Supelco®
Analytical Products

Application Note

Hydroxyproline in Meat, Meat Products and Sausages

(according to German Food and Feed Code §64 LFGB 06.00-8)

Note

Pursuant to the valid copyright regulations this application note contains only a rough description of the content of the official method followed by a detailed description of the specific measurement procedure with the Spectroquant® Prove Spectrophotometers. A detailed description of the method specific handling steps can be found in the official method of the German Food and Feed Code §64 LFGB 06.00-8 [1].

Method

In meat products Hydroxyproline occurs in connective tissue only. Therefore, the determination of Hydroxyproline can be used as an index for the content of connective tissue in these products especially for the less valuable content of hides, tendons, cartilage and bone parts [2]. The Hydroxyproline content is determined after acid hydrolysis of the sample followed by the separation of the fat content and oxidation with Chloramine T. The oxidized form of Hydroxyproline reacts with 4-(Dimethylamino) benzaldehyde to form a red colored product that is measured photometrically at 430 nm. This method is based on the official method of the German Food and Feed Code §64 LFGB 06.00-8 [1] and describes the determination of Hydroxyproline in meat, meat products and sausages.

Measuring range

	Description	
Method 2538	Hydroxyproline Meat §64 LFGB 06.00-8	0.000 – 1.000 g/100g

Sample

- Meat, meat products and sausages.

Reagents and auxiliaries

Cat. No.	Description
1.73026	Spectroquant® VIS Spectrophotometer Prove 100 plus
1.73027	Spectroquant® UV/VIS Spectrophotometer Prove 300 plus
1.73028	Spectroquant® UV/VIS Spectrophotometer Prove 600 plus
114946	Rectangular cells 10 mm
102426	Chloramine T Trihydrate for analysis
143007	Hydrochloric acid solution 6.0 mol/l (6.0 N)
101772	Petroleum benzine boiling range 40-60°C SupraSolv®
100244	Citric acid monohydrate for analysis EMSURE®
106498	Sodium hydroxide pellets for analysis EMSURE®
106268	Sodium acetate anhydrous for analysis EMSURE®
100518	Perchloric acid 60% for analysis EMSURE®
100997	1-Propanol for analysis EMSURE®
109634	2-Propanol for analysis EMSURE®

Also first generation Prove instruments are compatible and preprogrammed with this method.

Additional needs

- L-Hydroxyproline (optional)
- DURAN® Laboratory bottles
- Round-bottomed flask or Erlenmeyer flask with NS and return condenser
- Test tube, closable, 10 – 15 mL
- Heating block, drying cabinet or heating plate
- Water bath (temperature controllable)
- Vacuum
- Funnel
- Folded filter, diameter 15 cm
- Volumetric flasks, 50 mL, 100 mL
- Standard laboratory glassware (e.g glass beakers) and pipettes
- Analytical balance

Preparing the solutions

- **Buffer solution pH 6.8**: The solution must be prepared according to German Food and Feed Code §64 LFGB 06.00-8 [1].
- **Oxidizing reagent:** The solution must be prepared according to German Food and Feed Code §64 LFGB 06.00-8 [1].
- **Color reagent solution:** The solution must be prepared according to German Food and Feed Code §64 LFGB 06.00-8 [1].

Sample preparation

- Homogenize sample.

Procedure

Acid hydrolysis and fat separation

- Weigh approx. 2 g of the homogenized sample with an accuracy of 1 mg to a DURAN® laboratory bottle and follow the procedure according to German Food and Feed Code §64 LFGB 06.00-8, chapter 7.1 [1].
- Note the sample weight.
- Use filtrate for the preparation of the measurement solution.

Hydroxyproline determination

Reagent blank

- Mix 0.100 mL of distilled water with 5 mL Oxidizing reagent in a closable test tube and incubate for 20 min at room temperature.
- Add 2 mL of Color reagent, close the tube, mix and incubate in a water bath at 60 °C for 15 min.
- Cool down the vessel to room temperature with running water within 3 min.
- Incubate for 30 min at room temperature.

Sample

- Mix 0.100 mL of the resulted filtrate after acid hydrolysis and fat extraction of the sample with 5 mL Oxidizing reagent in a closable test tube and incubate for 20 min at room temperature.
- Add 2 mL of Color reagent, close the tube, mix and incubate in a water bath at 60 °C for 15 min.
- Cool down the vessel to room temperature with running water within 3 min.
- Incubate for 30 min at room temperature.

