Methods of analysis for specialty yeast products

The methodologies presented below are representative of those used by the various EURASYP members but are by no means the only methods available for such analyses. The instructions provided are for the equipment specified. The details are presented for the purpose of information only and to illustrate the care taken by members to carry out proper and appropriate analyses. It is not our intention to provide analytical support or other advice via this website.

1) Ash by ignition

The purpose of this work instruction is the determination of ash by ignition on yeast products according to a standard method.

Apparatus

- Furnace, adjusted to 550 ± 25 °C
- Analytical balance
- Porcelain crucibles
- Hotplate

Reagents

Hydrochloric acid 10% Slowly add, under continuous stirring, 240 ml hydrochloric acid 37% to approximately 250 ml water in a 1000 ml volume flask. Allow to cool to room temperature, make up to volume with water and mix.

Measurements

- Immerse a crucible in hydrochloric acid 10% for 1 hour, rinse with water and ignite for 1 hour at 550 ± 25 °C.
- Allow to cool and equilibrate in a dessicator and weigh accurately to within 0.1 mg (m0).
- Introduce an amount of sample (liquid or solid) sufficient for an amount of ash in a range of 0.1 - 1 g in the crucible, (V or m1).
- Carbonize the sample on a hotplate until no vapors are observed.
- Ignite the sample in the muffle furnace during at least 16 hours at 550 ± 25 °C. Or until no carbon residue is present
- Allow to cool and equilibrate the sample in a dessicator and weigh accurately to within 0.1 mg (m2).

Calculation

Liquid samples:
ash (g/l) = (m2-m0) x 1000/V
Solid samples:
ash (%) = (m2-m0) X 100/ (m1-m0)

2) Chloride using a Jenway chloride meter

The purpose of this work instruction is the determination of chloride by a silver titration method on yeast products according to a standard method.

Principle

A sample to be measured is added to a buffer solution. A constant current is passed between two silver electrodes, which then liberate silver ions at a constant rate into a solution. These silver ions combine with the chloride ions in solution and are precipitated as insoluble silver chloride. When all of the chloride has combined with the generated silver, free silver ions become available in the solution and the two further silver electrodes detect their presence. During the generation of the silver the titration time is displayed. When the presence of excess silver ions is detected, at this point the digital readout of the titration time is halted. The titration time is a measure of the chloride concentration in the original sample.

Apparatus

- Chloride meter, complete with glass beakers and stirring bars (eg Jenway PCLM 3)
- Range: 500 Ml-mg/l
- Dispenser, adjusted at 15 ml
- Dispenser, adjusted at 1 ml
- Drying oven, adjusted at 100 ± 2 °C

Or equivalent

Reagents

1) water: Distilled or equivalent
2) Acid Mixture: While stirring, slowly add 15 ml metric acid 65%, analytical reagent (see remarks), to 600 ml water in a 1 L volumetric flask. Add 100 ml acetic acid 100%, analytical reagent (see remarks), make up to volume and mix.
3) Gelatin solution: Weigh 4.0 g gelatin (240-260 Bloom) in 600 ml water in a 1 L volumetric flask. While stirring, dissolve the material by slowly warming to 90 °C. When dissolved allow to cool to ambient temperature. Make up to volume with water and mix.
4) Standard solution: place 1 g sodium chloride, analytical reagent grade, into a beaker and dry for 2 hours in a drying oven at 100 ± 2 °C. Cool in a desiccator to ambient temperature. Weigh exactly 500mg of this dried sodium chloride into a 1 L volumetric flask. Make up to volume with water and mix.
5) Polish: Scouring liquid, hosehold quality, e.g. Cif

Procedure

1) Standard: Use standard sample as such
2) Sample: Prepare sample solutions containing 100-900 mg sodium chloride per liter
3) Measurement:
Conditioning of the acid/gelatin solution Transfer 15 ml acid mixture and 1 ml gelatin solution into the supplied glass beaker. Place the beaker on the chloride meter platform and mix the solutions with the aid of a stirring bar. Depress the "condition" switch momentarily. After approximately 5 seconds the digital display starts. After a while it will stop (this value on the display must be ignored). Titration Add accurately to within 1 mg approximately 0.5 g of the prepared solutions in the "conditioned" acid/gelatin solution. Depress the "titrate" switch momentarily. After approximately 5 seconds the digital display starts. The value obtained is the titration time in seconds (see remarks).

Calculation

500 x titration weight standard (g)/titration time standard (s) = standard factor (see remarks)
Titration time sample (s) x standard factor/titration weight sample (g) = mg NaCl per liter in the sample solution

Remarks

1) Titrate the standard solution in duplicate
2) Before each series clean the electrodes with a tissue moistened with polish. Gently rub the exposed ends of the electrodes until they are clean and bright. Rinse the electrodes with water to remove all traces polish and dry with a tissue. Place the electrodes in the electrode head and make sure they are of equal length, straight and parallel.
3) After four titrations clean the beaker and continue with freshly conditioned acid/gelatin solution.
4) Never leave the electrodes in the titration solution after each series.
5) If the polish procedure is ineffective, place the electrodes for 1 minute in a mixture of equal part nitric acid 65% and water. Rinse the electrodes several times with water.
6) To give accurate results, the titration time should be between 100 and 900 seconds.
7) The standard factor should be between 0.805 and 0.845. If the factor is not correct, adjust the 500 Ml screw at the back of the apparatus and run again the standard in duplicate. Repeat the procedure until the standard factor is within the limit.

3) Dry matter

The purpose of this work instruction is the determination the dry matter content on yeast products according to a standard manner.

Principle

To determine the percentage of dry matter of a sample, the liquid part is removed by heating at 100-105 °C. By weighing the sample before and after treatment, the percentage of dry matter is determined. Sand is used to enlarge the surface of viscous residues, improving the drying process.

