Protocol 04	
Monitored ingredient	Starch grains
Foodstuff	Starch, pudding
Examination	Histochemistry Unstained sectiones
Short protocol/full version	Full version

1 Sample Description

According to this method are examined various samples of different types of starches and powders containing starch (puddings etc.). Using microscopic method we can prove the presence of starch, but also on the basis characteristic morphology we can determine the origin of the starch in the sample.
This procedure is appropriate for dry, powdered materials - starches, puddings etc.

2 Detection limit

Not determined.

3 Time Consumption

Sample preparation	Sample treatment
0,5 hour	20 min.

4 Sampling

4.1 Sample Amount

For the detection it is necessary to take samples in a sufficient amount.
Picked up:

bulk sample	at least 1 package

4.2 Sampling

Sampling of the investigated material can be carried out directly in production (from the line), or you can taken sample from retail market.

4.3 Sample treatment

Because that it is a powdery, dry material is not necessary fixation of sample. Sample can be kept in the original consumer packaging or pour it into sealable sample container that prevents the sample against pest infestation.

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5 Material and Equipment

5.1 Chemicals and Solutions

- xylene pure
- xylene p. a.
- hard paraffin grated

5.2 Equipment

(uses strictly in accordance of the appropriate manual)

- microtom
- fume hood
- staining cuvettes/staining automat
- distillation apparatus
- scales
- thermostat
- hotplates

5.3 Laboratory Tools

- tweezers
- scalpel
- knife
- cutting mat
- embeading cell
- markers
- filtration paper

5.4 *Laboratory Glass*

- pipettes
- beaker 200 ml
- slides for histochemistry
- cover sliips
- funnels

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6 Sample Treatment and Preparation

6.1 Embedding into Paraffin Blocks

We recommend preparing at least four blocks for each sample. For the purposes of embedding samples into paraffin blocks, commercial medium based on paraffin in combination with bee wax is used. This medium is insoluble in water. Sample mixed with paraffin are embedded in embedding cells by an embedding line. After cooling down, these blocks are prepared for cutting.

6.2 Cutting the Blocks

Tissues embedded in paraffin blocks are cut to 4 μm sections by a microtome. The samples are cut for examination according to the procedure described in the following scheme. The sections are then spread on the water surface and mounted to slides.

Cutting Scheme

block A

section 1 – cut off 50 μm – **section 2** – cut off 50 μm – **section 3** – cut off 50 μm – **section 4** – cut off 50 μm – **section 5** – cut off 50 μm – **section 6**

block B

section 7 – cut off 50 μm – **section 8** – cut off 50 μm – **section 9** – cut off 50 μm – **section 10** – cut off 50 μm – **section 11** – cut off 50 μm – **section 12**

block C

section 13 – cut off 50 μm – **section 14** – cut off 50 μm – **section 15** – cut off 50 μm – **section 16** – cut off 50 μm – **section 17** – cut off 50 μm – **section 18**

block D

section 19 – cut off 50 μm – **section 20** – cut off 50 μm – **section 21** – cut off 50 μm – **section 22** – cut off 50 μm – **section 23** – cut off 50 μm – **section 24**

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6.3 Stainig

For determining kind of starch is not necessary sectiones in no way modified by using histochemical staining. For determination kind of starch are the most appropriate unstained sections. It is only necessary sectiones get rid of paraffin.

6.4 Mounting Slides

Sections are mounted between a slide and a cover slip with. Usually, used synthetic resins are insoluble in water, so it is necessary to dewater the sections in an ascending sequence of alcohol and xylene.

Process for Manual Mounting:
1. put a drop of mounting medium on the margin of the slide.
2. place a cover slip on the margin of the slide under the angle of 45°.
3. carefully and slowly move the cover glass in order to avoid formation of bubbles.
4. put slides in thermostat with 60°C for the night.
5. carefully clean the edges using alcohol and a razor.

It is also possible to use a mounting automate.