Measurement

Note

It is advisable to measure the reagent blank and the sample using the same cell as the one used for the zero adjustment or else a cell with identical optical characteristics and an identical absorption (matched pair).

- Open the methods list (<Methods>) and select Method No. 2538 "Hydroxyproline Meat §64 LFGB 06.00-8".
- The instrument automatically prompts a "Zero adjustment".
- For the zero adjustment fill a clean and dry 10 mm rectangular cell with distilled water.
- After prompting, insert the filled rectangular cell into the cell compartment. The zero adjustment is performed automatically.
- Confirm the performance of the zero-adjustment procedure by clicking on <OK>.
- A window with an input field to enter the sample weight pops up.
- Enter the weight of the sample in grams (g), accurate to 0.001 grams (g), confirm with **<OK>** and click on **<START>** to switch to the measurement procedure.

Note

It is possible to enter a sample weight in a range of 0.010 to 5.000 g.

- Fill the prepared reagent blank into a clean and dry 10 mm rectangular cell. Insert the cell into the cell compartment. The measurement is performed automatically. A (✓) symbol appears behind the cue "Insert Reagent Blank".
- Confirm the measurement by clicking on <OK>.

- Finally fill the prepared sample solution into a clean and dry 10 mm rectangular cell. Insert the cell into the cell compartment. The measurement is performed automatically. A (✓) appears behind the cue "Insert Sample"
- Confirm the measurement by clicking on <OK>.
- Read off the result in g/100 g and the absorption for the reagent blank (A_{RB}) and the sample (A_{Sample}) from the display.
- Tap the **<START>** button to start the measurement procedure for the next sample.

Method control

- The method can be checked using L-Hydroxyproline as standard substance
- Prepare a stock solution with 600 mg/l resp. 60 mg/100 mL L-Hydroxyproline by dissolving 60.0 mg L-Hydroxyproline in approx. 80 mL distilled water. Transfer the solution completely to a 100 mL volumetric flask and fill up to the mark with distilled water.
- Dilute the stock solution to 4.8 mg/100 mL L-Hydroxyproline with distilled water (4 mL stock solution ad 50 mL in a 50-mL volumetric flask).
- Mix 0.100 mL of the prepared standard solution with 5 mL Oxidizing reagent in a closable test tube and incubate for 20 min at room temperature.
- Add 2 mL of Color reagent, close the tube, mix and incubate in a water bath at 60 °C for 15 min.
- Cool down the vessel to room temperature with running water within 3 min.
- Incubate for 30 min at room temperature.
- Measure this solution versus a reagent blank as described in the section "Measurement". Hereby enter a weight of 2.00 g.

Note

Due to the different sample preparation procedure and determination procedure of the standard solution compared to a sample analysis it is necessary to recalculate the displayed result manually as follows:

Measured Concentration standard [mg/100 mL] =

Displayed result [g/100 g] x F1 / F2 =

Displayed result [g/100 g] x 1000 / 50 =

Displayed result [g/100 g] x 20

F1 = 1000 = recalculation g/100 g to mg/100 mL

F2 = 50 = Factor sample preparation for real sample (acid hydrolysis and fat separation)

Adjustment

- In case of significant deviations in the method control procedure the preprogrammed factor of 10.55 or the current factor used in the calculation of the displayed results can be adjusted by the user.
- The corrected factor must be recalculated as follows: **Factor corrected = Current factor x (target value standard / measured and recalculated value standard)**
- To edit the preprogrammed factor, select method 2538 from <Methods>.
- Close the window for the "Zero adjustment" by clicking on <X>.
- Close the input field for the sample weight by clicking on <X>
- Click <Settings> and select the list "FACTORS".
- Tip on the input field "Factor", enter the corrected factor and confirm by clicking on <OK>.
- Close the window for the “Zero adjustment” by clicking on <X>.
- For the next measurement restart the method by selecting the method anew from <Methods>.

Note

- *To find the used factor, select Method 2538 from <Methods>.*
- *Close the window for the "Zero adjustment" by clicking on <X>.*
- *Close the input field for the sample weight by clicking on <X>.*
- *Click <Settings> and select the list "FACTORS".*

For more information visit,
SigmaAldrich.com/photometry

Literature

1. German Food and Feed Code §64 LFGB 06.00-8:2017 Bestimmung des Hydroxyprolingehaltes in Fleisch, Fleischerzeugnissen und Wurstwaren.
2. Matissek R., Steiner G., Fischer M.; (2010): Lebensmittelanalytik, 4.Auflage, Springer-Verlag, Berlin Heidelberg.

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