Apparatus

- Oven adjusted at 100 - 105 °C.
- Crucible, stainless steel or aluminum, diameter 4 - 5 cm
- Analytical balance
- Stirring rods

Or equivalent

Material

- Sand, grain size 0.4 - 0.8 mm

Or equivalent

Measurement

- In case of viscous samples introduce approximately 30 g sand and a stirring rod into a crucible. Dry for at least 15 minutes in the oven.
- Weigh the pre-dried and in the exicator equilibrated crucible accurately to within 1 mg (m0).
- Transfer the prescribed amount sample in the crucible (m1).
- Transfer the crucible in the drying oven for 5 hour
- Cool down the samples in the exicator for approximately 15 minutes and weigh accurately to within 1 mg (m2).

Calculation

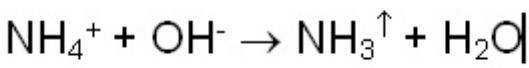
% dry matter = (m2 - m0)/ (m1 - m0) x 100%

4) Total Nitrogen by Kjeldahl methodology

The purpose of this work instruction is the determination of total nitrogen amount on yeast products according to a standard manner.

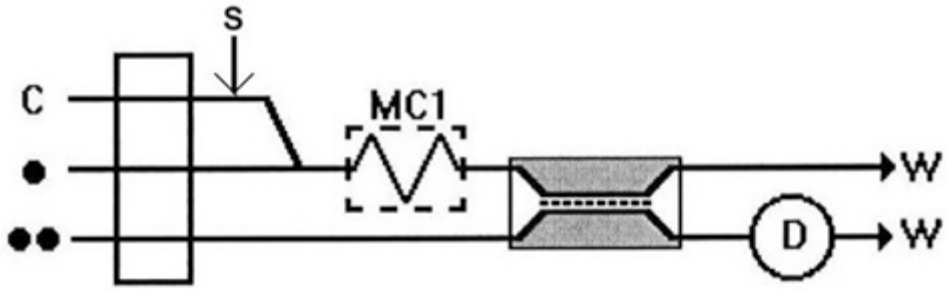
Principle

Using flow injection a fixed volume of the (diluted) sample solution is introduced into a carrier stream. The carrier stream is mixed with a sodium hydroxide solution in a thermo stated reaction coil.



The present ammonium is converted to ammonia, which passes through a gas permeable membrane into the indicator stream. The passed ammonia causes a local change of pH in the indicator stream, which realises a local change of the colour. The detector continuously measures the absorbance of indicator stream. The peak area or peak height detected is a measure for the concentration in the sample solution.

Apparatus
Tecator FIAstar 5000 Flow Injection System equipped with:
TKN Method Cassette 5000-040 see flow chart.



C	: Carrier tubing	red/red
S	: Sample tubing	grey/grey
.	: sodium hydroxide tubing	white/white
..	: indicator solution tubing	red/red
MC1	: thermostatic reaction coil	40°C
D	: detector path length	10 mm
	M-filter / R-filter	590 nm / 720 nm

- Pentium 4 computer with SOFIA software
- Tecator 5027 Autosampler
- Tecator 2040 digestion system connected with Tecator Autostep 2000 controller
- Dispenser Fortuna Optifix 10 ml
- Hamilton Microlab 500 diluter. Equipped with 2.5ml (sample) and10 ml (reagent) cylinder.
- Pipetman 500 - 5000 Ml
- Digestion tubes of 75 ml (calibrated)
- Membrane filter (Schleicher & Schuell RC 55 0.45 Mm)
- Spectrophotometer Hitachi U1500

Or equivalent.

SOFIA Program Conditions:
Wavelength :590 nm
Loop volume :40 Ml
Injection time :20 sec
Fill time :55 sec
Measuring time :75 sec
Thermostat temperature :40 °C
Integration mode :peak area
replicates :1
recalibration rate :off

Reagents

Description and purity Vendor Catalog number

Sulfuric acid p.a. 95-97 %	Merck	1.00731
Ammonium chloride p.a.>99.8%	Merck	1.01145
Kjeltabs, 1.5 g Potassium sulphate, 7.5 mg selenium	Thompson	AA11
Tecator Ammonia Indicator Mixture (5000 0295)	Tecator	5000 0295
Sodium hydroxide solution p.a. 32 %	Merck	1.05590
Potassium dihydrogen phosphate p.a.	Merck	1.04873
Sodium hydroxide 0.1 mol/l	Merck	1.09141
Hydrochloric acid 0.1 mol/l	Merck	1.09060

Water: demineralized water
Or equivalent.

- Standard Stock Solution Nitrogen 1.000 g/l Weigh 3.821 ± 0.010 g ammonium chloride p.a. accurately to 1 mg, transfer in a volumetric flask of 1000 ml, make up to volume with water and mix. Prepare 2 independent solutions A and B. Shelf life: 3 months at room temperature
- Control Stock Solution Commercially available certified Ammonium standard solution 1000 mg/l NH_4+ (=777.8 mg/l N)
- Dilute sulfuric acid Introduce in a 1000 ml volumetric flask approximately 500 ml water, using the optifix add 3 ml sulfuric acid 95 - 97 % make up to volume with water and mix. Shelf life: 3 months at room temperature
- Indicator stock solution: Weigh 0.500 ± 0.010 g Ammonia Indicator Mixture accurately to within 1 mg in a 100 ml volumetric flask, add respectively 50 ml water and 2.5 ml sodium hydroxide 0.1 mol/l, dissolve, make up to volume and mix. Shelf life: 2 months at room temperature
- Potassium dihydrogen phosphate 0.1 mol/l Weigh 1.360 ± 0.050 g potassium dihydrogen phosphate accurately to within 1 mg in a 100 ml volumetric flask, add respectively 50 ml water and 2.5 ml sodium hydroxide 0.1 mol/l, dissolve, make up to volume and mix. Shelf life: 2 months at room temperature
- Indicator solution Transfer 20 ml indicator stock solution in a 1000 ml volumetric flask; add 5 ml potassium dihydrogen phosphate 0.1 mol/l make up to volume and mix. Filter the solution over the membrane filter. Measure the absorbance at 590 nm (permitted range: 0.600 ± 0.050) If A < 550 add sodium hydroxide 0.1 mol/l in portions of 50 Ml and check A If A > 550 add hydrochloric acid 0.1 mol/l in portions of 50 Ml and check A This indicator solution needs a stabilization time of 24 h. Shelf life: 2 months at room temperature
- Sodium hydroxide solution 4 mol/l Weigh 500 ± 2.5 g sodium hydroxide solution 32% accurately to within 0.1 g in a 1000 ml volumetric flask, add approximately 100 ml water, cool to room temperature, make up to volume with water and mix. Shelf life: 3 months at room temperature

