Chemistry and Industry for Teachers in European Schools

THE "RAVIOLI IN A CAN"-PROJECT

OR: THE CHEMISTRY OF CANNED RAVIOLI

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Education and Culture

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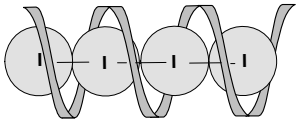
THE LABEL
DETECTION OF LIGNIN WITH PHLOROGLUCINOL

FUNDAMENTALS

Starch is not a uniform substance, instead it consists of two components, namely amylose and amylopectin. The amylose molecule has a helical structure. During the iodine test, iodine molecules assemble in the empty spaces the helical structure provides. The incorporation of iodine forms a soluble dark-blue complex, which breaks up at high temperatures.



Iodine molecules in the helical structure of an amylose molecule

TIME REQUIRED

5 min

APPARATUS NEEDED

scissors, 2 medium-sized test tubes, Pasteur pipette with bulb, test tube rack or a glass beaker, test tube holder, Bunsen burner, lighter

CHEMICALS

iodine-reagent (0,1g Iodine and 0,2g potassium iodide dissolved in 30 ml of water), wash bottle with demineralized water, label from a ravioli can

SAFETY HINTS

iodine (X_n, harmful; N, dangerous for the environment)

PROCEDURE

A small piece, ca. 0,2 – 0,3 g of the label (about 5 x 5 cm) is cut into smaller pieces and heated in a test-tube with 10 ml of water for 1 minute. After leaving to cool down the solution is decanted and some of the clear solution is poured into another test-tube, where one or two drops of the iodine reagent are added.

OBSERVATIONS

The solution turns dark blue/violet.

EVALUATION

Due to the dark blue/violet colour, starch is successfully detected in the label.

DISPOSAL

Repeat the procedure of heating the label and decanting the solution until the detection of starch is negative. The residual pieces of label pulp are kept for the following experiments: (hydrolysis of cellulose to sugar, Molisch's test and Fehling's test)

THE LABEL
HYDROLYSIS OF CELLULOSE INTO SUGAR (MOLISCH'S TEST)

FUNDAMENTALS

The reaction of concentrated sulfuric acid with cellulose gives both glucose and cellobiose. Glucose can be detected using 1-naphthol. This reaction was first utilized by the plant physiologist Hans Molisch. Since starch also leads to a positive reaction with 1-naphthol, it should have been removed before attempting to detect glucose.

TIME REQUIRED

10 min

APPARATUS NEEDED

2 test tubes, glass rod, Bunsen burner, lighter, test tube holder, tweezers, 2 pipettes, test-tube rack or glass beaker, label from a can (preferably the used pieces of the label left over from the first experiment)

CHEMICALS

Molisch-reagent (1,5g 1-naphthol dissolved in 3,5ml of ethanol), concentrated sulfuric acid (*w/w* = 96 %), demineralized water

SAFETY HINTS

1-naphthol, (X_n, harmful), ethanol (F, highly flammable), concentrated sulfuric acid (C, corrosive)

PROCEDURE

The starch-free residual pieces of label left over from the first experiment are put into a test-tube using tweezers. 2 or 3 ml of concentrated sulfuric acid are added before stirring for about two minutes. This mixture is then heated *gently* for about two minutes, to avoid carbonizing the contents. This solution has to cool down for a few minutes before continuing. After that, two drops of the solution are put into another test tube and diluted with approx. 1 ml of demineralized water. After adding three drops of the Molisch reagent, 2 ml of concentrated sulfuric acid are cautiously poured down the inner side of the test tube.

OBSERVATIONS

A blue-violet ring becomes visible at the boundary between the sulfuric acid and the less dense solution above it.

EVALUATION

The ring indicates the presence of cellulose.

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DISPOSAL The remaining mixture of sulfuric acid and pieces of the label are kept for Fehling's test (next experiment).

LITERATURE

Wöhrle, F.; Kirchhof, C.; Otto, B.; Schmidt, O.: Rund um's Papier, NiU-Chemie **6** (1995) Nr. 29, 26 ff.

THE LABEL
HYDROLYSIS OF CELLULOSE INTO SUGAR (FEHLING'S TEST)

FUNDAMENTALS

Cellulose can be hydrolytically split into glucose and cellobiose, as well as starch, Glucose can be detected using Fehling's test. The Fehling's reagent is used to indicate the presence of reducing agents such as mono- and disaccharides, aldehydes and others. Since aldehydes can easily be oxidized to carboxylic acids, they are strong reducing agents.

