

Proline in Fruit and Vegetable Juices

(according to EN 1141 and German Food and Feed Code §64 LFGB 31.00-7)

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 EN 1141 ^[1] and German Food and Feed Code §64 LFGB 31.00-7 ^[2].

Method

Each type of fruit and vegetable have its own specific amino acid composition characteristic. Therefore, adulteration of fruit and vegetable juices can be identified by the determination of the amino acid composition. For example, in natural orange juice proline is the amino acid with the highest concentration. The proline determination can be used to indicate if the juice be of synthetic or natural origin ^[3].

The Proline content is determined after extraction of a colored complex built by the reaction of Proline with Ninhydrin. The extract is measured photometrically at 509 nm. This method is based on the official method EN 1141 ^[1] and German Food and Feed Code §64 LFGB 31.00-7 ^[2] and describes the determination of Proline in fruit and vegetable juices.

Measuring range

Description		
Method 2539	Proline Juice EN 1141	0 – 1200 mg/l

Sample material

- Fruit and vegetable juices



Instruments, Reagents and auxiliaries

Cat. No.	Description
1.73026	Spectroquant [®] VIS Spectrophotometer Prove 100 plus or
1.73027	Spectroquant [®] UV/VIS Spectrophotometer Prove 300 plus or
1.73028	Spectroquant [®] UV/VIS Spectrophotometer Prove 600 plus
114946	Rectangular cells 10 mm
106762	Ninhydrin GR for analysis
100859	Ethylene glycol monomethyl ether for analysis EMSURE [®]
109652	n-Butyl acetate for analysis EMSURE [®]
100264	Formic acid 98-100 % for analysis EMSURE [®]
106649	Sodium sulfate anhydrous for analysis EMSURE [®]
107434	L-Proline for biochemistry

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

Additional needs

- Test tubes, closable, approx. 25 mL
- Water bath (temperature controllable)
- Funnel
- Folded filter, hydrophobic 110 mm
- Volumetric flasks, 20 mL, 500 mL
- Standard laboratory glassware (e.g. glass beakers) and pipettes
- Preparing solutions.

Preparing the solutions

Ninhydrin solution:

The solution must be prepared according to EN 1141^[1] resp. German Food and Feed Code §64 LFGB 31.00-7^[2].

Sample preparation

- Light colored samples with Proline concentration below 50 mg/l can be analyzed directly
- Deep colored samples with Proline concentration below 50 mg/l needs to be diluted in a range of 1-part sample + 1-part distilled water up to 1-part sample + 4-parts distilled water
- Samples with Proline concentration 50 mg/l to 499 mg/l needs to be diluted with distilled water 1+9 (1-part sample + 9-parts distilled water)
- Samples with Proline concentration 499 mg/l to 1200 mg/l needs to be diluted with distilled water 1+19 (1-part sample + 19-parts distilled water)
- Note the dilution ratio.

Procedure

Hydroxyproline determination

Reagent blank

- Mix 1.0 mL of the prepared sample with 1.0 mL Formic and 2.0 mL Ethylene glycol monomethyl ether in a closable test tube.
- Incubate the mixture in a water bath according to EN 1141^[1] resp. German Food and Feed Code §64 LFGB 31.00-7^[2].
- Cool down the mixture according to EN 1141^[1] resp. German Food and Feed Code §64 LFGB 31.00-7^[2].
- Add 10 mL n-Butyl acetate and extract the colored complex into the organic phase according to EN 1141^[1] resp. German Food and Feed Code §64 LFGB 31.00-7^[2].
- Filtrate the total solution through a hydrophobic folded filter according to EN 1141^[1] resp. German Food and Feed Code §64 LFGB 31.00-7^[2].
- Use the filtrate for the photometric measurement

Sample

- Mix 1.0 mL of the prepared sample with 1.0 mL Formic and 2.0 mL Ninhydrin solution in a closable test tube.
- Incubate the mixture in a water bath according to EN 1141^[1] resp. German Food and Feed Code §64 LFGB 31.00-7^[2].
- Cool down the mixture according to EN 1141^[1] resp. German Food and Feed Code §64 LFGB 31.00-7^[2].

- Add 10 mL n-Butyl acetate and extract the colored complex into the organic phase according to EN 1141^[1] resp. German Food and Feed Code §64 LFGB 31.00-7^[2].
- Filtrate the total solution through a hydrophobic folded filter according to EN 1141^[1] resp. German Food and Feed Code §64 LFGB 31.00-7^[2].
- Use the filtrate for the photometric measurement.

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. 2539 "Proline Juice EN 1141".
- 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 dilution ratio (1+x) pops up.
- Enter the number of used parts distilled water for the sample dilution, confirm with **<OK>** and click on **<START>** to switch to the measurement procedure.

Example

For undiluted samples the dilution ratio is 1+0, enter "0"

For samples with a dilution ratio of 1+9 enter "9"

For samples with a dilution ratio of 1+19 enter "19"

- 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 mg/l 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.

Evaluation

- Statement of the results:
- Proline [mg/l]
- Absorption of reagent blank A_{RB}
- Absorption of sample A_{Sample}

Method control

- The method can be checked using Cat. No. 107434 L-Proline for biochemistry as standard substance
- Prepare a stock solution with 100 mg/l L-Proline by dissolving 50.0 mg L-Proline in approx. 450 mL distilled water. Transfer the solution completely to a 500-mL volumetric flask and fill up to the mark with distilled water.
- Dilute the stock solution to 25 mg/l L-Proline with distilled water (5 mL stock solution ad 20 mL in a 20-mL volumetric flask).
- Analyze the prepared standard as described in section "Procedure" and "Measurement"
- Hereby enter a value of "0" for the dilution ratio of 1+0.

Adjustment

- In case of significant deviations in the method control procedure the preprogrammed factor of 5.612 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 2539 from <Methods>.

- Close the window for the "Zero adjustment" by clicking on <X>.
- Close the input field for the dilution by clicking on <X>
- Click <Settings> and select the list "FACTORS".
- Tap 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 2539 from <Methods>.*
- *Close the window for the "Zero adjustment" by clicking on <X>.*
- *Close the input field for the dilution by clicking on <X>.*
- *Click <Settings> and select the list "FACTORS".*

For more information visit,
[SigmaAldrich.com/photometry](https://www.sigmaaldrich.com/photometry)

Literature

1. Fruit and vegetable juices - Spectrometric determination of proline content; EN 1141:1994.
2. German Food and Feed Code §64 LFGB 31.00-7:1997 Spektralphotometrische Bestimmung des Prolingehaltes in Frucht- und Gemüsesäften.
3. Matissek R., Steiner G., Fischer M.; (2010): Lebensmittelanalytik, 4.Auflage, Springer-Verlag, Berlin Heidelberg.

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