation.

Analysis

Titer

2 to 8 mL standard are pipetted into a titration vessel and diluted to 30 mL with deion. H_2O. Then 5 mL complexing agent and 2 mL glacial acetic acid are added and after a pause of 20 s the solution is titrated with the Al/K titrant until after the endpoint.

Titrate at least 5 different amounts of standard in an ascending order.

Method blank

20 to 50 mL serum are pipetted into a titration vessel and diluted to 50 mL with deion. H_2O, where necessary. Then 5 mL complexing agent and 2 mL glacial acetic acid are added and after a pause of 20 s the solution is titrated with the Al/K titrant until after the endpoint.

Titrate at least 5 different amounts of serum in an ascending order.

Sample

50 mL serum (corresponds to 45.45 mL sample) are pipetted into a titration vessel then 5 mL complexing agent and 2 mL glacial acetic acid are added and after a pause of 20 s the solution is titrated with the Al/K titrant until after the endpoint.

Parameters

Titer

Pause	20 s
Dosing rate	4 mL/min
Filter factor	30
Damping until	0.5 mL
Stop volume	5 mL
Evaluation start	0.5 mL
Reaction type	exothermic
EP criterion	-15

Method blank and Sample

Pause	20 s
Dosing rate	4 mL/min
Filter factor	30
Damping until	0.5 mL
Evaluation start	0.5 mL
Reaction type	exothermic
EP criterion	-15

Calculation

Titer

A linear regression of the mL of titrant consumed versus the different sizes of the standard in mL is plotted automatically by ***tiamo***™. The titer is then calculated from the slope. For more details on the calculation of the titer for thermometric titration have a look at AN-H-131.

$$f = \frac{c_{NaCl}}{a \times c_{Al/K}}$$

f:	Titer of the selected titrant
c_{NaCl}:	Exact concentration of the standard solution in mol/L
a:	Slope of the liner regression
$c_{Al/K}$:	Concentration of the titrant in mol/L; here $c(Al(NO_3)_3)$ = 0.5 mol/L

Blank

A linear regression of the different sizes of the sample in mL against the mL of titrant consumed is plotted automatically by ***tiamo***™. The method blank is defined as the intercept of the linear regression line with the y-axis. For more details on the calculation of the blank for thermometric titration have a look at AN-H-131.

Sample

$$\beta_{Na} = \frac{(V_{EP1} - Blank) \times c_{Al/K} \times f \times M_A \times 100}{V_s}$$

β_{Na}:	Sodium content in mg / 100 mL
V_{EP1}:	Titrant consumption until the first endpoint in mL
$c_{Al/K}$:	Concentration of the titrant; $c(Al(NO_3)_3)$ = 0.5 mol/L
f:	Titer of the selected titrant
M_A:	Molecular weight of sodium; 22.990 g/mol
V_S:	Sample size in mL (45.45 mL)
100:	Conversion factor

Example determination

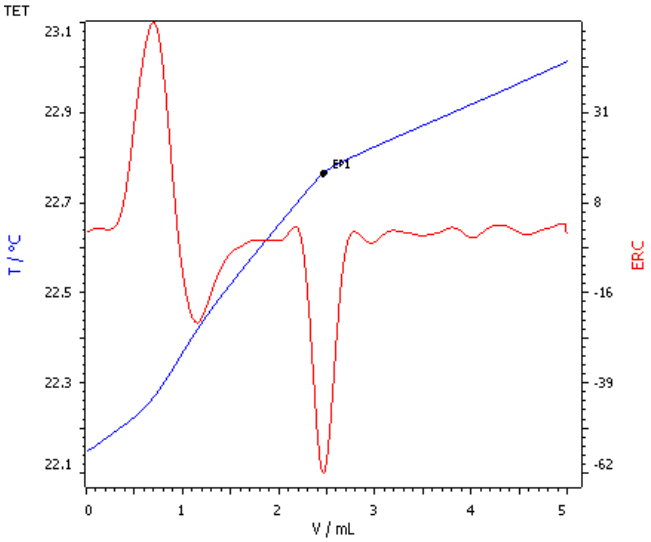


Fig. 5: Titration curve for the determination of sodium in milk using thermometric titration