7 Microscopic Examination and Evaluation of Results

The stained sections are examined by the light microscope with a lower magnification (e.g. 32x or 40x), for the study of detail is used higher magnification. The identification of starch grains must be based on data from the literature. For comparison use samples prepared in the laboratory and also the schematic pictures and photos from the literature.

8 Documentation

It is recommended to make a laboratory protocol for each sample with the following information:
- number of sample
- date of sampling
- type of product
- producer
- ingredients (if known)
- sample treatment and preparation (fixation, staining, etc.)
- examination results

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For clear identification, it is also recommended to label sample container by sample name and number (the same as written in the protocol). Blocks and slides should be labeled in the same way. It is possible to label samples by bar-codes.

All samples with photo documentation are archived.

9 Results

Based on morphological characteristic, you can determine the type of starch.

Potato starch
Potato starch has the largest grain of all domestic starches (70 - 100 μm). They are either oval or elliptical, core is eccentrically stored in the narrow end and with an obvious eccentric layering. Sometimes there are grains of double and triple.

Wheat starch
Grains are twofold - large and small, both are lenticular shape. Large grains have a size of 12 - 41 μm. In the centre is slightly obvious nucleus and almost infinitesimal concentric layering. Small grains are large 2 - 8 μm.

Corn starch
Corn starch has a multilateral grains with star-shaped forked cavity. Layering is usually not apparent, grain size is 8 - 20 μm.

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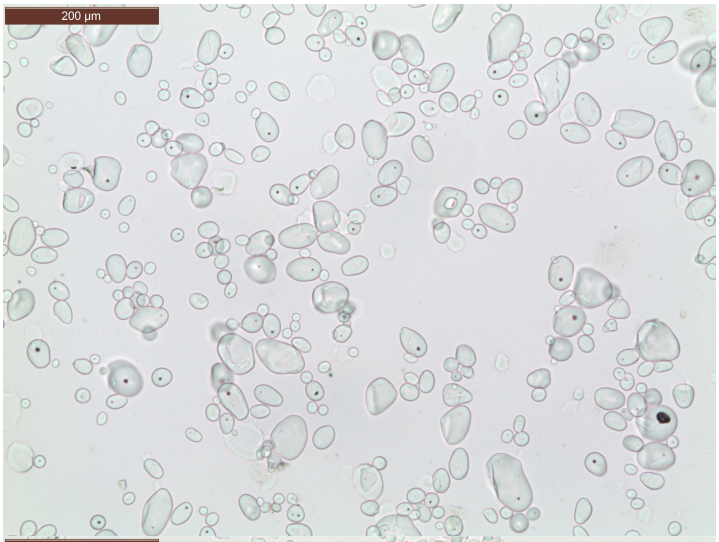
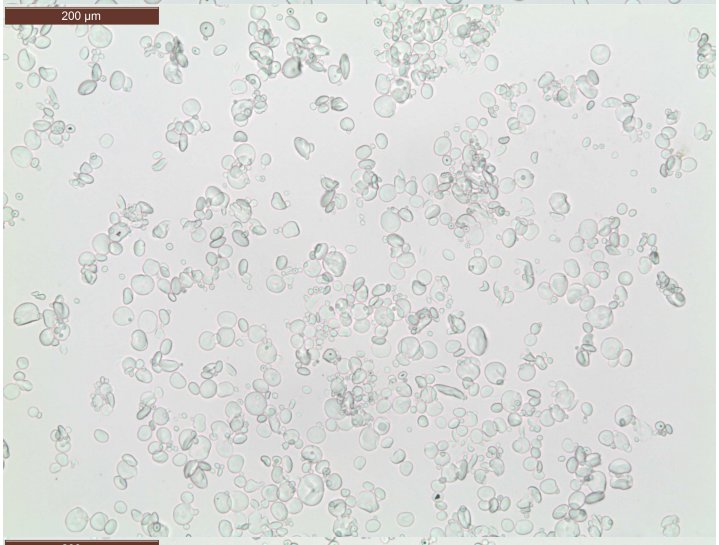
10 Photo Documentation

Potato starch unstained
(magnification 100x)



Wheat starch unstained
(magnification 100x)



Corn starch unstained
(magnification 100x)