TIME REQUIRED

10 min

APPARATUS NEEDED

measuring cylinder (25 ml), glass beaker (100 ml), Bunsen burner, lighter, 2 medium-sized test-tubes, test tube rack, glass rod, 2 glass pipettes (1 ml) with pipette filler

CHEMICALS

remaining mixture of sulfuric acid and pieces of the label from the previous experiment (Molisch's test); sodium hydroxide solution (*w/w* = 30 %); Fehling's solution: Fehling's I (7 % solution of copper (II) sulfate), Fehling's II (30 g of Rochelle salt (potassium sodium tartrate-4-water crystals) diluted to 100 ml with a 10 % (w/w) aqueous solution of sodium hydroxide), demineralized water, indicator paper

SAFETY HINTS

copper (II) sulfate (X_n, harmful; N, dangerous for the environment), sodium hydroxide solution (C, corrosive); sulphuric acid (C, corrosive)

PROCEDURE

25 ml of water are added to the suspension of paper and sulfuric acid left from the previous experiment. Leave the pulpy residue and decant 5ml of the clear solution above it into a test-tube. Neutralize with 30 % (w/w) sodium hydroxide solution. Use another test-tube to mix together 1 ml of Fehling's I and Fehling's II. Shake until the sediment disappears. Now this solution is added to the first test-tube and boiled.

OBSERVATIONS

The clear solution initially turns blue. During boiling an orange/reddish sediment appears.

EVALUATION

The positive Fehling's-test indicates the presence of glucose.

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LITERATURE

Bansa, H.: Papierzerfall und Gegenmaßnahmen PdN-Chemie **41** (1992) 7, 8 - 12

THE CAN
DETACHMENT OF THE LACQUER AND REMOVAL OF THE TIN FROM COATED TINPLATE CANS

FUNDAMENTALS

Cans of tinned food are basically made of tinned steel plates (tinplate). Due to improper handling of acidic foods in tinplated cans, several cases of tin-poisoning have occurred. Organic tin compounds can be harmful or even toxic. That's why two or three layers of varnish are applied to the inner side of the cans. It also prevents a negative impact on the taste of the food. Stoving varnishes, based on epoxy resin, are commonly used for this purpose.

TIME REQUIRED

30 min

APPARATUS NEEDED

washed can with lacquer layer inside, tweezers, metal cutters, measuring cylinder (25 ml), 2 glass beakers (10 ml), watch glass, Bunsen burner, tripod stand with glass-ceramic plate, lighter

CHEMICALS

half-concentrated hydrochloric acid, [c(HCl) = 6 mol/l]

SAFETY HINTS

half-concentrated hydrochloric acid, [c(HCl) = 6 mol/l] (C, corrosive)

PROCEDURE

A piece of 5 x 5 cm is cut out of the can with metal shears and divided into 1 cm^2 squares. These are put into a glass beaker. Add the half-concentrated hydrochloric acid and cover with the watch glass. The solution is heated until the hydrogen evaporates vigorously. When this point is reached, the Bunsen burner is turned off. The beaker is left on the tripod for another 10 minutes. After being cooled down, the acidic solution is decanted and saved for use in the experiment "Detection of tin and iron in tinplate". Now the residual pieces of metal are washed until the washings are neutral. After drying thoroughly, the de-tinned metal pieces and the removed layers of lacquer are separated with the tweezers and set aside for the experiments "Detection of varnish layer" and "Proof of corrosion-preventive effect of tin layer".

OBSERVATIONS

Shortly after heating, the varnish layer on the inner side of the pieces peels off like a foil. The solution turns to a pale bluish-green colour.

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EVALUATION

Tin and iron dissolve in concentrated hydrochloric acid, forming Fe^{2+} and Sn^{2+} ions. The solution has to be heated to dissolve the tin. The iron dissolves because there are a lot of cut surfaces on the pieces of metal, even if the tin layer is still intact.

$$Sn + 2\ H_3O^+ \longrightarrow Sn^{2+} + 2\ H_2O + H_2\uparrow$$

$$Fe + 2\ H_3O^+ \longrightarrow Fe^{2+} + 2\ H_2O + H_2\uparrow$$

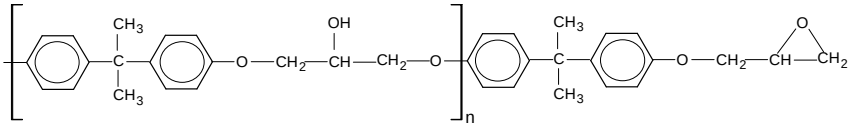
HINTS

The hydrochloric acid has to be heated in the fume cupboard.

THE CAN
DETECTION OF VARNISH LAYER (INDOPHENOL TEST)

FUNDAMENTALS

The indophenol test (devised by Gibb) is used for the detection of phenol in phenolic resins as well as substances splitting off phenol or phenol derivatives whenthey are heated. For instance, epoxy resins based on Bisphenol A, which are commonly used as excipients for varnishes, split off phenols when heating them. Therefore the test for phenols using the indophenol test is expected to be positive here.