Comments

- The toxic and corrosive NH_4·HF can be replaced by a NH_4F solution. In order to ensure the sample is sufficiently acidic 2 mL glacial acetic acid has to be added.
- For the analysis of sodium in cheese the addition of trichloroacetic acid is not necessary. For more information on this analysis please refer to AN-H-133.
- It is essential that the titration assembly must be adjusted in such a way that the Thermoprobe and the buret tips should be 1 mm above the propeller. The direction of rotation of the stirrer must be set in a way to carry the titrant delivered away from the sensor of the Thermoprobe to minimize noise.

- The linear regression for the titer and the blank can be determined automatically from the results using ***tiamo***™.
- For more information about the titer and blank determination using ***tiamo***™, see also Metrohm Application Note AN-H-131.
- CCl_3COOH (trichloroacetic acid) and its solutions are toxic and corrosive. Wear appropriate protective clothing. Adhere to recommendations in MSDS documentation. Disposal of CCl_3COOH solutions and residues containing CCl_3COOH should be in accordance with local regulations.

References

- AN-H-131
  Determination of titer and blank value for thermometric titrations using ***tiamo***™
- AN-H-133
  Automatic sodium determination in cheese

Oxidation stability

Summary

Susceptibility to rancidness can be tested with the Rancimat method, which is an accelerated aging test. For this purpose air passes through a sample at elevated temperature. The reaction products of the oxidation of the fatty acids, particularly low molecular organic acids, are absorbed in the measuring solution (deionized water). The conductivity increases due to volatile organic acids. This is used as a measure for the progress of the oxidation. At the point where the conductivity rises rapidly the induction time is reached, which characterizes the oxidation stability of the sample.

The analysis regulation corresponds with the following generally acknowledged methods:

- AOCS Cd 12b-92 - Sampling and analysis of commercial fats and oils. Oil Stability Index (OSI)
- ISO 6886 / GB/T 21121 – Animal and vegetable fats and oils – Determination of the oxidative stability

Instruments

- Rancimat
- Laboratory balance (resolution ± 0.01 g)

Reagents

- Deionized water (ISO 3696 Type II)

Sample preparation

No sample preparation is required. Butter can be weighed in directly.

In case of problems weighing solid fat into the bottom part of the reaction vessel, the sample can be previously melted on a water bath. Care has to be taken that the water bath temperature is not far beyond the melting point of the sample. Otherwise deterioration of the sample can be expected.

Analysis

Before the determination can be started, the temperature of the heating block has to be stable. Fill each measuring vessel with 60 mL deionized water and place it on the Rancimat together with the measuring vessel cover containing the integrated conductivity cell. Use a new and clean reaction vessel. Weigh in 3 g of sample into the bottom part without touching the side walls and close it with the reaction vessel cover with the air inlet tube attached. Connect the two tubing for the air supply, place the reaction vessel in the heating block and start the data recording immediately.

Parameters

Sample	Clarified butter (butter oil, ghee, …)
Sample size	3.0 g ± 0.1 g
Measuring solution	60 mL deionized water
Temperature	120 °C
Gas Flow	20 L/h
Evaluation	Induction time
Evaluation sensitivity	1.0

Example determination

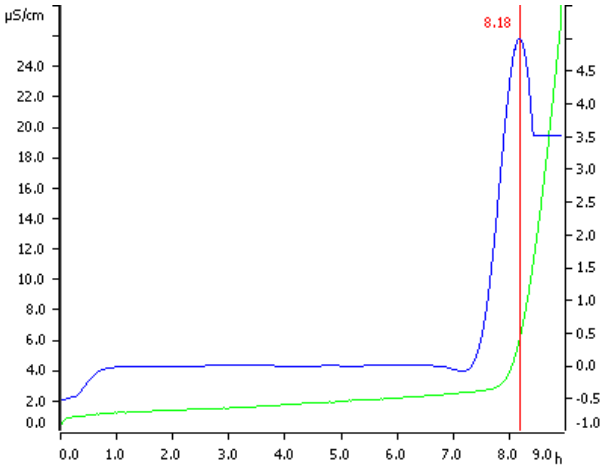


Fig. 6: Oxidation stability of clarified butter determined by the Rancimat method (green: measurement curve, blue: 2nd derivative used for automatic evaluation of induction time)

