

Protocol 05	
Monitored ingredient	Starch grains
Foodstuff	Meat products
Examination	Histochemistry, Lugol Calleja stainig
Short protocol/full version	Short version

1 Detection Limit

The detection limit is 0.1% addition of starch.

2 Time Consumption

Sample preparation
24 hours.

Sample treatment
60 minutes

3 Sample Treatment

3.1 Stainig

Sectiones are necessary before staining get rid of paraffin. Paraffin sections dissolves in the solvent (xylene) and through ethanol are the incisions converted into water because histological dyes are mostly soluble in water. We can dyed by hand in special cuvettes or in the staining automat. Dyes and dye mixtures are applied according to the procedure described below.

Lugol Calleja

Objective: highlight the starch granules

Procedure:

Getting rid of paraffin	xylene.....	10 min.
	100 % alcohol + ether (2/3+1/3).....	10 min.
Nuclear fast red.....		15 min.
Distilled water.....		Wash
Lugol's solution.....		5 min.
Distilled water.....		Wash
Solution B Calleja.....		5 min.
Distilled water.....		Wash
Dewatering (alcohol)	96%.....	Wash
	100%.....	Wash
xylene I (pure)	5 min.
xylene II (p. a.)	5 min.

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Preparation of Solutions:

Nuclear fast red:

10 g	aluminum sulphate
0,1 g	nuclear fast red
100 ml	distilled water

- dissolve over low heat, simmer briefly. After chilling filtered.

Solution B Calleja:

100 ml	indigocarmine 1% (1,0 g indigocarmine+ 100 ml distilled water)
200 ml	acid picric (saturated solution)

Lugol's solution:

300 ml	distilled water
2,0 g	potassium iodide
1,0 g	iodide

Stainig results:

core	– red
muscle	– green
elastic connective tissue	– yellow
fibrous connective tissue, bone, cartilage	– blue
starch grain	– brown

3.2 Microscopic Examination and Evaluation of Results

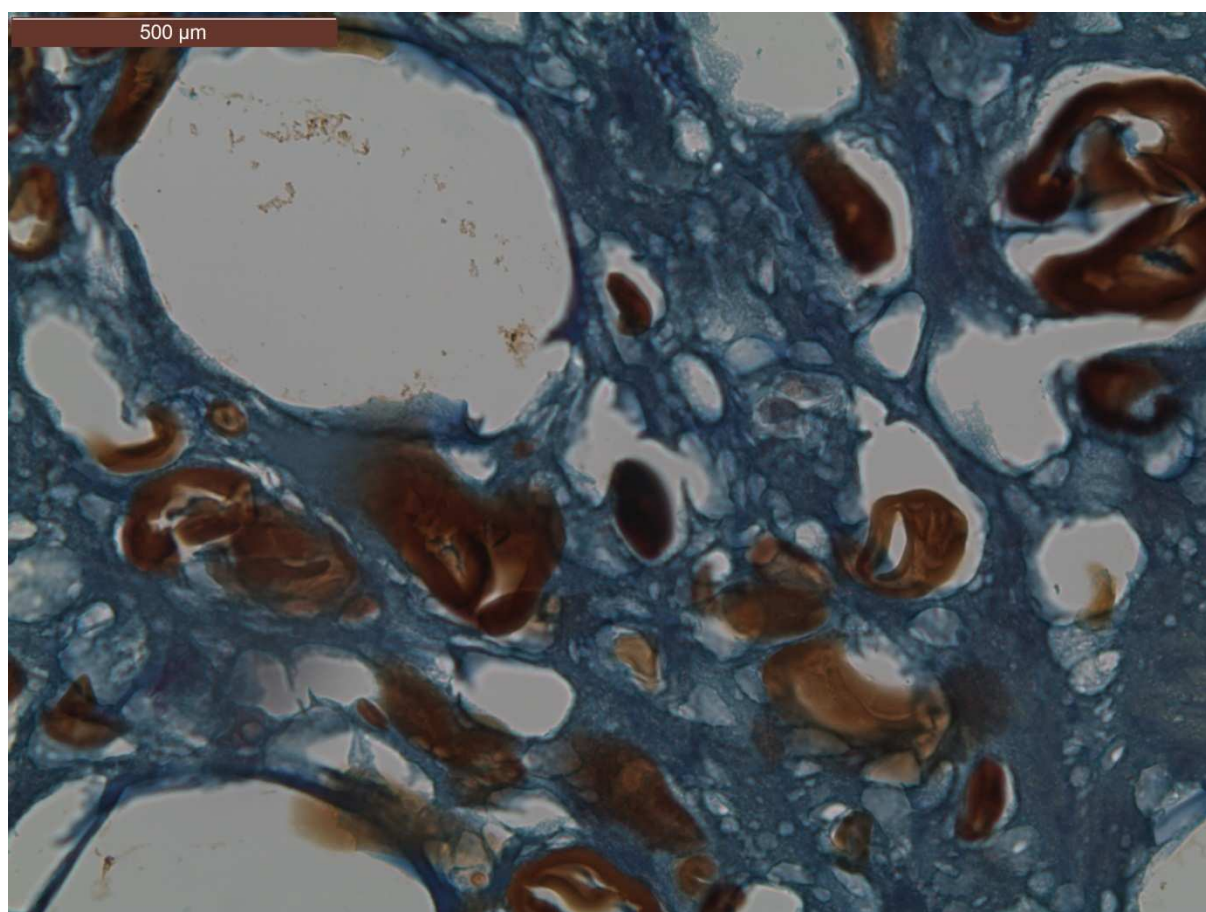
The stained sections are examined by the light microscope with a lower magnification (e.g. 32x or 40x), for the study of detail is used higher magnification. Usually, only qualitative examination is performed. Describes the presence of different types of tissues in the examined samples. It is possible to focus only on identification of selected tissues highlighted by special staining. The identification of tissues of animal and vegetable origin 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.

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3.3 Results

Starch granules are stained brown. Due to the loss characteristics can not determine the type of starch grains.

4 Photo Documentation



Starch granules in a meat product – “Kabanos”, staining Lugol - Calleja, magnification 200x

5 List of Abbreviations

LC – Lugol – Calleja staining