Section of an epoxy resin molecule

TIME REQUIRED

10 min

APPARATUS NEEDED

removed varnish layer (previous experiment), tweezers, ignition tube, Bunsen burner, lighter, glass tube holder, filter paper, 2 glass beakers (100ml), magnetic stirrer with stirring bar

CHEMICALS

2,6-dibromoquinone-4-chloroimide (BQC), ammonia solution (c = 2mol/l), ethoxyethane

SAFETY HINTS

2,6-dibromoquinone-4-chloroimide (X_n, harmful), ammonia solution (C, corrosive; N, dangerous for the environment), ethoxyethane (F+, highly flammable, X_n, harmful),

PROCEDURE

PREPARED FILTER PAPER

Firstly a saturated solution of 2,6-dibromoquinone-4-chloroimide in ethoxyethane is prepared by adding 1 g of 2,6-dibromoquinone-4-chloroimide to 10 ml of ethoxyethane, stirring for ten minutes and then decanting afterwards. A filter paper is dipped into the solution and dried. Repeat this procedure three times to make sure the filter paper is saturated with the reagent.

INDOPHENOL TEST

Pieces of the peeled-off lacquer layer left from the previous experiment are put in an ignition tube.
The ignition tube is placed diagonally in a flame and heated for max. 1 minute. Using tweezers, the top of

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the tube is covered with a piece of the previously prepared filter paper, which is then moistened with one or two drops of ammonia solution.

OBSERVATIONS The filter paper turns blue.

EVALUATION The blue filter paper indicates the presence of phenols (xylenols, cresols). Epoxide resins show a positive test for phenols.

LITERATURE

Braun, D.: Erkennen von Kunststoffen, Qualitative Kunststoffanalyse mit einfachen Mitteln; 2 Auflage (1986); Carl Hanser-Verlag München Wien

THE CAN
DETECTION OF TIN AND IRON IN TINPLATE

FUNDAMENTALS

Dissolving tin and iron in half-concentrated hydrochloric acid forms ions (Sn^{2+}, Fe^{2+}/Fe^{3+}) which can be analytically detected.

TIME REQUIRED

10 min

APPARATUS NEEDED

3 test-tubes, test-tube rack, test-tube holder, glass beaker (100 ml), Bunsen burner, lighter, trivet with glass ceramic plate, drying oven

CHEMICALS

Residual acidic solution from experiment "Detachment of the lacquer and removal of the tin on coated tinplate cans", potassium hexacyanoferrate (III) solution (w/w = 10 %), hydrogen peroxide solution (w/w = 30 %), aqueous solution of ammonium thiocyanate (w/w = 5 %), ice or cold water, molybdophosphoric acid hydrate (w/w = 5 %), concentrated ammonia solution (w/w = 25 %), circular filter paper (diameter 70 mm)

SAFETY HINTS

ammonium thiocyanate (X_n, harmful), hydrogen peroxide (C, corrosive, O, oxidising), half-concentrated hydrochloric acid (C, corrosive), molybdophosphoric acid hydrate (C, corrosive), concentrated ammonia solution (T, toxic, N dangerous for the environment)

PROCEDURE

In this experiment the acidic solution from experiment "Detachment of the lacquer and removal of the tin on coated tinplate cans" is used:

a) Detection of iron:

- In a test-tube, add a few drops of potassium hexacyanoferrate (III) solution to a small sample of the resulting solution.
- Another sample is prepared in a test-tube. A few drops of hydrogen peroxide solution are added and the sample is heated Now add a few drops of an ammonium thiocyanate solution.

b) Detection of tin:

- A circular filter paper is soaked with a 5% solution of molybdophosphoric acid hydrate, causing the filter paper to turn yellow. A glass beaker, whose diameter is slightly smaller than the one of the filter paper, is filled with concentrated ammonia solution. Then the filter paper is placed on the top of the filled

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glass beaker, which is now heated on the heating plate. The filter loses its yellow colour during the heating. The filter paper is dried in a drying oven and afterwards is stored in a closed brown flask.

- *Test with the previously prepared filter paper:* One drop of the ion solution is dripped on the filter paper.
- *Fluorescence test:* Dip a test-tube filled with ice or cold water in the solution. In a dark room hold the test-tube into the reducing part of the Bunsen burner flame

OBSERVATIONS

a) Detection of iron:
When the potassium hexacyanoferrate (III) solution is added the solution turns blue.
When the ammonium thiocyanate solution is added the solution turns blood-red.

b) Detection of tin:
The filter paper turns blue when a drop of the solution is added.
A blue fluorescence can be seen on the test-tube in the flame.

EVALUATION

In hydrochloric acid iron and tin from the tinplate dissolve to produce aqueous Fe^{2+} and Sn^{2+} ions.

a) Detection of iron:
With the first reaction Fe^{2+} ions are detected as Berlin blue.

$$Fe^{2+} + K_3[Fe(CN)_6] \longrightarrow K[FeFe(CN)_6] + 2\ K^+$$

(blue)

With the second reaction Fe^{3+} ions are formed by oxidising Fe^{2+} ions with hydrogen peroxide. This ion can be detected using a solution of ammonium thiocyanate, which together with Fe^{3+} forms the blood-red complex cation pentaaqua (thiocyanato-*N*) iron (III), $[Fe(NCS)(H_2O)_5^{2+}.]$

$$2\ Fe^{2+} + H_2O_2 + 2\ H^+ \longrightarrow 2\ Fe^{3+} + 2\ H_2O$$

$$Fe^{3+} + SCN^- + 5\ H_2O \longrightarrow [Fe(NCS)(H_20)_5]^{2+}$$

(blood-red)

b) Detection of tin:
Tin is detected through the blue fluorescence in the first test.
In the second test Sn^{2+} is used as a reducing agent to reduce the molybdophosphoric acid to molybdenum blue. The blue colour of molybdenum blue indicates the concurrent presence of both Mo(IV) and Mo(VI) ions. A detailed description of the structure of molybdenum blue has not yet been published.