Comments

- The induction times are approximately doubled with a temperature decrease of 10 °C.
- Make sure that the reaction and measuring vessels are very clean! Dirt particles (e.g. dust, residue from a washing agent) catalyze the reaction.
- The initial conductivity of the measuring solution should be less than 20 µS/cm.

References

- AOCS Cd 12b-92
  Sampling and analysis of commercial fats and oils: Oil Stability Index
- ISO 6886 / GB/T 21121
  Animal and vegetable fats and oils – Determination of the oxidative stability (accelerated oxidation test)
- AB 204
  Oxidation stability of oils and fats – Rancimat method

Determination of lactose in lactose free milk

Summary

The determination of lactose in lactose free milk and other products requires, in some cases, the separation of lactose from lactulose. The lactulose peak can overlap the lactose peak. This can increase the lactose concentration, which will lead to wrong positive results.

The method described here is optimized to achieve better separation of lactose and lactulose. The sugar separation includes also other compounds which have to be determined in other lactose-free food products.

Instruments

- IC instrument
- Sample changer
- Amperometric detector
- IC equipment for dialysis

Columns

Metrosep Carb 2 – 150/4.0	6.1090.420
Metrosep Carb 2 Guard/4.0	6.1090.500
Metrosep CO3 Trap 1 – 100/4.0	6.1015.300

Reagents

- Sodium hydroxide, NaOH, suprapur 30%
- Glucose, galactose, sucrose, fructose, maltose, lactose, and lactulose standard solutions = 1 g/L in ultrapure water, prepared from commercially available standards, p.a ≥ 98%
- Sodium acetate anhydrous, $NaCH_3CO_2$, puriss. p.a ≥ 99.0%
- Acetone, C_3H_6O, puriss. p.a ≥ 99.5%
- Ethanol, C_2H_5OH, puriss. p.a ≥ 99.8%
- Ultrapure water, resistivity >18 MΩ·cm (25 °C), type I grade (ASTM D1193)

Solutions

Eluent	c(NaOH) = 5 mmol/L and $c(NaCH_3CO_2)$ = 2 mmol/L in carbon dioxide free ultrapure water
Rinsing solution 1	Ultrapure water/ethanol Φ(EtOH) = 50% (V/V)
Rinsing solution 2	Ultrapure water

Standard solutions

β [mg/L]	Sucrose	Galactose	Glucose	Fructose
Std. 1	0.25	0.25	0.25	0.25
Std. 2	0.50	0.50	0.50	0.50
Std. 3	1.0	1.0	1.0	1.0
Std. 4	5.0	5.0	5.0	5.0
Std. 5	15	15	15	15
Std. 6	30	30	30	30
Std. 7	60	60	60	60

β [mg/L]	Lactose	Lactulose	Maltose
Std. 1	0.25	0.25	0.25
Std. 2	0.50	0.50	0.50
Std. 3	1.0	1.0	1.0
Std. 4	5.0	5.0	5.0
Std. 5	15	15	15
Std. 6	30	30	30
Std. 7	60	60	60

Sample preparation

The milk samples were diluted 1:100 in ultrapure water/ acetone solution 90:10 (v/v). The diluted milk samples were analyzed directly using Inline Dialysis before injection.

Analysis

The samples and standards were injected after the Inline Dialysis.

Column regeneration

Rinse the pre-column and trap-column every two weeks for 13 h with a 300 mmol/L NaOH solution with 1 mL/min at 40 °C. The column itself needs only to be rinsed when necessary.

Column conditioning

Rinse the columns with the eluent for minimum 12 h to remove the excess of the NaOH.

Depending on the sample load, the columns can be regenerated periodically.

