# NEW APPROACH TOWARD α-AMYLASE ELECTROPHORESIS AND ISOAMYLASE DETECTION

**Mohammad Mehrabadi and Ali Reza Bandani***

* Insect Physiology Lab., Plant Protection Dep., University of Tehran, Karaj, IRAN. E-mail: abandani@ut.ac.ir

**[Mehrabadi, M. & Bandani, A. R.** 2010. New approach toward α-amylase electrophoresis and isoamylase detection. Munis Entomology & Zoology, 5, suppl.: 1085-1087**]**

ABSTRACT: Native polyacrylamide gel electrophoresis has been a useful method for analysis of α-amylases and their isozyme(s). Routinely, for detection of enzyme activity soluble starch (1%) is added at the end of electrophoresis, before staining the gel, which is time consuming and in some cases minor isozyme(s) could not be detected. Thus, we developed a convenient and efficient native page by adding the soluble 0.5% starch at the beginning of electrophoresis as a part of gel components and running at 4 °C. This simple modification should be beneficial for analyzing the both purified and unpurified.

KEY WORDS: α-amylase electrophoresis, isoamylase detection.

A simple and short in gel assay method with improved sensitivity for the detection of α-amylases and their isozyme(s) in complex mixtures by polyacrylamide electrophoresis, which starch is a component of gel, is described. All previous α-amylase native electrophoresis have been carried out using the procedure described by Laemmli (1970) and Campos et al. (1989). According to this method, before staining the gel, it should be exposure to soluble starch for detection of enzyme activity zoon about more than 1 h. This time needs for penetration of starch into the gel and hydrolyzing of it by α-amylase. Furthermore, in order to having better result, the dish contains the gel should be shaking gently during this stage. The rate of enzyme denaturizing is increased when electrophoresis run in room temperature, then minor isozymes would not be detectable (Fig. 1B). So, we conducted modified method for simplifying electrophoresis and reducing the time as well as improved sensitivity for the detection of α-amylases and their isozyme(s) (Fig. 1A).

## RESULTS AND DISCUSSIONS

Our experiments showed that adding of 0.5 % starch to gel not only was reliable but also in some cases, was more efficient for detection of isoamylases. For this purpose starch should be added to mixture before adding of TEMED and APS, because solving of starch giving time and if these material is added to the mixture, it would be polymerized before starch solving. Furthermore, for prevention of having smear tail in gel, α-amylases should be in a temporary/reversibly low activity/inactive mode during the loading stage. It could be achieved by running the electrophoresis at 4 °C or adding trace amount of SDS to sample buffer.

We used Cowpea weevils, *Callosbruchus maculatus* (Fabricius) (Col: Bruchidae) as a source of α-amylase. Adults of *C. maculatus*, were randomly selected, cold-immobilized, dissected under a stereoscopic microscope, and the midguts removed in distilled water. The midguts were placed in a pre-cooled homogenizer and ground in one ml of universal phosphate buffer 0.02M at pH 6.5. The homogenates were centrifuged at 15 000 (×g) at 4 °C for 15 min. The

resulting supernatants were transferred to a new tube and frozen at -20 °C for further use. The protein concentration was determined according to method of Bradford (1976) using BSA as standard.

Native-PAGE is performed in 12% (w/v) gel with 0.05% SDS and 0.5% starch for separating gel and 5% for stacking gel with 0.05 % SDS and without starch at pH 7.5 (Mehrabadi et al., 2009). The electrode buffer was prepared based on the method of Laemmli (1970) but SDS was not used. The sample buffer contained 25% stacking buffer (0.5 M Tris-Hcl ,pH 6.8), 20% Glycerol, 2% SDS, 0.005%(w/v) bromophenol blue, but without mercaptoethanol and heating. Electrophoresis was conducted at 4°C with a voltage of 120V until the blue dye reached the bottom of the slab gel. To prepare gels for α-amylase assay, the gel was rinsed with water and washed by shaking gently with 1.5% (v/v) Triton X-100 in phosphate buffer (0.02 M) for 20 min. After washing the gel with sterile water, it placed into phosphate buffer (0.02 M) contained 2mM $CaCl_2$ and 10mM NaCl for 30min. Then, the gel was rinsed with water and treated with a solution of 1.3% $I_2$, 3% KI to stop the reaction and to stain the un-reacted starch background. Zones of α-amylase activities appeared at light band against dark background (Fig. 1A).