Procedure

1.2. Pretreatment standard
1.3.

- Calibration solutions Using the dilutor dilute respectively 0.00, 0.50, 1.25, 2.50, 3.75, and 5.00 ml standard stock solution in volumetric flasks of 250 ml. Add respectively 100 ml water and using the Optifix add 10 ml sulfuric acid 95 -97%. Cool to room temperature, make up to volume with water and mix. Shelf life: 1 month at room temperature
- Reagent blank Transfer approximately 25 ml water into a digestion tube and, using the Optifix, add 3.0 ml sulphuric acid 95 -97%. Cool to room temperature, make up to volume with water and mix. Shelf life: prepare a daily fresh solution
- Continuous Calibration Verification solution (CCV-standard) Using the dilutor transfer 0.75 ml standard stock solution into a digestion tube. Using the Optifix add 3.0 ml sulfuric acid 95 - 97%. Cool to room temperature, make up to volume with water and mix. Prepare two independent solutions A and B Shelf life: prepare a daily fresh solution

1.4. Pretreatment control

Using the dilutor dilute 1.00 ml control stock solution with 4 ml diluted sulfuric acid into a digestion tube. Using the Optifix add 3 ml sulfuric acid 95 - 97%, add a Kjeltab and perform the digestion procedure as described in table 1. Cool to room temperature, make up to volume and mix. Use 2 independent control solutions (A en B).

1.5. Pretreatment samples

Weigh or pipette such an amount of sample in the digestion tube that the nitrogen content is within the dynamic range of the method: 0.5 - 20 mg N/l in the measuring solution. (See application methods). Add respectively 3 ml sulfuric acid 95 - 97%, add a Kjeltab and perform the digestion procedure as described in table 1. Cool to room temperature, make up to volume and mix.

Table 1 Digestion temperature program

Step	**Temperature[°C]**	**Time[h:min]**
1	200	0:30
2	360	1:30

1.6. Measurement

Inject the obtained solutions into the FIASTAR 5000 system and measure the nitrogen peak areas with the aid of the data processing system.

Element	**Dynamic range [mg/l]**	**CCV concentration [mg/l]**
N	0.5 - 20	10

Calculation A x (B-C) / (D-C) = mg Ammonium Nitrogen per liter measuring solution

where:
A = concentration in the CCV-standard [mg/l] B = measured concentration N in the measuring solution [mg/l]
C = measured concentration N blank [mg/l] D = measured concentration N in the CCV-standard [mg/l]

Remarks
- Samples containing a lot of sugars can give digestion problems (black foam), in this case avoid water and place the digestion tubes directly in the digester at 360°C
- Dilute the measuring solution with the reagent blank whenever the concentration in the measuring solution exceeds the upper limit of the dynamic range.

5) Amino Nitrogen using TNBS

The purpose of this work instruction is the determination of amine groups on yeast products according to a standard manner.

Principle
α-amino nitrogen groups react with trinitrobenzenesulphonic acid (TNBSA) to give a yellow complex. The optical density of this complex is a measure for the α-amino nitrogen content.

Apparatus
- Water bath provided with a lid and adjusted at 40 ± 1 °C.
- Spectrophotometer, adjusted at 420 nm and provided with a 10 mm cell
- Dispenser adjusted at 1 ml
- Diluter, provided with 0.5 and 5 ml cylinders
- Pipette adjusted at 1 ml
- Glass centrifuge tubes
- Drying oven adjusted at 110 ± 2 °C.

Or equivalent Reagents

1) Distilled water or equivalent
2) Sodium dodecyl sulphate (SDS) solution: Dissolve 10.00 g SDS, analytical reagent, in approximately 80 ml water in a 100 ml glass beaker. Quantitatively transfer this solution into a 100 ml volumetric flask, make up to volume with the water and mix. Always use a freshly prepared solution.
3) Sodium phosphate buffer: Dissolve 17.78 g disodium hydrogen phosphate dehydrate, analytical reagent, in approximately 80 ml water in a 100 ml volumetric flask. Make up to volume with the same and mix.
4) TNBSA solution: Dissolve 0.1 g trinitrobenzene sulphonic acid (sigma grade II or equivalent), in approximately 80 ml water in a 100 ml volumetric flask. Make up to volume and mix. Always use a freshly prepared solution.
5) Sulphuric acid 1 mol/l: introduce 5.5 ml sulphuric acid 96%, analytical reagent, in a 100 ml volumetric flask containing approximately 80 ml water. Make up to volume and mix.
6) Standard solution tyrosine: Weigh accurately to within 1 mg in duplicate (standard solution A and B) approximately 180 mg tyrosine, analytical reagent, in a 100 ml volumetric flask and dissolve in 20 ml sulphuric acid 1 mol/l. Make up to volume with water and mix. Always use a freshly prepared solution.
7) Hydrochloric acid 1 mol/l
8) Hydrochloric acid 6 ml/l: Slowly and while stirring 50 ml of hydrochloric acid 37%, analytical reagent, to approximately 50 ml water in a 400 ml glass beaker.
9) Sodium hydroxide 6 mol/l: Dissolve 24.0 g sodium hydroxide, analytical reagent, in approximately 100 ml water in a 400 ml beaker.

Procedure
1) Preparation of the tyrosine calibration line solutions: With the diluter prepare the series of dilutions with water as indicate in the table below in centrifuge tubes and mix.