Parameters

Flow	0.8 mL/min
E1	0.05 V
E2	0.55 V
E3	-0.1 V
t1	300 ms
t2	50 ms
t3	200 ms
Measurement duration	100 ms
Range	200 µA
Channel	current
Column temperature	40 °C
Detector temperature	35 °C
Sample Loop	20 µL
Dialysis time	10 min
Transfer time	32 s

Calculation

Automatic integration with MagIC Net software using peak area.

Example determination

For example chromatograms of the calibration and the sample see *Appendix*.

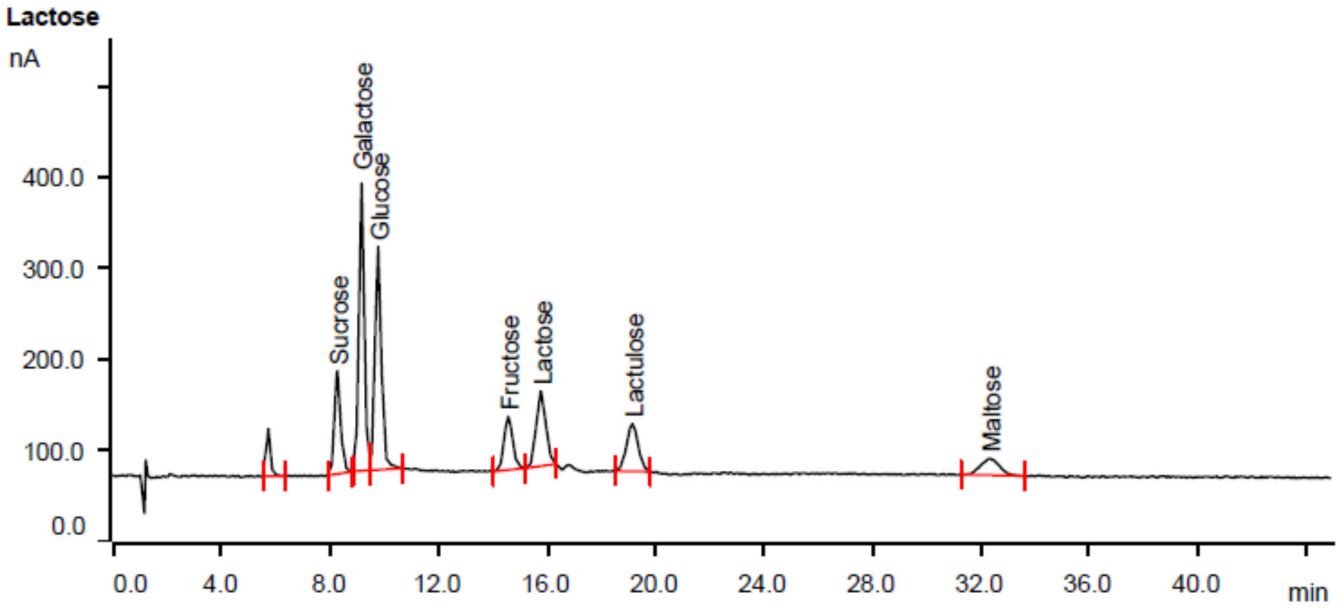
Comments

- For the eluent preparation a 30% NaOH solution was used because of its lower viscosity (compared to 50% NaOH).
- The carbonate from the eluent has to be removed by a carbonate trap column. This avoids additional loading of carbonate onto the column during the daily analysis, which would lead to a strong peak shift.
- The carbonate trap column was installed between the inline filter and the pulsation absorber.
- The column regeneration with a concentrated sodium hydroxide solution removes the carbonate from the column. Therefore a used column has to be preconditioned prior to analysis.
- To improve the peak stability the milk samples were diluted with ultrapure water/ acetone solution 90:10 (v/v). After every tenth milk sample a rinsing sample containing ethanol/ ultrapure water (1:1, v/v) and a control standard were injected. This helped to follow the expected peak shift. After analysis of 58 diluted milk samples nearly no peak shift was detected.
- The lactose content in the milk was below the lowest standard. Therefore the milk was spiked at 100 mg/L level lactose which refers to the new target limit.

