Electrophoresis of Potatoe-Proteins

EDC 08/15

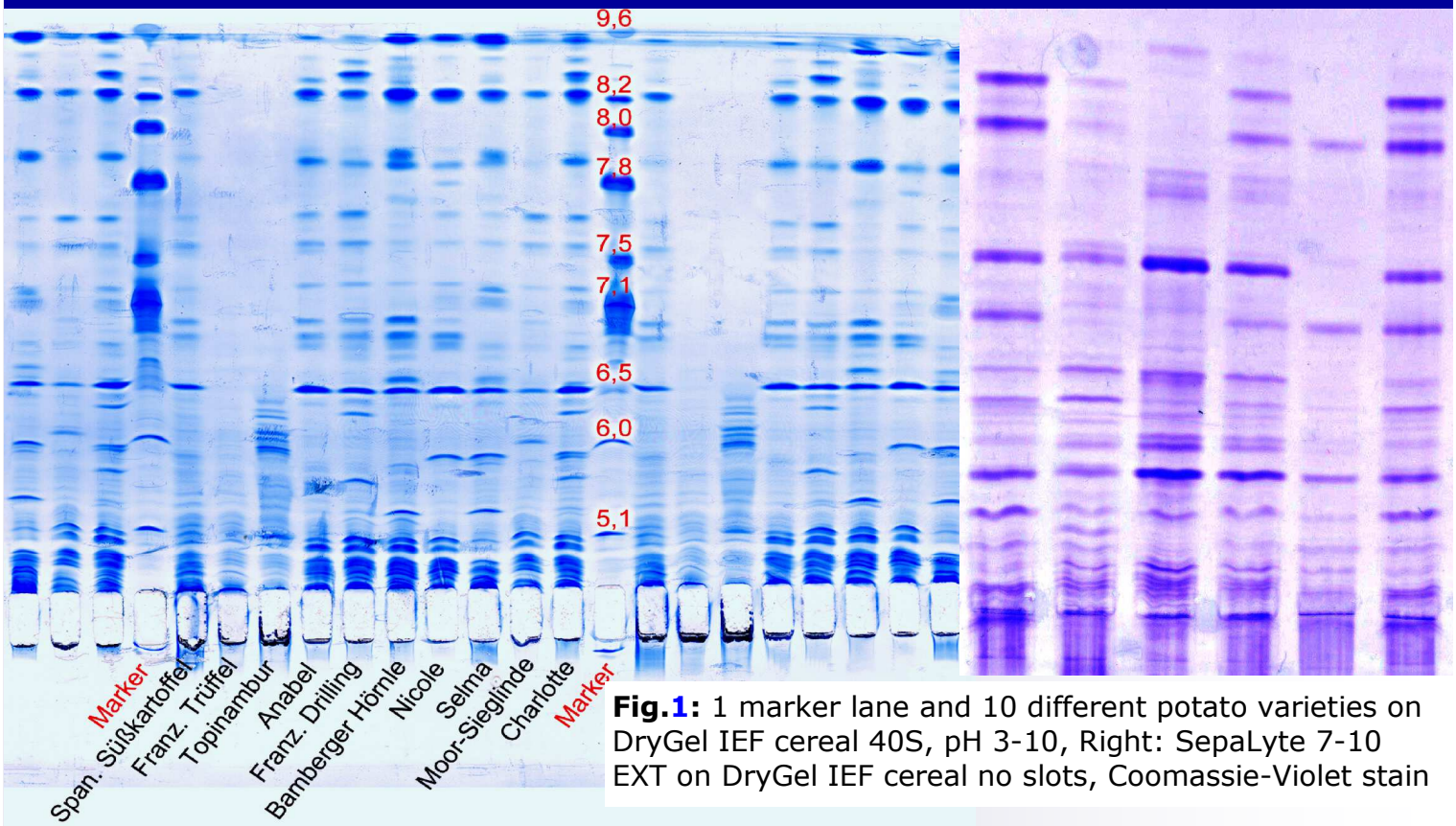

Fig.1: 1 marker lane and 10 different potato varieties on DryGel IEF cereal 40S, pH 3-10, Right: SepaLyte 7-10 EXT on DryGel IEF cereal no slots, Coomassie-Violet stain

***General**:* To display all potato-proteins and to type their molecular weights the SDS-electrophoresis is the most suitable method.
Different potato-varieties show no protein differences in their molecular weights because normally only amino acid exchanges take place in the breeding process. In the SDS-electrophoresis potatoes can be discriminated only from other vegetable-species but not other potato varieties.
To type different potato-varieties electrophoretic methods discriminating the charge-differences should be chosen:

1. Isoelectric Focusing (IEF): This method is the official way of typing potatoes. This technique will discriminate between different potato-varieties.
2. Anodal native electrophoresis also shows differentiations between the varieties. The advantages are the quicker run and the easier staining procedure.