Dilution table calibration line solutions

Approximate tyrosine content of the calibration line solution to be incubated (mg/ml)	Standard solution	Standard solution (ml)	Water to be added (ml)
0.036	A	0.100	4.900
0.072	B	0.200	4.800
0.108	A	0.300	4.700
0.144	B	0.400	4.600
0.180	A	0.500	4.500

2) Colour reaction:
Using the pipettor, transfer 1.00 ml of the diluter sample solution and/or of the calibration line solutions into centrifuge tubes already containing 1.00 ml SDS solution. Add 1.00 ml sodium phosphate buffer. At time = 0 minutes, in order of the series and at regular time intervals place the tubes in the water bath at 40.0 ± 0.1 oC and allow to equilibrate for exactly 5 minutes. At time = 5.0 minutes with the dispenser add 1.00 ml TNBSA solution and mix. Replace the tubes in the water bath at 40.0 ± 0.1 oC and leave for exactly 60 minutes with the lid closed. At time = 65 minutes, in the same order of the series and with the same regular time intervals terminate the reaction by adding 1.00 ml hydrochloric acid 1 mol/l and mix. Cool to ambient temperature. Measure the absorbance with the spectrophotometer at 420 nm, zeroing the instrument with water.

3) Reagent blank:
Use 1.00 ml water as reagent blank. Perform the colour reaction as prescribed under item 2 "colour reaction".
4) Sample blanks:
Using the pipettor transfer 1.00 ml of the diluted sample solution into centrifuge tubes and add 4.00 ml water. Place in the water bath at 40.0 ± 0.1 oC for 60 minutes. Allow to cool to ambient temperature and measure the absorbance as mentioned under item 2.

Calculation
Carry out the calculation with the aid of the computer program available for this analysis. If this is impossible carry out the calculation as follows:

- For the calibration line calculate the absorbance as follows: Absorbance calibration line solution - absorbance reagent blanks
- For the samples calculated the absorbance as follows: Absorbance sample- (absorbance blank reagent + absorbance sample blank)
- Plot the relation between the absorbance and the exactly calculated tyrosine content in mg/ml of the calibration line solutions. Maximal allowed difference for each calibration point is 5%.
- Read the tyrosine content of the sample solutions to be determined in mg/ml from the calibration line produced.

Tyrosine content (mg/ml) x 14/181.19 = mg α-amino nitrogen calculated as N per ml sample solution used in the colour reaction.

Where:
14 = molecular weight of Nitrogen
181.19 = molecular weight Tyrosine

6) Nucleotides by HPLC

Apparatus 1. High pressure liquid chromatograph, consisting of:
a pump: Spectra-Physics P1000, flow rate 1.50 ml/min
b detector: Spectra-Physics Spectra 100, adjusted at 254 nm
c injector : Spectra-Physics AS 100 with 20 μl sample loop, oven temperature 40 gr. C
d integrator: Software Chrom Quest
e column: Whatman Partisil 10 SAX, length 25 cm, inner diameter 4.6 mm
2. Thermostat bath: Julabo TW 20
3. Dilutor: Hamilton Microlab 500
4. Balance: PM 200
or equivalent

Reagents

1. Milli-Q water
2. Mobile phase: Dissolve 6.8 g potassium dihydrogen phosphate, analytical reagent, in 0.9 l water. Add 100 ml acetonitrile and mix. Adjust the pH to 3.35 by adding phosphoric acid 10 %. De-aerate this solution with helium. (expired date: 2 week)
3. Diluted phosphate buffer: Dissolve 6.8 g potassium dihydrogen phosphate, analytical reagent, in 1 l water. Adjust the pH to 3.35 by adding phosphoric acid 10 %. Mix 100 ml of this solution and 100 ml acetonitrile with 900 ml water. (Expired date: 1 month)
4. Guanosine-5'-monophosphate.Na2 6.2H2O (Sigma G-8377)
5. Inosine-5'-monophosphate. Na2 8H2O (Sigma I-4625)
6. Adenosine-5'-monophosphate. Na 4H2O (Sigma A-1752)
7. Cytidine-5'-monophosphate. Na2 6.5H2O (Sigma C-1006)
8. Uridine-5'-monophosphate. Na2 1.5H2O (Sigma U-6375)

Procedure

1. Sample: Prepare sample dilutions containing 0.2 to 20 mg of each of the components per liter measuring solution. Preferably these solutions should contain less than 400 mg sodium chloride per liter.
2. Standard: Weigh, in duplicate, approximately 20 mg inosine accurately to within 0.01 mg, in a volumetric flask of 50 ml. Dissolve in diluted phosphate buffer, make up to volume with same and mix. Weigh, in duplicate, approximately 20 mg guanosine accurately to within 0.01 mg, in a volumetric flask of 50 ml. Dissolve in diluted phosphate buffer, make up to volume with same and mix. Dilute 0.160 ml of each solution with 1.440 ml diluted phosphate buffer with the aid of the dilutor into an injection vial, encapsulate and mix.
3. Measuring operation: Inject the solutions obtained into the liquid chromatograph and measure the peak areas with the aid of the software of Chrom Quest. The retention times to be expected are, in minutes:

Cytidine : 2.85
Adenosine : 3.32
Uridine : 3.59
Inosine : 4.43
Guanosine : 5.36

Calculation

1. For the standardized components (guanosine and inosine) :

A x C x D x E / (B x 50) = mg component per liter measuring solution

Where:
A = peak area component in sample solution B = peak area component in standard solution C = mg standard weighed D = purity of the standard in weight % E = conversion factor (see remark 4) : Guanosine = 1.028 Inosine = 0.966

2. For the non-standardized components:

F x H x I x J x K / (G x 50) = mg component per liter measuring solution

Where:
F = peak area component in sample solution G = peak area guanosine in standard solution H = mg guanosine weighed I = purity of guanosine in weight % J = response factor K = conversion factor (see remark 3) : Adenosine = 1.052 Cytidine = 1.000 Uridine = 1.000

Report the components as follows:

5'-AMP.Na2 $4H_2O$
5'-CMP.NA2 $6.5H_2O$
5'-GMP.NA2 $7H_2O$
5'-IMP.NA2 $7H_2O$
5'-UMP.NA2 $1.5H_2O$

Determination of response factors
1 Guanosine solution: Weigh approximately 20 mg guanosine, accurately to within 0.01 mg, in a volumetric flask of 50 ml. Dissolve in diluted phosphate buffer, make up to volume with same and mix. Introduce 5.00 ml of this solution in a volumetric flask of 50 ml, make up to volume with diluted phosphate buffer and mix.
2 Component solution: Weigh approximately 20 mg of the component concerned, accurately to within 0.01 mg, in a volumetric flask of 50 ml. Dissolve in diluted phosphate buffer, make up to volume with same and mix. Introduce 5.00 ml of this solution in a volumetric flask of 50 ml, make up to volume with diluted phosphate buffer and mix.
Measuring oration