DISPOSAL remnants of metal: as normal household refuse; solutions: neutralise and add to aqueous heavy metal waste

THE CAN
PROOF OF CORROSION-PREVENTIVE EFFECT OF TIN LAYER

FUNDAMENTALS

Cans are made of tin plate. It is a steel sheet coverered with pure tin. This layer of tin works as a corrosion preventive.

TIME REQUIRED

20 min

APPARATUS NEEDED

de-tinned metal from experiment "Detachment of the lacquer and removal of the tin on coated tinplate cans", washed can, metal-cutting shears, glass beaker (150ml), magnetic hot plate stirrer, tinned iron nail, Petri dish (10 cm)

CHEMICALS

ethanol, Agar (for example DIFCO-AGAR 0140-01), potassium hexacyanoferrate (III) ($K_3[Fe(CN)_6]$)

SAFETY HINTS

ethanol (F, flammable)

PROCEDURE

From the non-coated parts of the can two relatively small pieces (2 x 2 cm) are cut out. Use an iron nail to scratch the tin layer's surface roughly in several areas on one of the pieces. 1,5 g of agar are added to 50ml of water in a 150 ml glass beaker. Heat and stir on a hot-plate with magnetic stirrer, until the solution is clear. Now a small amount of potassium hexacyanoferrate (III) solution is added. The solution is once again stirred and then put into a Petri dish. The pieces of tin-plate and one piece of the de-tinned metal are placed in the solution.

OBSERVATIONS

After about 10 minutes a blue colouring appears in areas where the tin layer has been damaged. No change occurs where the tin layer is intact.

EVALUATION

Fe^{2+} ions dissolve and form "soluble" Berlin blue:

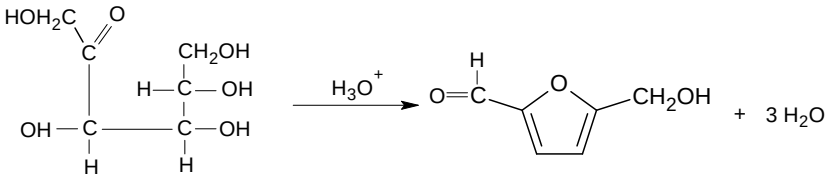
$$Fe \longrightarrow Fe^{2+} + 2e^-$$

$$Fe^{2+} + K_3[Fe^{+III}(CN)_6] \longrightarrow K[Fe^{+II}Fe^{+III}(CN)_6] + 2\,K^+$$

THE NOODLES
DETECTION OF STARCH IN AQUEOUS PASTA EXTRACTS WITH MOLISCH'S REAGENT

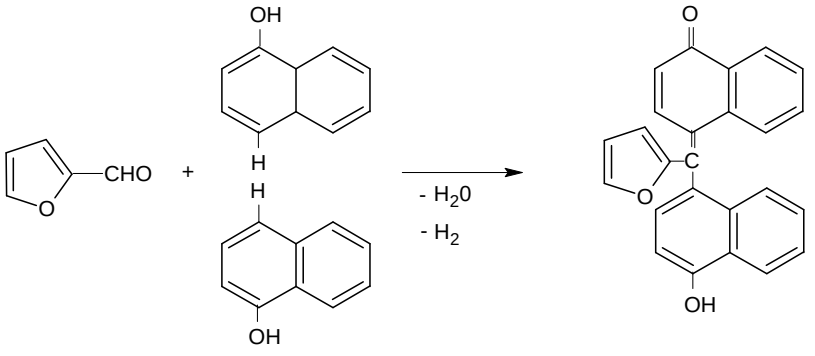
FUNDAMENTALS

Carbohydrates containing pentose and hexose sugars can be detected using Molisch's test. This works for oligo- and polysaccharides as well, which enables us to detect starch. Due to the impact of concentrated sulfuric acid, pentoses convert to furfural, hexoses convert to 5-Hydroxymethylfurfural. Only after these conversions is the typical Molisch dye formed. Here for example, furfural reacts with 2 1-naphthol molecules under elimination of water and dehydrogenation, forming an intensely violet-coloured dye.