Method #1: Isoelectric Focusing (IEF)
This method displays excellently all different potato varieties, see figure 1.
Samples need only be diluted before being applied to the gel.
Sample treatment: Press out the potato-liquid using a garlic-press. Add 10 mM fresh ascorbic acid. Dilute this press-sap with water at least 1 + 3. Dilute the marker 10 + 290 µl. Apply 20 µl of each sample at the anode after the prefocusing. *Do not heat native samples!*

Consumables IEF:

DryGel IEF cereal no slots (4 gels)	EDC-1120
DryGel IEF cereal 30S (4 gels)	EDC-1128
DryGel IEF cereal 40S (4 gels)	EDC-1123
DryGel IEF cereal 104S (4 gels)	EDC-1126
SepaLyte 7-10 EXTENDED	ProTec
Sample Application Strips (27 à 20 µl)	Biostep: BS146.667
Standard (IEF Marker from Serva)	Serva 39212.01

Method #2: SDS-Electrophoresis
SDS-electrophoresis displays all proteins, they run negatively charged by the tenside towards the anode according to their molecular weights. Different varieties show no real differences, see figure 2.

Sample treatment: Press out the potato-liquid using a garlic-press. Add 10 mM fresh ascorbic acid. Dilute this press-sap with the sample-buffer at least 1 + 3. Pipette to the LMW-marker vial 400 µl sample buffer.
After the samples dilution is done add 5% (v/v) DTT-solution (Dithiothreitol) to the vials (reduction!) and heat 3 min at 95°C. After the vials are cooled down add 5% (v/v) IAA (Iodoacetamide) to the samples (alkylation!). Apply 15 µl of each sample.

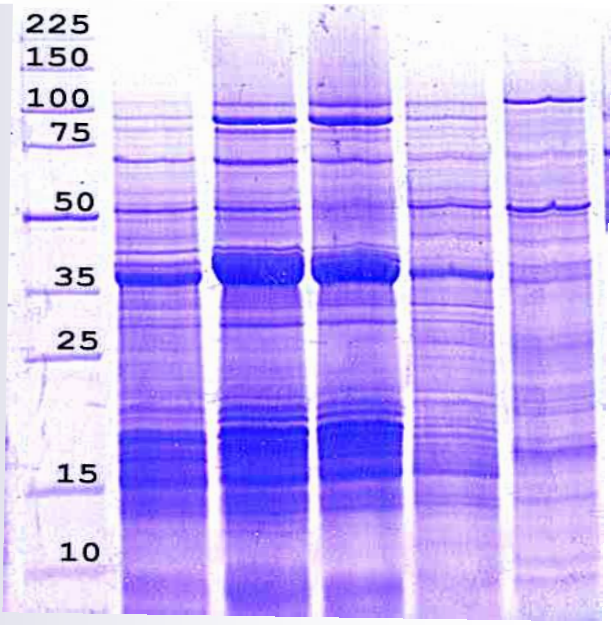

Fig 2: SDS-electrophoresis of 1 marker and different potato-varieties.

Consumables native anodal electrophoresis:

DryGel Elpho 12.5% 52S (4 gels)	EDC-4113
SDS Buffer Kit (for 4 gels)	EDC-5002
Standard (LMW from GE)	GE 17-0446-01

Method #3: Native, anodal Electrophoresis
In native electrophoresis the different mutations of the varieties show different patterens in this electrophoretic method, see figure 3. The same easy „Hot Coomassie Staining" as in the SDS-electrophoresis can be applied.

Native Sample preparation

Sample buffer: 25 ml rehydration buffer + 100 µl Bromophenolblue solution (1%).+ 100 µl Xylene Cyanol (1%)
Sample treatment:
Press out the potato-liquid using a garlic-press. Add 10 mM fresh ascorbic acid.
Dilute this press-sap with the sample buffer at least 1 + 4. Apply 15 µl of each sample.
Pipette 400 µl sample buffer.to the HMW-marker vial. *Do not heat native samples!*

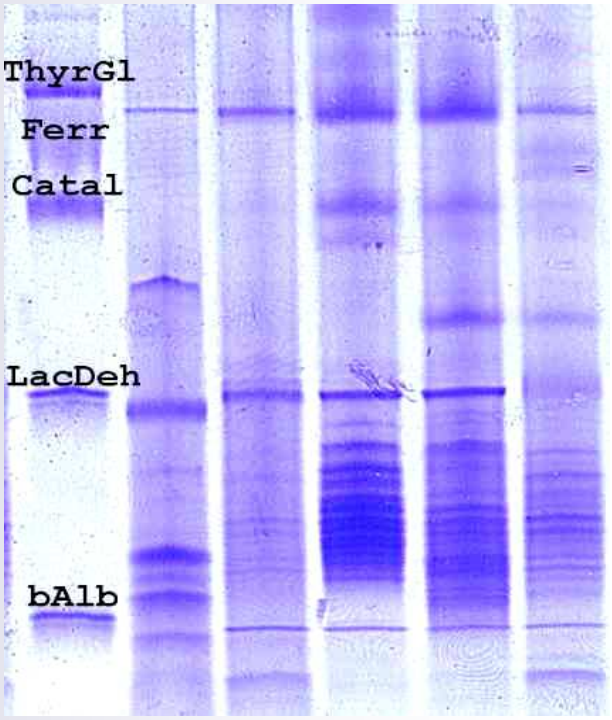

Fig.3: Native, anodal electrophoresis Samples (from left to right): marker, 5 different potato varieties: Granola, Aula, Linda, Rosario, Sweet potato