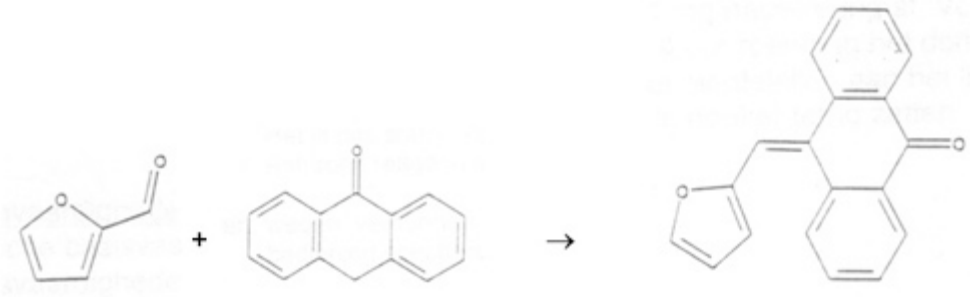
See procedure, 3.

Calculation
L x M x N / (O x P x Q) = response factor

Where:
L = peak area guanosine O = mg guanosine weighed P = purity guanosine in weight % Q = peak area component M = mg component weighed N = purity component in weight %
Remarks
1 Carry out the analyses in duplicate. Maximum difference allowed between duplicates: 2 % relative.
2 The response factor is the ratio of the molar absorbance coefficients of a nucleotide and guanosine determined with the current method. Response factors must be checked every six months and after modification of any one of the analyses parameters (e.g. flow rate, column type).
3 Conversion factors are physical constants to translate the molecular form used for the standardization into the one desired for the calculation. Conversion factors have been calculated using the water content of the components exactly as stated under 'Reagents'. When using components with a different water content, the conversion factor must be recalculated.

7) Carbohydrates using anthrone

Principles
Anthrone in concentrated sulphuric acid is a reagent for the determination of saccharides [2,3]. The carbohydrates are hydrolysed with sulphuric acid 67 % into monosaccharides. In this acidic environment, the monosaccharides are subsequently converted into 2-furaldehydes, also called furfurals (Figure 1). These compounds condense with anthrone (9,10-dihydro-9-oxoanthracene) at its C-10 methylene group to form a chromogene (furfurylidene-anthrone). The intensity of the blue-green colour of this compound corresponds to the amount of glucose present and can be detected at a wavelength of 610-630 nm. The content of glucose is given in mg anhydroglucose per g original material.
1. Glucose polymer + H2SO4 ->Glucose
2. Glucose + H2SO4-> Furfural



3. Furfural Anthrone Furfurylidene-anthrone
Figure 1: Reaction of furfural into furfurylidene-anthrone Apparatus and conditions

Spectrophotometer: Lamda 10 or Lambda 11 (spectral band width <= 2 nm) Analytical balance: 1213 MP Mettler AT201 (readable to within 0.01 mg) and Sartorius 1405 MP (readable to within 1 mg) Dilution apparatus: Hamilton ML1000 or Dilutrend (Boehringer) Water bathes: Julabo type 19 and 20B, adjusted at 30 and 100 °C(+-0.1 °C) Pipet and Multipipet: Eppendorf Plastic cuvets: Sarstedt, 67.742, 10x4x45 mm Magnetic stirrer: Combimag RCO Glass beads diameter 2 mm or equivalent apparatus.

Materials
Demineralised water: Distilled water which has been purified over a milliQ purification filter system (Millipore)
Concentrated sulphuric acid: 95-97 %, Merck, 100731.250, p.a., store at room temperature under fume cupboard in a separate plastic can, R 35, S 1/2-26-30-45
Anthrone: Merck, 1438.0010, p.a., store at room temperature
or equivalent quality.

References, standards and controls
Glucose.H2O standard: Fluka, 49159, p.a., store at room temperature Glucose polymer control: Purified glycogen of known purity, store at room temperature or equivalent quality.

Reagents
Sulphuric acid 67 %: Add 100 ml concentrated H2SO4 to 43 ml water. The water must be stirred and H2SO4 must be added in small amounts. Cool the mixture using cold streaming water.

Anthrone reagent: Weigh approximately 500 mg anthrone accurately (to within 1 mg) and add 250 ml concentrated H2SO4. Stir the solution for 4 hours in the dark. The solution is only stable after 4 hours. Keep the reagent in the dark.

Procedure
Weigh approximately 100 mg glucose.H2O accurately (to within 0.1 mg) in a 100 ml volumetric flask. Fill up with water to volume. Dilute this solution 50, 25, 17, 13 and 10 times with water. Use water as the standard blank.

1) Pretreatment control
The control is treated the same way as the samples.

2) Pretreatment samples
Weigh approximately 50 mg sample accurately (to within 0.01 mg) and in duplicate in a glass tube (150 mm, diameter 16 mm), which contains approximately 10 glass beads. Add 5.0 ml sulphuric acid 67 % while stirring. Be sure that no sample remains on the bottom of the tube. Leave the tubes for 1 hour in a water bath at 30 °C and stir the solutions on a regular basis. Add 5.0 ml water slowly to the solutions while stirring. Dilute the sample solutions 100 times with water.

3) Preparation measurement
Turn on the spectrophotometer and click twice on the 'Lamba 11' icon. Click on 'Sample' and fill in the results filename, the number of samples (sum of standard, control and sample solutions) and the sample identity. Click on 'Utilities' and then on 'Configuration'. Fill in the data direction. Click 'OK'. Put a cuvet containing water in the cuvet holder and click on 'Autozero'.