D-Fructose 5-Hydroxylmethylfurfural

Formation of 5-Hydroxymethylfurfural



α-Naphthol violet dye

Forming the "Molisch dye" with Furfural

TIME REQUIRED

15 to 20 min

APPARATUS NEEDED

magnetic stirrer with hotplate, stirring bar, large crystallizing dish (14 cm) (water bath), mortar with pestle, Erlenmeyer flask (200 ml) with rubber stopper, 2 test-tubes, test-tube rack, glass beaker (150 ml),

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pipette (1 ml), spatula, spoon, graduated cylinders (10 ml and 50 ml), glass rod, Pasteur pipette with bulb

CHEMICALS Molisch-reagent (0,5 g 1-naphthol dissolved in 10 ml of ethanol), concentrated sulfuric acid, Ravioli, washing-up liquid

SAFETY HINTS 1–naphthol (X_n, harmful), ethanol (F, flammable), concentrated sulfuric acid (C, corrosive)

PROCEDURE *Preparation of the Ravioli-extract:*
4 Ravioli-noodles are cleaned to remove sauce and meat by washing with water and a little washing-up liquid. The cleaned noodles are broken down into small pieces in the mortar. One half of the pulp is mixed with 50ml of water in a 200 ml Erlenmeyer flask, which is then stoppered and immediately shaken for 30 seconds. The stopper is removed and the sample is immediately put into a boiling water quench for 2 minutes and then cooled under running water. Wait until the sediments precipitate and then decant sufficient of the solution necessary to proceed.

TEST 3 drops of Molisch's reagent are added to 2 ml of the Ravioli-extract in a test-tube. After shaking, some concentrated sulfuric acid is cautiously poured down the inner side of the test tube to create two layers of liquids. Observe the test-tube for 30 seconds without shaking.

OBSERVATIONS A blue-violet ring appears at the boundary layer between the two layers.

EVALUATION This is the positive Molisch test and hence pasta contains starch.

HINTS Keep the Ravioli-extract for the following experiments. The Molisch reagent can be stored in a fridge for a longer period of time.

DISPOSAL Any extract contaminated with Molisch's reagent and sulfuric acid is neutralised and added to organic waste.

THE NOODLES
DETECTION OF REDUCING SHUGARS IN THE HYDROLYSATE (FEHLING'S TEST)

FUNDAMENTALS

This experiment proves that both uncooked and cooked noodles, as well as the pasta in canned Ravioli do not contain any reducing sugars. They are not detectable until acid causes the starch to decompose.

TIME REQUIRED

20 to 30 min

APPARATUS NEEDED

magnetic stirrer with hotplate, stirring bar, crystallizing dish (14 cm) (water bath), measuring cylinder (50 ml) Erlenmeyer flask (Pasteur pipettes (200 ml) with bulbs, 2 glass beakers (100 ml), mortar with pestle, 10 test-tubes with 2 stoppers, 4 graduated pipettes (2ml), 2 Pasteur pipettes with bulbs, graduated cylinders (10 ml), test-tube rack, 3 glass rods, test-tube holder

CHEMICALS

Fehling's solution: Fehling's I (7% solution of copper(II) sulfate), Fehling's II (30 g of Rochelle salt (potassium sodium tartrate-4-water crystals) diluted to 100 ml with a 10 % aqueous solution of sodium hydroxide), half-concentrated hydrochloric acid (c = 6 mol/l), sodium hydroxide solution (w/w = 10 %), Ravioli, noodles, washing-up liquid, indicator paper

SAFETY HINTS

copper (II) sulfate (X_n, harmful; N, dangerous for the environment), sodium hydroxide solution (C, corrosive), hydrochloric acid (C, corrosive)

PROCEDURE

Preparation of the Ravioli-extract (alternatively you may use the extract from the previous experiment):
4 Ravioli-noodles are cleaned to remove sauce and meat by washing with water and a little washing-up liquid. The cleaned noodles are broken down to small pieces in the mortar. One third of the pulp is mixed with 50 ml of water in a 200-ml-Erlenmeyer flask, which is then stoppered and immediately shaken for 30 seconds. The stopper is removed and the sample is put into a boiling water quench immediately for 2 minutes and then cooled under running water. Wait until the sediments precipitate and decant enough of the solution necessary to proceed.

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Preparation of the dry-noodle-extract
5 g uncooked noodles are broken down to small pieces and ground in a mortar. An Erlenmeyer flask is filled with the resulting powder. After adding 50 ml of water, the Erlenmeyer flask is stoppered and immediately shaken for 30 seconds before putting it into a boiling water quench (remove the stopper) for two minutes.

Preparation of the Ravioli-hydrolysate
1 Ravioli-noodles are cleaned as described above, transferred into a glass beaker and 20 ml half-concentrated hydrochloric acid is added. The solution is heated for 3 minutes. The excess solution is decanted into a second test-tube and neutralised with sodium hydroxide solution.

Preparation of the dry-noodle-hydrolysate
2 uncooked noodles (ca. 2 g) are heated with 20 ml of half-concentrated hydrochloric acid for 3 minutes. The excess solution is decanted into a second test-tube and neutralised with sodium hydroxide solution.

FEHLING'S TEST

5 ml each of Fehling's I and Fehling's II are put into a test-tube. The solution is shaken until the sediment disappears. 2 ml of each of the two of the previously prepared hydrolysate solutions are put into test-tubes and mixed with the equal amount of Fehling's reagent. The solutions are heated for 5 minutes in a boiling water bath.

