



ESTERASE ACTIVITY FROM THE GERMINATED *JATROPHA CURCAS* SEEDS IN DIFFERENT EXTRACTION BUFFERS

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ABSTRACT

The buffer solution plays a major role in protein stability and activity, thereby making the selection of a buffer to achieve maximum activity for a protein will be a formidable challenge. The present work constitutes an extension of this investigation to esterases from germinated *Jatropha curcas* seeds. The 0.1 M NaCl solution, 0.1 M phosphate buffer pH 7.0, 0.1 M citrate buffer pH 5.0 and 0.1 M Tris-HCl buffer pH 8.5, 0.1 M NaOH and distilled water were used to extract protein from germinated *Jatropha curcas* seeds. The esterase activity and specific activity for NaCl solution, phosphate buffer, citrate buffer, Tris-HCl buffer, NaOH and Distilled water was 9.07, 8.6, 8.2, 6.46, 0.07 and 4.98 $\mu\text{moles}/\text{min}/\text{gm}$ and 0.09258, 0.0905, 0.088, 0.0715 0.0003 and 0.081 IU/mg, respectively. The Native-PAGE analysis showed the esterase enzyme activity in different extraction buffer. Among 13 esterase bands, 8 esterolytic bands were major bands (band no 1, 3, 6, 7, 8, 11, 12 and 13) and remaining were minor bands. The amount of proteins and esterase activity were found to be the highest when extracted with 0.1 M NaCl solution.

KEY WORDS: Phosphate, Citrate, Tris-HCl, NaCl, Esterases, *Jatropha curcas*.

INTRODUCTION

The buffers have profound effects on the tertiary and quaternary structures of proteins. It is important to realize that buffers perturb protein conformational stability because of a complex interplay between various effects rather than by stand-alone mechanisms [1]. They should exhibit little or no change in pH with temperature, show insignificant penetration through biological membranes, and have maximum buffer capacity at a pH where the protein exhibits optimal stability. The mechanisms or combinations thereof by which buffers may cause protein stabilization (or destabilization) are complex and not well understood.

Esterases are a group of hydrolytic enzymes that catalyze the hydrolysis of various types of esters and are widely distributed in nature. Germination brings out the synthesis or activation of enzymes responsible for the degradation of seeds reserves. Among these enzymes, esterases are involved in the metabolic processes of germination and maturation of plants. They are constitutively expressed in seeds during germination to release the reserve materials for the growing embryo [2]. They are involved in fruit ripening, abscission, cell expansion, somatic embryogenesis, stomatal movements, reproduction as well as detoxification of xenobiotics. *Jatropha curcas* is one of the drought resistant, oil yielding plant, belonging to the family Euphorbiaceae and is a common plant in most arid areas of Asia, South America and Africa. Since *Jatropha* constitutes one of the potential sources of various phytochemicals and esterases might be involved in transesterification, detoxification and insecticide or pesticide scavenging activity.

The method used for protein extraction and purification may affect how a particular buffer subsequently modulates its stability. In this context, the objective of our work was

the comparison of different extraction buffers for extraction of esterases from *Jatropha curcas* and protein pattern study from crude extracts.

MATERIALS AND METHODS

Materials: The seeds of *Jatropha curcas* were collected from Upparahally, Kolar District, Karnataka, India.

Chemicals: NaCl, NaH_2PO_4 , Na_2HPO_4 , Sodium citrate, Citric acid, Tris base, Acrylamide, N, N, Methylene bisacrylamide, 1-Naphthyl acetate, 1-Naphthyl propionate, Bovine Serum Albumin, fast blue RR salt, fast blue B salt (diazobase B) were obtained from Sigma Chemical Company, St. Louis, MO, USA. All other chemicals were analytical grade.

Germination: The germination was carried out for 4 days. The endosperm collected and 10% enzyme extract was prepared and protein and esterase activity were determined as described below.

Preparation of acetone powder and enzyme extract: The acetone powder (10%) was prepared by blending soaked and dehulled seeds of *Jatropha* in chilled acetone for 1 -3 minutes, it was then filtered by suction pump, dried at 37°C and stored at 4°C until further use. A 10% acetone powder of *Jatropha* seeds was used to extract esterase with 0.1 M NaCl solution and different buffers, 0.1 M sodium phosphate buffer pH 7.0, 0.1 M citrate buffer pH 5.0 and 0.1 M tris-HCl pH 8.5 by stirring over a magnetic stirrer for 1 hr at 4°C . The extract was then centrifuged at 10,000 rpm for 20 min at 4°C . The supernatant was collected and used for qualitative and quantitative analysis of total protein and esterase activity.