4) Measurement
Pipet 1.0 ml of the standard blank, standard, control and sample solutions in glass tubes. Add every 10 s 2.0 ml anthrone reagent to a subsequent tube, stir this solution and put it in a water bath at 100 oC for exactly 5 min. Put the tube in an ice water bath in order to stop the reaction (after exactly 5 min reaction time). The colour of the solution will become blue-green. Transfer the obtained standard blank, standard, control and sample solutions to cuvets and determine (within 1 hour) the absorbance at a wavelength of 620 nm using the spectrophotometer (manually, one cuvette after the other).

Calculation
The glucose concentration of the standard solutions (XST, in µg/ml) is calculated from the glucose.H2O concentration by multiplying it by 180/198 (MW glucose is 180 and MW of glucose.H2O is 198), using the following formula:

A linear calibration line is calculated using the glucose concentration (XST, calculated as described above) and the absorbance (YST) of the standard blank and the 5 diluted standard solutions:
YST = a*XST + b a = slope of calibration line b = intersection with y-axis
The glucose concentration of a sample (XSA, in Mg/ml) is calculated by filling in its absorbance (YSA) in the following formula:

$$x_{ST} = \frac{\text{weighed amount of glucose.}H_2O\text{ (mg)}}{\text{dilution factor} * 100\text{ ml}} * \frac{180}{198} * 10^3$$

XSA = (YSA - b)/a Furthermore, the concentration of free glucose (MW 180) has to be converted into the concentration of anhydroglucose (glucose residue, MW 162) as it is present in the glycogen polysaccharide. Therefore, the concentration of free glucose has to be multiplied by 162/180. The amount of anhydroglucose present in glycogen samples is calculated as follows:
Anhydroglucose in carbohydrate (mg/g) =

$$x_{SA} * \text{dilution factor} * \frac{10\text{ ml (volume added water and }H_2SO_4)}{\text{weighed amount of sample (mg)}} * \frac{162}{180}$$

8) Glutamic acid/MSG using an enzymological method

Principles
This method describes the spectrophotometrical determination of L-glutamic acid in research and production samples and is based on the Boehringer L-glutamic acid test-combination kit nr 139 092 [1]. The analysis consists of two reactions:
Glutamate dehydrogenase (GDH) catalyses the reaction of L-glutamic acid into 2-oxoglurate by means of oxidation with nicotineamide-adenine dinucleotide (NAD).

Catalysed by diaphorase, the NADH formed transforms iodonitrotetrazolium chloride (INT) in the red coloured chromogene formazan. The absorption is measured at 492 nm.

Remarks:
Reducing substances such as SO2 (>50 Mmol), cysteine (>80 Mmol), and ascorbic acid (>10 Mmol) may disturb the incubation: this can be prevented by cooking the sample in an alkaline environment using hydrogen peroxide (references 1 and 2).
The measurement is performed by means of the Konelab analyser.

Unit definition
The L-glutamic acid content is expressed in mg L-glutamic acid per gram sample.

Measuring range
The measurement range is 0.02 - 0.12 mg L-glutamic acid per mL sample solution.

Apparatus and conditions
Konelab Arena 30 : Thermo, adjusted at 492 nm
Konelab sample cups : Contents 1.5 mL
Konelab reagent vessels : 10 mL
Konelab reagent vessels : 20 mL
Balances : Sartorius model 1405MP
or 1213MP and Mettler model AT201
Diluter : Hamilton model ML 500
Purification apparatus : Milli Q UF (Millipore ZF MQ 24010)
Centrifuge : Eppendorf 5417R
Magnetic stirrer : Variomag HP15
1.5 and 2.0 mL test tubes : Eppendorf
Water bath : Julabo 20B
1.0 and 25 mL multipipette : Eppendorf
pH meter : Radiometer, PHM 83 Autocal

Or equivalent equipment.

Materials
Phosphate/Triethanolamine buffer pH 8.6 : Boehringer, glutamate kit, cat.nr.139092
Nicotiamide-adenine dinucliotide, NAD : Boehringer, glutamate kit ,cat.nr.139092
Iodonitrotetrazolium chloride, INT : Boehringer, glutamate kit ,cat.nr.139092
Diaphorase : Boehringer, glutamate kit ,cat.nr.139092
Glutamate dehydrogenase, GIDH : Boehringer, glutamate kit ,cat.nr.139092
Or equivalent quality.

Standard:
Sodium glutamate monohydrate : Merck 1.06445
The concentration [%] of L-glutamic acid in sodium glutamate monohydrate is (147.13 / 187.13) x certificate value [%].

Control: The control sample for the L-glutamic acid method is a standirized yeast solution. The stock and amounts for daily use are stored at -20°C. L-glutamic acid is released from the cells by incubating at 100°C (see 5.4).

Reagents
- Water: Ultra High Quality (UHQ) water, conductivity <= 0.10 MS.cm
- The reagents are directly derived from the ready-made glutamate kit, consisting of five reagent bottles.

Bottle 1: Buffer solution
Bottle 2: NAD/diaphorase
(solid matter)
Bottle 3: INT solution
Bottle 4: Substrate GLDH
Bottle 5: Standard L-glutamic
acid (not applicable)

Preparation: For at least 140 measurements.
GlutR1 reagent (20 mL vial) Undiluted buffer (reagent kit bottle 1). Transfer part of the content of bottle 1 into a 20 mL Konelab vial.

GlutR2 Diaphorase / NAD reagent (10 mL vial) Add 2.5 mL water to one bottle (reagent kit bottle 2), mix gently en transfer into a glass tube and rinse the bottle with another 2.5 mL of water, add to the glass tube and mix. Transfer the mixture into a 10 mL Konelab vial.
GlutR3 INT reagent (10 mL vial) Add 6 mL water to the volume of bottle 3 and swirl well. Transfer the content of bottle 3 into a 10 mL Konelab vial.

GlutR4 GLDH substrate reagent (10 mL vial) Transfer the volume of bottle 4 into a glass tube and rinse, without spilling, with 4.8 mL water, add to the glass tube and mix. Transfer the content of the glass tube into a 10 mL Konelab vial.