OBSERVATIONS

The hydrolysate solutions show a positive test for reducing sugars. The Ravioli-extract as well as the dry noodle-extract show negative results.

EVALUATION

Through hydrolysis the starch has decomposed, forming glucose and maltose.
Fehling's reagent, which is a mixture of Fehling's I and Fehling's II, is used to detect reducing sugars such as glucose. When heating them in a basic medium, divalent Cu^{2+} ions of the copper (II) sulfate solution are reduced to the monovalent ions Cu^{+}. Copper (I) oxide (Cu_2O) is formed and precipitates as a red sediment. The reducing agent is the aldehyde unit of the open-chain sugar molecule. This unit is itself oxidised to a carboxyl group. The simplified reaction equation is as follows:

$$2Cu^{2+} + 4OH^{-} + R\text{-}CHO \longrightarrow Cu_2O\downarrow + H_2O + H_3O^{+} + R\text{-}COO^{-}$$

blue red

The potassium sodium tartrate present in Fehling's II prevents the formation of $Cu(OH)_2$, which would precipitate immediately. It forms a complex with the copper (II) ions and therefore keeps them dissolved in the solution.

As may be seen from the equation above, a free aldehyde group is necessary for Fehling's test. The structure of starch reveals that α-D-glucose molecules are bound together with 1,4- or 1,6-glycosidic bonds, which makes a conversion of the cyclic hemiacetal into the open-chain aldehyde impossible. As the results of the experiment clearly show, a positive test for reducing sugars in unprocessed noodles is therefore impossible. Starch molecules can not decompose under these conditions. On the other hand, strong acids such as hydrochloric acid induce hydrolysis of the starch, forming the core components from which starch is made, namely glucose and maltose molecules. Since they are reducing agents, they can easily be detected with Fehling's test, as the red sediment shows.

HINTS

If the Ravioli-extract shows a slightly positive reaction, this is to be ascribed to possible contaminations of the Ravioli-noodle with tomato-sauce absorbed by the noodle. This is not a positive test for decomposed starch.

The hydrochloric acid should only be heated in the fume hood.

DISPOSAL

acidic solutions: neutralise and pour down the drain; solutions contaminated by Fehling's solutions: neutralise and add to aqueous heavy metal waste.

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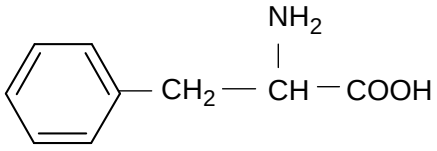
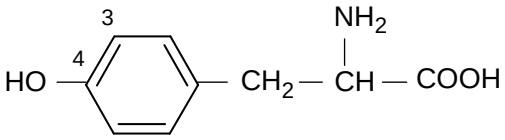
THE MEAT
TEST FOR PROTEINS WITH XANTHOPROTEIC REACTION

FUNDAMENTALS

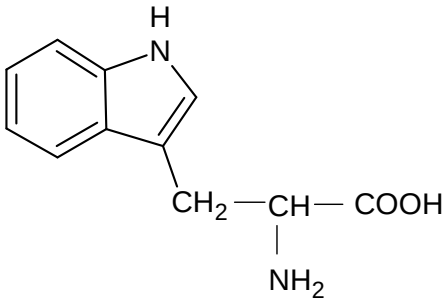
The xanthoproteic reaction indicates the presence of proteins. The term derives from the change in colour to yellow when proteins react with concentrated nitric acid. In this reaction the cyclic aromatic systems of the aromatic amino acids phenylalanine, tyrosine and tryptophane are nitrated.



Phenylalanine



Tyrosine



Tryptophane

TIME REQUIRED

10 min

EQUIPMENT

test-tube, test-tube rack, test-tube holder, Bunsen burner, spatula, glass rod, Pasteur pipette, graduated pipette (5ml)

CHEMICALS

nitric acid (*w*/*w* = 20%), ammonia solution (/*w* = 25%), (pork) meat (Ravioli filling)

SAFETY HINTS

Nitric acid (C, corrosive), ammonia solution (C, corrosive)

PROCEDURE

A pea-sized amount of meat is put into a test-tube, to which 3 ml of nitric acid are added. The meat is broken into smaller pieces with a glass rod. The sample is heated gently until the solution turns light yellow. A small amount of solid residue remains. This mixture is cooled under running cold water and ammonia solution is then added.

OBSERVATIONS

The solution turns orange after the ammonia solution is added.

EVALUATION

Proteins containing aromatic amino acid groups form yellow nitration products with nitric acid. In a basic medium the yellow colour intensifies, resulting in an orange-coloured product.

HINTS

The xanthoproteic reaction is not a specific test for proteins. Nevertheless it works well here, since nearly every kind of protein compound contains a certain amount of aromatic amino acid groups.

This experiment should be carried out in a fume hood.

DISPOSAL

Excess solutions are neutralised and poured down the drain.