Detection of isoamylases by using new method is more accurate than common technique through loading the same amount of protein. Low mobility band was not detectable in common procedure whereas it was clearly detected by using new method at all protein concentrations (Fig. 1A,B). These results showed that new method was more efficient than common procedure especially in samples contain low protein concentration. The mobility of bands was the same in both methods indicated that adding of starch into the gel had no deleterious effects on protein electrophoresis.our results showed that loading the samples contains total protein concentration between 20-30 mg had the best results; in the cases loading more than 50 mg protein a smear may appear on the gel (data not shown). The most α-amylase activity was obtained in the case of 30 mg protein loading, too (Fig. 1C,D).

In conclusion, we have developed a convenient and efficient method of α-amylase electrophoresis. Thus, application of the new method for α-amylase activity detection permitted simple, detailed and rapid information on individual amylases and their isoformes in crude complex mixtures. The improvement of previously described α-amylase electrophoresis resulted in enhanced detection of α-amylase. We also demonstrated that the new method had no deleterious effects on the α-amylase characteristics in the mixture. This protocol will, in general, reduce experimental time and reduce the consumption of precious protein samples.

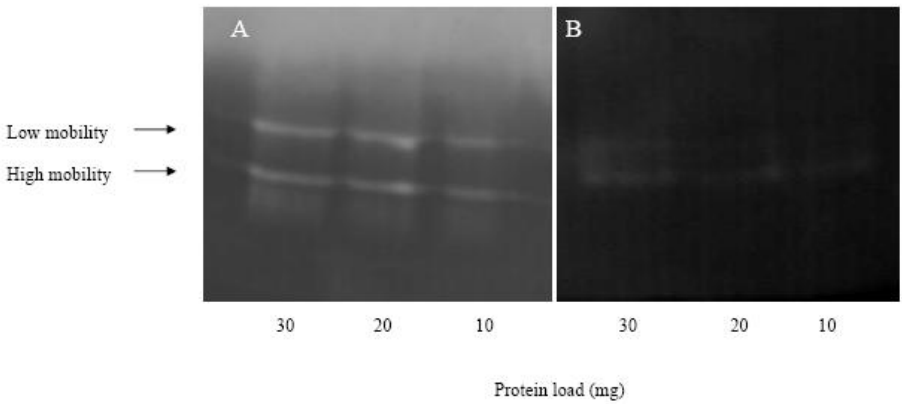
## LITERATURE CITED

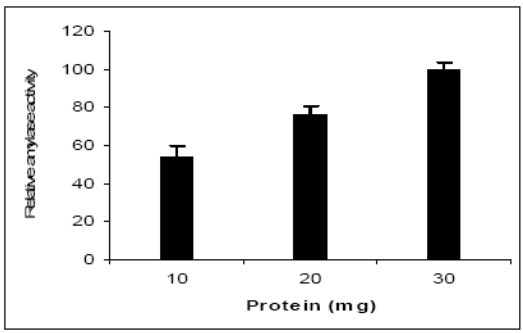
**Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.

**Campos, F. A. P., Xavier-Filho, J., Silva, C. P. & Ary, M. B.** 1989. Resolution and partial characterization of proteinases and α-amylases from midgut of larvae of the bruchid beetle *Callosobruchus maculatus*. Comp. Biochem. Physiol. Part B., 92: 51-57.

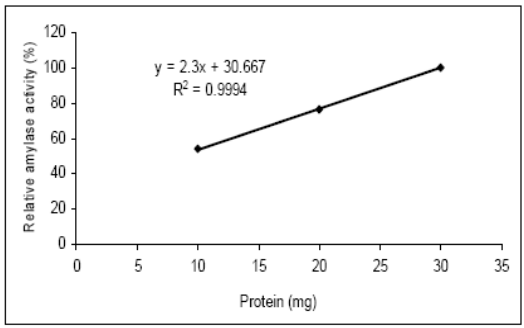
**Bradford, M.** 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.

**Mehrabadi, M., Bandani, A. R., Saadati, F. & Ravan, S.** 2009. Sunn pest, *Eurygaster integriceps* Put (Scutelleridae: Hemiptera) digestive α-amylase, α- glucosidase and β-glucosidase. J. Asia Pac. Entomol., 12: 79-83.




**C**




**D**

Figure 1. SDS-polyacrylamide gel electrophoresis (Native Page) of the *C. maculates* extract. SDS-PAGE was performed in 12% (w/v) gel, 0.05% SDS with 0.5% starch (A) and without starch (B) for separating gel and 5% for stacking gel with.0.05% SDS as a function of protein loading (C, D). The sample buffer contained 25% stacking buffer (0.5 M Tris–HCl, pH 6.8), 20% Glycerol, 2% SDS, and 0.005% (w/v) bromophenol blue. Gel was stained with a solution of 1.3% I2, 3% KI.