Procedure
1) Pre-treatment standard
Weigh approximately 150 +/- 5 mg sodium glutamate monohydrate, with an accuracy of 0.01 mg, in a 100 mL volumetric flask. Make up to volume with water and mix. Dilute the solution with water according to the data from the following table: encoding standard solution [mL] water to be added [mL] Total dilution factor concentration Glutamic acid [mg/mL] approx.

S 1	0.100	4.900	5 000	0.02
S 2	0.200	4.800	2 500	0.04
S 3	0.300	4.700	1 667	0.06
S 4	0.380	4.620	1 316	0.08
S 5	0.500	4.500	1 000	0.10
S 6	0.500	3.500	800	0.12

2) Pre-treatment control
Place the 50-mL-greiner tube containing the frozen control sample for 30 minutes in a boiling water bath. Prevent the Greiner tubes from floating in the water bath. After cooling down to room temperature, weigh approximately 10 gram sample, to an accuracy of 0.001 gram, in a 50 mL volumetric flask and dissolve in approximately 40 mL water. Make up to volume with water and mix. Adjust the solution to an L-glutamic acid concentration of approximately 40 - 80 mg/L by diluting with water. Centrifuge the diluted solution for 5 minutes at 14.000 rpm in an Eppendorf centrifuge and use the supernatant for the analysis.

3) Pre-treatment liquids
Weigh approximately 10 gram liquid sample, to an accuracy of 0.001 gram, in a 50 mL volumetric flask and dissolve in approximately 40 mL water. Make up to volume with water and mix. Adjust the solution to an L-glutamic acid concentration of approximately 40 - 80 mg/L by diluting with water.

4) Pre-treatment spray dried samples
Weigh approximately 10 gram spray dried sample, with an accuracy of 0.001 gram, in a 50 mL volumetric flask and dissolve in approximately 40 ml water. Make up to volume with water and mix. Adjust the solution to an L-glutamic acid concentration of approximately 40 - 80 mg/L by diluting with water.
Measurement
Using the Konelab Arena 30 analyser:
Requesting samples: -Press the Samples button on top of the main screen. -Press F8-more -Press F4-batch entry -Type the sample name and press Enter. -Select the used segments and used positions from the pull-down menus. -Press F2-Save changes -Introduce the sample segments in the Konelab one by one by opening and closing the sample segment door. The Konelab automatically detects which segments are inserted.

Inserting reagents: -Press the Reagents button on the top of the main screen. -Press on a free reagent position number -Press F2-Insert reagent -Select GlutR1 reagent from the list -Press OK and follow on-screen instructions -Repeat the last 4 steps for the GlutR2,GlutR3 and GlutR4 reagent

Starting analysis: -Go to the main screen and press the green start button, which is located on the keyboard at the right of the Home button. This is the only way the analyser can be started.

Results: -When analysis is complete, press the Results button on the top of the main screen -Press F8-more, F4 to see all analysed samples -Press F8-more twice, F1 to see details of a selected sample. Press F1 once more to turn details off. -Press F5-Print results to print the results

Generating peak files: -When the sample series is completely finished, go to the main screen and press F4-Reports and F8-more. -Select items to report "samples" and "all" from the pull down menus -Press F4-Results to file -Select "one row per result" and press OK -Always enter "results" as the filename Subsequently use the available program to generate the actual peak file.

Calculation
A calibration curve is produced from the six standards by means of linear regression. The concentration of L-glutamic acid is calculated using the coefficients of this curve and taking into account the weighting, dilution and proportion.
Linear regression
The slope (a) and intercept (b) for the calibration line are calculated via linear regression:
Ystd = a Xstd + b
With
Ystd = Abs(492) of the standard solutions
Xstd = Concentration L-glutamic acid of the standard [mg/mL]
The L-glutamic acid concentration of a standard is calculated using formula
xstd = (W(NaGlu) x MW(L-Glu) x C) / (Df x MW(NaGlu)) [mg L-glutamic acid per mL]

Where:
W(NaGlu) = Weight of Sodium glutamate monohydrate [mg]
Df = Dilution factor of Sodium glutamate (monohydrate) solution
C = Content of Sodium glutamate (monohydrate) of standard batch [g/g]
MW(L-Glu) = Molar mass of L-glutamic acid [147.13 g/mol]
MW(NaGlu) = Molar mass of sodium glutamate monohydrate [187.13 g/mol]
The L-glutamic acid content of a sample is calculated using formula:
L-glutamic acid = {(Ysample - b) x Df } / (a x W) [mg L-glutamic acid/g sample]

Where:
a = Slope of calibration line
b = Intercept of calibration line
ysample = Abs(492) of the sample solutions
W = Weight of sample [g]
Df = Dilution factor of the sample solution

9) Salmonella

Principles
Salmonellae are usually motile, Gram-negative, aerobic or facultatively anaerobic rods. Most strains are aerogenic, produce H2S and do not ferment lactose. Genetically all Salmonellae of the genus Salmonella belong to 1 species but biochemically they can be divided into 5 "subgenera". Clinical important Salmonellae were given in the past a second ("species") name which indicated the disease and/or the name of the affected animal. However, all Salmonellae should be considered as potential pathogens of man and many animal species. Multiplication of Salmonella can be very fast in protein-rich foods at temperatures between 25°C and 45°C. The common route of infection is by the alimentary tract; the (sometimes life threatening) disease is called enteric fever. Normally Salmonella in food, feed and water appear together with and are outnumbered by competitive organisms of faecal and non-faecal origin. In food and feed Salmonellae have usually been subjected to processing (heating, drying, freezing etc.) often resulting in injured or debilitated surviving cells. It is therefore necessary to resuscitate the Salmonella cells in the first isolation step, to increase the Salmonella cells and to suppress the proliferation of competitive organisms such as Coliforms, Proteus and Pseudomonas in the second selective enrichment step and to restrict the outgrowth of non-Salmonellae.