LITERATURE

Buktasch, F.; Glöckner W.; Experimentelle Schulchemie – Organsische Chemie II, Aulis-Deubner Verlag Köln (1975)

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THE MEAT
TEST FOR NITROGEN ATOMS IN PROTEINS OF THE MEAT FILLING

FUNDAMENTALS If enough heat is supplied, strongly alkaline reagents decompose proteins. This reaction forms gaseous ammonia, proving the presence of nitrogen atoms in the original meat sample.

TIME REQUIRED 5 min

APPARATUS NEEDED test-tube, test-tube holder, test-tube rack, Bunsen burner, spatula

CHEMICALS sodium hydroxide, universal indicator paper, meat (Ravioli filling)

SAFETY HINTS sodium hydroxide (C, caustic)

PROCEDURE A pea-sized amount of meat is put into a test-tube. 3 pieces of solid sodium hydroxide are added. The test-tube is cautiously heated using a small flame. The resulting gases are tested with a piece of moistened universal indicator paper. Also try to identify the smell of the gases (cautiously!)

OBSERVATIONS The resulting gases cause the indicator paper to turn blue. A noticeably fishy smell is detected.

EVALUATIONS Proteins decompose when they are heated together with alkali metal hydroxides. The product of this reaction is gaseous ammonia.

DISPOSAL Neutralise and pour down the drain.

LITERATURE Buktasch, F.; Glöckner W.; Experimentelle Schulchemie – Organsische Chemie II, Aulis-Deubner Verlag Köln (1975)

THE MEAT
DETERMINATION OF THE FAT CONTENT IN THE MEAT FILLING

FUNDAMENTALS

The fat content in the meat filling can be determined through extraction with petroleum ether and subsequent vaporisation of the solvent in a previously weighed flask.

TIME REQUIRED

30 to 45 min (without drying of the meat)

APPARATUS NEEDED

balance, small crystallizing dish, desiccator with phosphorus pentoxide (only necessary if the meat is not dried in a drying oven), mortar with pestle, 2 round-bottom flasks (100 ml), spatula, reflux condenser, distiller (Claisen column), funnel, folded filter paper, Erlenmeyer flask (200 ml) (use for filtration), Pasteur pipette, test-tube, stand material, lifting platform, magnetic stirrer with hotplate, stirring bar, crystallising dish (14 cm, water bath), thermometer, 4 clamps

CHEMICALS

petroleum ether (bp. 40 – 60 °C), bromine water (w/w = 0,5 %)

SAFETY REMARKS

petroleum ether (F, flammable)

PROCEDURE

Determination of the fat content of dry meat
Exactly 10 g of meat are dried in a drying oven at 80 °C overnight and subsequently reweighed. To prevent unpleasant smells, you could alternatively use a desiccator with phosphorus pentoxide or a similar desiccant.

The dried meat is ground in a mortar. Exactly 2 g of the ground meat is transferred into a round-bottomed flask, to which 20 ml of petroleum ether are added. With a reflux condenser, the sample is heated in a water bath for ten minutes at 80 °C. The resulting solution is decanted through a folded filter and kept in an Erlenmeyer flask. The residue is once again mixed with 20 ml of petroleum ether and heated for 10 minutes using a reflux condenser. The solution is again decanted through a folded filter. The filtrate is transferred into a second round-bottomed flask. Make sure you have weighed the flask before filling it. The

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solvent is now evaporated by distillation. Use a water bath at 80 °C to keep the sample boiling. The cooled round-bottomed flask is weighed once more to determine the fat content.

OBSERVATIONS

The meat becomes remarkably darker when drying it. After the distillation an oily, yellowish residue remains. The fat content of the dry meat should amount to approximately 25 %.

EVALUATION

The meat contains fat which can be extracted with petroleum ether. This way the fat content (here: of dry meat) can be determined.

HINTS

The residue can be tested for unsaturated fatty acids (C = C double bonds).

THE MEAT
DETECTION OF C = C DOUBLE BONDS WITH BROMINE WATER

FUNDAMENTALS

Fats and oils contain two different types of fatty acids. Saturated fatty acids contain a hydrocarbon chain only made of single C – C bonds, whereas unsaturated fatty acids have at least one C = C double bond (monounsaturated fatty acid) located in the hydrocarbon chain. If more than one double bond is present in the chain, the fatty acid is said to be polyunsaturated.

TIME REQUIRED

5 to 10 min

APPARATUS NEEDED

2 test-tubes, glass rod, test-tube rack

CHEMICALS

petroleum ether (bp 40 – 60 °C), bromine water (w/w = 0,5 %), Ravioli meat (or residue from the previous experiment)

SAFETY HINTS

petroleum ether (F, flammable)

PROCEDURE

The meat from about half a piece of Ravioli is mixed with 5 ml of petroleum ether in a test-tube and stirred. The solution is decanted into another test-tube and 1 to 2 ml of bromine water are added. Shake the sample.

OBSERVATIONS

The liquid phase at the bottom of the test-tube is colourless. The bromine water has been decolourised.