Author

Competence Center Titration
Competence Center Voltammetry and Stability
Competence Center Ion Chromatography
Metrohm International Headquarters

Appendix



Peak number	Component name	Height	Area	Concentration	Retention time
		nA	(nA) x min	ppm	min
2	Sucrose	113.120	29.983	1.001	8.28
3	Galactose	315.324	74.032	0.999	9.16
4	Glucose	244.845	63.818	1.000	9.78
5	Fructose	58.505	21.604	0.997	14.57
6	Lactose	82.031	34.002	0.999	15.78
7	Lactulose	52.162	26.036	1.000	19.15
8	Maltose	18.370	14.730	0.999	32.32

Fig. 7: Standard 3 with 1 mg/L sugars.

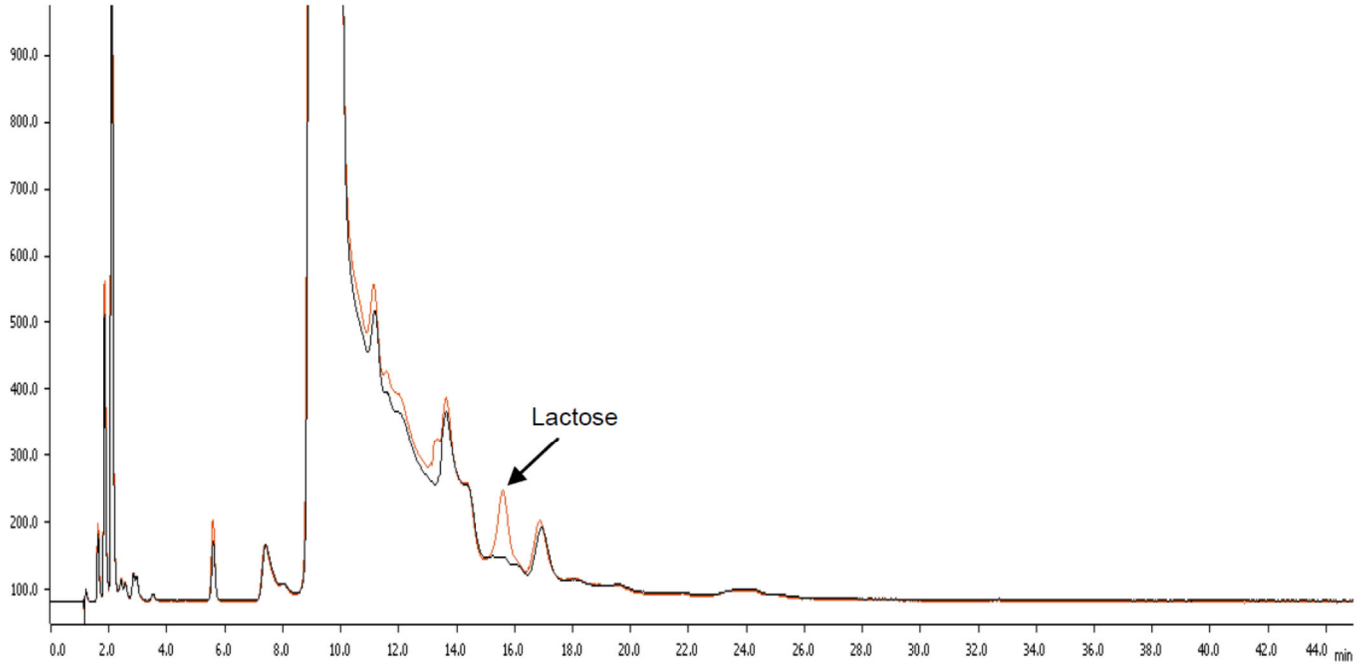


Fig. 8: Lactose free milk sample diluted (1:100) and spiked with 100 mg/L lactose (red); Inline Dialysis
Comparison with an unspiked milk sample diluted (1:100), black chromatogram; Dialysis