Apparatus
1. Petri dishes, plastic (90 x 15 mm), sterile 2. Bacteriological pipettes, 2 ml, 5 ml, sterile 3. Tubes, sterile 4. flaskes or bottles, sterile 5. Water bath for tempering agar, 46 -50°C 6. Heater block 100 +/- 1°C 7. Stomacher or shaking machine 8. Incubator, 42.5 +/- 1°C 9. Incubator, 37 +/- 1°C 10. Inoculating loop, 1 Ml, 10 Ml, sterile 11. Marking pencil 12. Stopwatch 13. Balance, minimum 0.1 decimal 14. Mini Vidas 15. Safety glove 16. Automatic pipettor 17. mediumpreparator (used as incubator)
Or equivalent

Media and Reagents

1. Water:
Distilled water or equivalent

2. Buffered Trypticase Soy Broth without Dextrose (BTSB w/o D): Dissolve 0.89 g potassium dihydrogen phosphate anhydrous (KH_2PO_4), analytical reagent and 1.81 g disodiumhydrogen phosphate anhydrous (Na_2HPO_4), analytical reagent in 1 liter water and measure the pH; it should be 7.3 +/- 0.2. Then add 27.5 g TSB w/o Dextrose (DIFCO 0862-17-8) under agitation and heating. Sterilise for 15 minutes at 121°C and cool to ambient temperature. Check the final pH, which should be 7.3 +/- 0.2. The medium may be kept at 15 - 20°C for 2 months.

3. Buffered Peptone water (BPW): Dissolve 34 g monopotassium dihydrogen phosphate (KH_2PO_4), anhydrous reagent, and 43.6 g dipotassium hydrogen phosphate anhydrous (K_2HPO_4), analytical reagent and 10 g Bacto-peptone in 1 liter water and measure the pH; it should be 6.8 +/- 0.1. Fill into a 2 liter bottle, make up to volume with water. Sterilise for 15 minutes at 121°C. The medium may be kept at 15 - 20°C for 2 months.

4. Selenite Cystine Broth (SC): Dissolve 4 g of sodium biselenite (Oxoid L121) in 1 liter water and than add 19 g Selenite Broth base (Oxoid CM 395) warm to dissolve under agitation and dispense into suitable quantities. Sterilise for 10 minutes in a boiling water bath (Do not autoclave). Final pH should be 7.0 +/- 0.2.

5. Rappaport-Vassiliadis Enrichment Broth (RV): Dissolve 30 g Rappaport-Vassiliadis Enrichment Broth (Oxoid CM 669) in 1 liter water under agitation and heating. Dispense into suitable quantities and sterilise for 15 minutes at 115°C. The final pH should be 5.2 +/-0.2.

6. Brain Heart infusion Agar (BHIA): Dissolve 52 g BHIA (Difco 0418-01-5) in 1 liter water. Bring to boil to dissolve completely, and dispense into suitable quantities in tubes bottles or flasks. Sterilise for 15 minutes at 121°C and cool to ambient temperature. The final pH should be 7.4 +/-0.2. The medium may be kept for 2 months.

7. M. Broth: Dissolve 36.2 g M Broth (Difco 0940) in 1 liter water under agitation and heating. Dispense into suitable quantities and sterilise for 15 minutes at 121°C. The final pH should be 7.0 +/- 0.2. The medium may be kept at 15 -20°C for 2 months.

8. Salmonella species Vidas kit (SLM): Biomeriéux code 30702

9. Rambach agar: Ready for use medium bought at Biotrading

10. API 20E: Biomeriéux code 20100

Selective enrichment and incubation

25g sample Incubate the flask, which contains the inoculated BTSB w/o D at 37 +/- 1°C for 18-24 hrs. Gently mix the incubated BTSB w/o D. flask and transfer aseptically 2 ml each in a jar containing 20 ml pre-warmed RV and 20 ml pre-warmed SC. Incubate the jar containing 20 ml inoculated RV at 42.5 +/- 0.5°C for 18 -24 hrs. Incubate the jar containing 20 ml inoculated SC at 37°C +/- 1°C for 18 - 24 hrs.
500g samples (Media preparatory) Incubate the inoculated BPW at 37 +/- 1°C for 18 - 24 hrs. Transfer in duplicate aseptically 10 ml from the inoculated BPW into a jar containing 100 ml pre-warmed RV and 100 ml pre-warmed SC. Incubate the jar containing 100 ml inoculated RV at 42.5 +/- 0.5°C for 18 -24 hrs. Incubate the jar containing 100 ml inoculated SC at 37°C +/- 1°C for 18 - 24 hrs.

M.Broth incubation
Homogenise gently the incubated SC and RV jars. Transfer aseptically from each RV and SC jar 1 ml into a tube containing 10 ml pre-warmed M. Broth (MSC and MRV). Incubate the inoculated M.Broth at 42.5 +/- 0.5°C for 4 - 6 hrs.

Vidas SLM test
Homogenise *the* incubated M. Broth. Select the tubes containing MSC and MRV from the same sample. Transfer aseptically 2ml from both tubes into 1 empty sterile tube. Put the tube into a heater block at 100 +/- 1°C for 20 minutes. Cool the tube immediately after performing the heating step in water at ambient temperature. Perform the Vidas SLM test (see the Vidas manual). If the result of the test is positive transfer 1 loopful from the incubated but NOT heated MSC and MRV onto Rambach agar plates. Incubate the plates at 37+/-1°C for 24 hrs.

Reading of the Rambach agar plate
Salmonella colonies are red; the edge of the colony appears greasy.

API20E test
Isolate a single Salmonella suspected colony from the Rambach agar plate onto a BHIA agar plate. Incubate the BHIA agar plate at 37°C for 24 hrs. Perform The API20E test as written in the manual.

Calculation
Salmonella is absent in a sample when the result of the SLM test is negative. A sample is Salmonella suspected when the result of the SLM test is positive but no further investigation has been performed. A sample is Salmonella positive when the SLM test is positive, suspected colonies were observed on the Rambach agar plate, T he API20E result was reliable and a second identification by an independent laboratory of the suspected isolate was confirmed as Salmonella.

10) Other Microbiological testing

Apart from Salmonella, other relevant testing is carried out using the ISO published methodology or a closely related method. Microorganisms which are commonly tested for include:

- Total plate count
- Total coliforms
- E. coli
- Yeasts
- Moulds