

# Annatto in Cheese (according to German Food and Feed Code §64 LFGB 03.00-37)

## 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<sup>®</sup> 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 03.00-37 <sup>[1]</sup>.

## Method

Annatto extracts are natural yellow colorings obtained from the outer coats of the seeds of the tropical shrub *Bixa orellana*. Main components are the carotenoids bixin (C<sub>25</sub>H<sub>30</sub>O<sub>4</sub>) and norbixin (C<sub>24</sub>H<sub>28</sub>O<sub>4</sub>). Depending on the conditions of extraction either the mono methyl ester bixin (extractable with organic solvents or plant oils) or the sodium or potassium salts of the water-soluble dicarboxylic acid norbixin prevails. For more than a hundred years annatto extracts have been added to numerous foods such as sweets, ice cream, dressings, and dairy products to produce an intensive color. Because of its use in butter, margarine, and cheese, annatto was formerly declared as "butter color" or "cheese color". Annatto extracts are food additives with an E-number (E 160b) <sup>[2]</sup>.

The Annatto content in cheese is determined after acetone extraction of the sample followed by solid-phase extraction (SPE) to separate the annatto components from fat and β-carotene. The resulted sample extract is measured photometrically at 488 nm.

This method is based on the official method of the German Food and Feed Code §64 LFGB 03.00-37 <sup>[1]</sup> and describes the determination of annatto in cheese.

## Measuring range

Method 2540  
Annatto Cheese §64 LFGB 03.00-37  
0.0 – 10.0 mg/kg

## Sample material

Cheese

## Reagents and auxiliaries

Cat. No.	Description
1.73026	Spectroquant <sup>®</sup> VIS Spectrophotometer Prove 100 plus or
1.73027	Spectroquant <sup>®</sup> UV/VIS Spectrophotometer Prove 300 plus or
1.73028	Spectroquant <sup>®</sup> UV/VIS Spectrophotometer Prove 600 plus
114946	Rectangular cells 10 mm
107712	Sea sand
100014	Acetone for analysis EMSURE <sup>®</sup>
109063	Hydrochloric acid 2 mol/l (1N) Titripur <sup>®</sup>
100983	Ethanol for analysis EMSURE <sup>®</sup>
104367	n-Hexane for analysis EMSURE <sup>®</sup>
100921	Diethyl ether for analysis EMSURE <sup>®</sup>
106009	Methanol for analysis EMSURE <sup>®</sup>
100063	Acetic acid (glacial) 100% for analysis EMSURE <sup>®</sup>
54059-U	Supelclean <sup>™</sup> LC-NH <sub>2</sub> SPE Tubes (bed wt. 500 mg, volume 6 mL, pk of 30)

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

## Additional needs

- Nitrogen, gaseous
- Mortar and pestle
- Centrifuge and centrifuge tubes 100-ml
- Funnel (approx. 10-cm diameter) and folded filters
- Rotary evaporator and thermostable water bath
- Ultrasonic bath
- Flask, pear shaped 100-ml
- Cheese grater
- Water bath
- Graduated cylinders, 10-ml
- Standard laboratory glassware (e.g. glass beakers) and pipettes
- Analytical balance

## Sample preparation

- Grate the cheese with a cheese grater

## Procedure

### Annatto extraction

- Mix approx. 5 g of the grated cheese sample with sea sand in a mortar and follow the extraction procedure according to German Food and Feed Code §64 LFGB 03.00-37, chapter 9.1.1 <sup>[1]</sup>.
- Note the sample weight.
- Prepare a blank extract with sea sand in the same manner.

### Solid-phase extraction (SPE)

- follow the SPE procedure according to German Food and Feed Code §64 LFGB 03.00-37, chapter 9.1.2 <sup>[1]</sup>.
- Prepare a reagent blank with the blank extract in the same manner.

## Annatto determination

## 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. 2540 „Annatto Cheese §64 LFGB 03.00-37“.
- 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 10.000 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 mg/kg Annatto and the absorption for the reagent blank (ARB) and the sample ( $A_{\text{Sample}}$ ) from the display.
- Tap the **<START>** button to start the measurement procedure for the next sample.

## Evaluation

Statement of the results:

Annatto [mg/kg]

Absorption of reagent blank  $A_{\text{RB}}$

Absorption of sample  $A_{\text{Sample}}$

## Literature

1. German Food and Feed Code §64 LFGB 03.00-37:2006 Bestimmung des Annattogehaltes in Käse
2. Bareth A., Strohmair W., Kitzelmann E., HPLC and spectrophotometric determination of annatto in cheese, EUR. Food Res. Technol. (2002) 215: 359-364

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