Consumables native anodal electrophoresis:

DryGel Elpho 12.5% 52S (4 gels)	EDC-4113
Protein Buffer Kit Native Anodal (for 4 gels)	EDC-5003
Standard (HMW from GE)	GE 17-0445-01

Rehydration of DryGels

The volume of rehydration buffer is poured in the DryPool Combi. Then the gel is layed on this volume with the gel-side down. Avoid air-bubbles. Figure 4, above.

Volumen, handling and times see the manuals:
`EDC –-> rehydration pools or download`

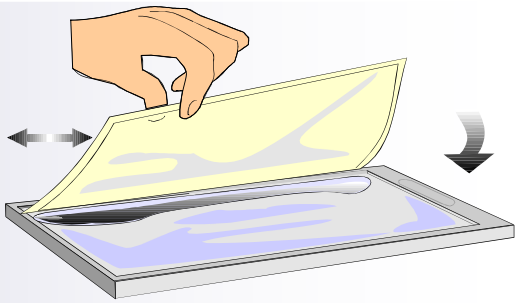

Fig.4: DryPool Combi, side #1

Soaking and Arranging of the Electrodes

The fleeze electrodes are placed in the reverse of the Dry-Pool Combi, fig.5. The buffers are pipetted onto the strips.

Volumen, handling and times see the manuals:
`EDC –-> rehydration pools or download`

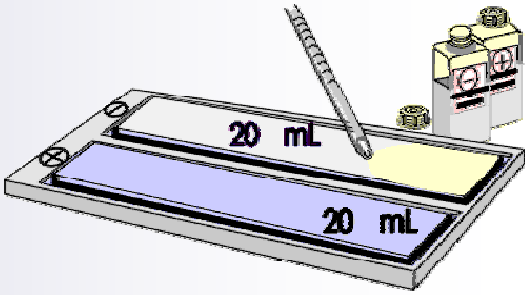

Fig.5: DryPool Combi, side #2

Electrophoresis Chamber

EDC´s flatbed professional (fig. 6) or GE´s Multiphor used as the horizontal chamber for all these procedures described here.

Detailed information see our website:
`EDC –-> chambers –-> flatbed professional`

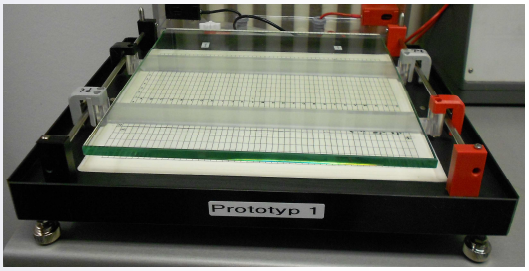

Fig.6: flatbed professional

Visualization of the electrophoretic bands

SDS-electrophoresis and Native electrophoresis:
General protein staining with Coomassie R-350, in hot acetic acid, see fig 7.
This hot Coomassie-staining is staining and fixing simultanously! The acetic acid for staining and destaining can be of technical quality.

IEF: General protein staining with Coomassie-Violet:
(Acid Violet; CI 42650) Sigma #210579-50g
20% TCA have to be used as fixing solution.

Recipes and handling see our website:
`EDC –-> staining –-> dye-staining –-> Protein-Staining`

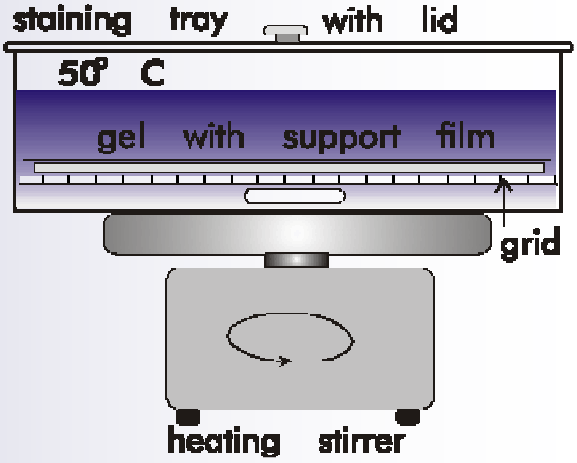

Fig.7: flatbed professional

Hardware from EDC:

DryPool Combi:	EDC-me-d
flatbed professional, horizontal chamber	EDC-prof2836
Staining Tray, standard size	EDC-wm-n1