DAL QUALITY CLASSIFICATION BY SOLUBLE PROTEIN CONTENT MEASUREMENTS

D. Trau, Puay Yen Yap and Michael Cheng, Tip Biosystems Pte Ltd, Singapore

- *Photopette® enables fast dal quality classification by protein measurements in the field.*
- *The method can be performed by a layman within 10 minutes.*

OBJECTIVE

The objective of this application note is to provide a fast method to classify dal quality based on its water soluble protein content. The method can be carried out in the field; only boiling water is required.

INTRODUCTION

Dal contains proteins and dal with high protein content is preferred. A method to access the dal quality in the field, when purchasing dal from the farmer is needed. The dal protein is extracted and the protein concentrations is directly measuring at 280 nm using Photopette® Protein.

A typical UV absorbance spectrum for a protein is shown in Figure 1. Aqueous solutions of proteins have absorbance maxima at 220 nm and 280 nm. Amino acids with aromatic rings (such as tyrosine and tryptophan) and/or Cys-Cys di-sulphide bonds within the proteins are the primary reason for the absorbance peak at 280 nm. Peptide bonds are primarily responsible for the peak at ~220 nm [1, 2].

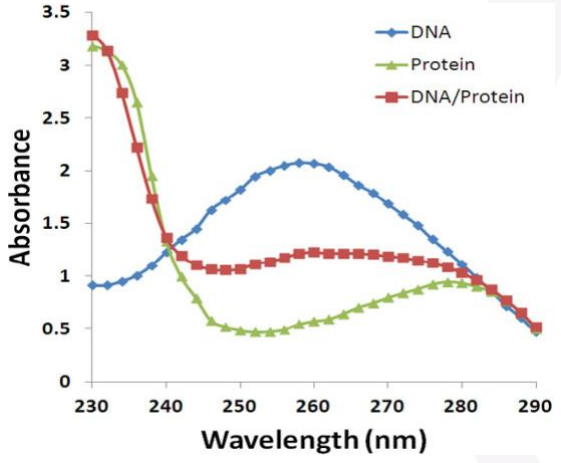

Figure 1: Typical UV absorption spectrum for a protein and a DNA sample and DNA/protein mixture [3]

Other factors such as pH, ionic strength and the 3D-structure of protein etc. can also affect the absorbance spectrum of proteins [5].

MATERIALS AND APPARATUS

Instruments:

- Photopette® Protein with 280 nm wavelength.
- Electrical coffee grinder
- Weighing balance with 0.01 g accuracy or better
- Membrane filter 0.22 micrometer with 10 ml syringe
- Water kettle
- Falcon tubes of 50 mL
- Reaction tubes
- Spatula

Reagents:

- Deionized water

METHOD

EXPERIMENTAL PROCEDURE

Protein Extraction: Clean the grinder to remove any leftover powder in the grinder. With a spatula, fill in ~3 g of dal into the electrical coffee grinder and grind the dal. Weigh exactly 0.50 g of grinded dal powder into a 50 ml of falcon tube. Add boiling deionized water to the 20 ml mark. Shake and mix the content for 5 mins. Add cold water deionized water to the 50 ml mark and mix to cool the extract down to room temperature. Filter through a 0.22 micrometer syringe filter into a 2 ml reaction tube.

Measurement: Turn-on the Photopette® device and connect to the Photopette® iOS/Android app. Refer to the "Photopette® User Manual" for operating [4]. Select "Dal Quality" as the measurement type. Select dataset and set additional settings (if needed) before selecting *'Start Measurement'*. Please follow the video-tutorials on the Tip

Biosystems webpage (www.tipbiosystems.com) to get familiar with the measurement process.

Perform an Autozero with deionized water. Ensure that there is no air-bubble trapped in the CuveTip™ cavity as presence of air-bubbles disrupt the optical-path and result in erratic values. Remove the CuveTip™ from the deionized water and tap the tip onto a paper tissue to remove trapped water. Insert the CuveTip™ into the dal protein extract to fill the cavity; remove and tap the tip onto a paper tissue to remove the sample. Insert into the dal protein extract again and press "Measure".

The dal quality will be displayed as "High Protein", "Medium Protein" and "Low Protein".

DISCUSSIONS

EXPERIMENTAL RESULTS

Four dal samples with different amount of protein have been measured by the method described above.

Dal Sample	Conc. mg/ml	Avg. absorbance (AU)	Standard Deviation (SD)	1st Data	2nd Data	3rd Data
White	0.854	0.569	0.002	0.568	0.568	0.572
Mong	0.927	0.618	0.002	0.62	0.616	0.618
Red	0.352	0.235	0.001	0.234	0.236	0.234
Yellow	0.942	0.628	0.000	0.628	0.628	0.628
Control	0.014	0.009	0.008	0	0.012	0.016

The "Dal application" will automatically qualify the dal regarding to the soluble protein into "High Protein", "Medium Protein" and "Low Protein". For the samples in the table above, samples "White", "Mong" and "yellow" are "High Protein" and sample "Red" is "Low Protein".

SUMMARY

Photopette® Protein can be used for fast and direct *protein measurements to classify dal quality in the field.* The method can be performed by a layman within 10 minutes.

LIMITATIONS

The method is determining the water soluble protein in dal but not the total protein concentration. However, the water soluble protein is the most valuable for human nutrition and therefore can be used to reflect the dal quality.

To prevent bias of the data, grinding of the dal into its powder form must be done on the same day that the measurement is taken.

During the extraction of the protein using hot deionized water, ensure that the style of the hand shaking is uniform for all the dal samples and all the dal powder is uniformly mixed in the water. The falcon tube must be kept closed throughout the shaking as any heat escaped may affect the amount of protein extracted.

REFERENCES

[1] M. H. Simonian and J. A. Smith, "Spectrophotometric and Colorimetric Determination of Protein Concentration," in *Current Protocols in Molecular Biology*, vol. Chapter 10, Hoboken, NJ, USA: John Wiley & Sons, Inc., 2006, p. Unit 10.1A.

[2] E. Layne, "Spectrophotometric and turbidimetric methods for measuring proteins," 1957, pp. 447–454.

[3] C. M. Stoscheck, "Quantitation of protein.," *Methods Enzymol.*, vol. 182, pp. 50–68, 1990.

[4] F. Omar, "Photopette User Manual V1.0.0," Tip Biosystems Pte Ltd, Singapore, 2016.

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