EVALUATION

Meat is not soluble in the organic solvent, but the fat is. Oleic acid is an unsaturated fatty acid, which means that it contains C = C double bonds. Commercially-available pork is known to contain varying amounts of oleic acid. In an addition reaction, bromine undergoes addition to the double bonds of the alkene, forming an alkyl halogenoalkane. Due to the fact that colourless bromide ions are formed when the addition takes place, the yellowish colour of the bromine water gradually dis-appears and the solution turns colourless.

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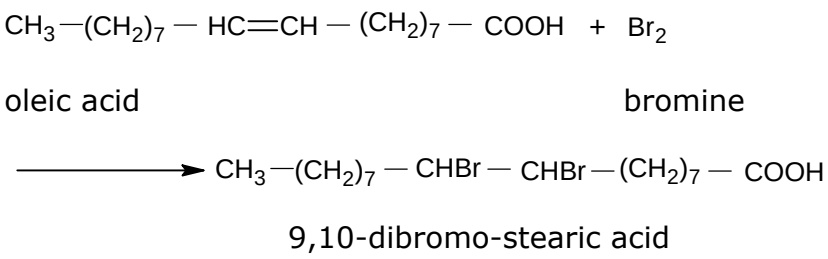

oleic acid bromine

9,10-dibromo-stearic acid

DISPOSAL Add to halogen-free organic waste.

THE SAUCE
ANALYSIS OF TOMATO PIGMENTS IN RAVIOLI SAUCE BY THIN LAYER CHROMATOGRAPHY

FUNDAMENTALS

Tomatoes contain different carotenes as colouring components, but the percentage varies. For instance ß-carotene, which is found in carrots, is found in tomatoes of the sort "high beta" at 36 ppm. Tomatoes of other varieties contain phytoene or lycopine, which chemically differ only slightly from ß-carotene.

TIME REQUIRED

25 min

APPARATUS NEEDED

4 test-tubes with stoppers, TLC-jar, chromatographic paper with silica gel layer (e.g. Macherey and Nagel Polygram SIL G/UV254), Pasteur pipette, 4 small glass cylinders with caps, filter paper, glass capillaries (made from glass tubes), 3 measuring cylinders (10 ml), graduated pipette (2 ml)

CHEMICALS

ravioli sauce, petroleum ether, boiling limits 60 – 80 °C, propan-2-ol, tomato puree, carrot juice, paprika powder

SAFETY HINTS

petroleum ether (F+, highly flammable), propan-2-ol (F+, highly flammable)

PROCEDURE

A) *Pigment extract:* 10 ml Carrot juice, 3 g tomato puree with 10 ml water, 5 ml ravioli sauce with 5 ml water and 1 g powdered paprika with 10 ml petroleum ether are poured in each of the test tubes and mixed well. 2 ml of petroleum ether are poured into each of the test tubes which contain the aqueous solutions. After the phases have separated the upper red-coloured phase can be extracted with a Pasteur pipette and transferred to a glass cylinder for storage.

B) Chromatography: The obtained solutions are separated by chromatography on the plates specified above with a mixture of petroleum ether and propan-2-ol (9 : 1). 10 ml eluent are poured into the TLC-jar, including a paper filter to saturate the vessel with the eluent's vapours. The spots should be applied with glass capillaries (made from glass tubes). They should be applied at least 10 times, after letting them dry in-between. The spots should have a small diameter(2 – 3 cm). The

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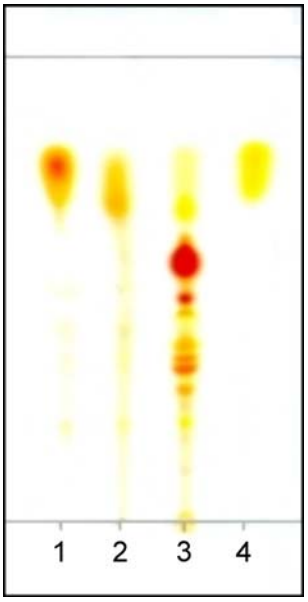
retention time is about 5 to 10 minutes. After letting it dry thoroughly, the chromatogram can be analysed.

OBSERVATIONS

The dried chromatogram shows for all samples yellow to red orange marks at R_f values of 0,7 to 0,8.



Thin layer chromatogram of the petrol extracts

(1) Ravioli sauce
(2) tomato puree
(3) red paprika powder
(4) carrot juice

EVALUATION

The tomato pigments are mixtures of different carotenes. Capsanthin, the red paprika pigment found at the highest percentage, is seen as a red mark.

HINTS

The coloured spots on the chromatogram fade quickly. A photograph can be taken to document the results.

DISPOSAL

Solvents are added to organic wastes.

LITERATURE

Albrecht, U.; Escher, M.; Hartnagel, S.; Heinz A.; Knapp J., Kohlenberger, A.; Leibold, M.; Lesniak, B.; Ludwig, J.; Rust, N.; Schwanzer, C.; Solleder, O.; Vogt, T. und Bader H. J.: Chemie der Raviolidose, NiU-Chemie **13** (2002) Nr. 69, 12 - 16