Protein assay: The protein content in the enzyme extract and fraction was determined by the method of Lowry [3] using bovine serum albumin as standard.

Esterase assay: Esterase activity was monitored quantitatively according to the method of Gomori [4] as modified by VanAsperen [5]. A typical assay mixture contained 5 ml of 0.3 mM substrate solution (a stock solution of 30 mM 1-Naphthyl acetate was prepared in acetone and diluted to 100 fold with 0.05 M sodium phosphate buffer, pH 7.0) and 1 ml of suitably diluted enzyme extract. The reaction mixture was incubated for 15 minutes at 27°C and the reaction was arrested by the addition of 1 ml DBLS reagent (2 parts of 1% diazo blue B and 5 parts of 5% sodium lauryl sulphate). The reaction mixture was allowed to stand for 30 minutes and the intensity of the color formed was measured at 600 nm. A calibration curve was prepared using 1-naphthol.

Staining and Destaining of proteins: The proteins were stained on polyacrylamide gels, according to the method of Davis [6] using 0.5% solution of Coomassie Brilliant Blue R-250 in 25% methanol and 7.5% acetic acid in

water for 1 hour and was destained in 25% methanol and 7.5% acetic acid in water for overnight.

Staining for esterase activity: Esterase activity on polyacrylamide gels was detected essentially according to the method of Hunter and Markert [7]. Fast-blue RR was used for simultaneous coupling with 1-naphthol moiety released upon hydrolysis of 1-Naphthyl acetate. The gels were removed from plates and stained for esterase activity using 100 ml of 0.05 M phosphate buffer pH 7.0 containing 40 mg of fast-blue RR and 20 mg of 1-naphthyl acetate (dissolved in 2 ml of acetone) for 20 minutes at room temperature.

RESULTS AND DISCUSSION

The crude esterase was extracted from germinated *Jatropha curcas* seeds with different extraction buffers as described above. The protein concentration and esterase activity were examined and the results are shown in Table 1.

TABLE 1: Crude extracts of germinated *Jatropha curcas* seeds using different extraction buffers

Sl. No	Extraction Buffer	Total activity (μmoles/min/gm)	Total protein (mg/gm)	Specific activity (1U/mg)
1	0.1 M NaCl solution	9.073	98.01	0.09258
2	0.1 M Phosphate buffer pH 7.0	8.6	95	0.0905
3	0.1 M Citrate buffer , pH 5.0	8.2	92.82	0.088
4	0.1 M Tris-HCl buffer, pH 8.5	6.46	90.32	0.0715
5	0.1 M NaOH	0.070	210.60	0.0003
6	Distilled water	4.98	61.42	0.081

The excellent review showed that charge–charge interactions were better optimized in the enzymes than in proteins without enzymatic functions, relative to randomly distributed charge constellations obtained by the Monte Carlo technique [8]. Proteins belonging to the mixed type were electrostatically better optimized than pure helical or strand structures. Proteins stabilized by disulfide bonds showed a lower degree of electrostatic optimization. Finally, the electrostatic interactions in a native protein were effectively optimized by rejection of the conformers that lead to repulsive charge–charge interactions.

The highest esterase activity achieved in 0.1 M NaCl extract and next in 0.1 M phosphate and citrate buffer, this indicates possible charge-charge interaction optimized the esterase enzyme. Another study revealed that Na⁺, K⁺, Ca⁺², and Mg⁺² increased the stability of the acidic protein apoflavodoxin (pI = 4.0) at neutral pH [9]. The cation stabilizing effect extended to 500 mM, and the researchers

speculated that the unusual increase in stability at neutral pH was likely to be a common property among highly acidic proteins. Maximum esterase activity found in citrate buffer at pH 5.0 and Distilled water compared to Tris-HCl buffer pH 8.5, this infers neutral pH and acidic pH solution optimized the esterase enzyme activity than in basic pH solution. The protein extracted with NaOH solution was high but the stability and activity of esterase enzyme is negligible due to extreme basic pH 13.0, it precipitated the membrane protein and soluble cellular protein therefore the protein content was high in NaOH extraction.

The Native-PAGE analysis showed the esterase enzyme activity in different extraction buffer.. Among 13 esterase bands, 8 esterolytic bands were major bands (band no 1, 3, 6, 7, 8, 11, 12 and 13) and remaining were minor bands as shown in Fig.1.

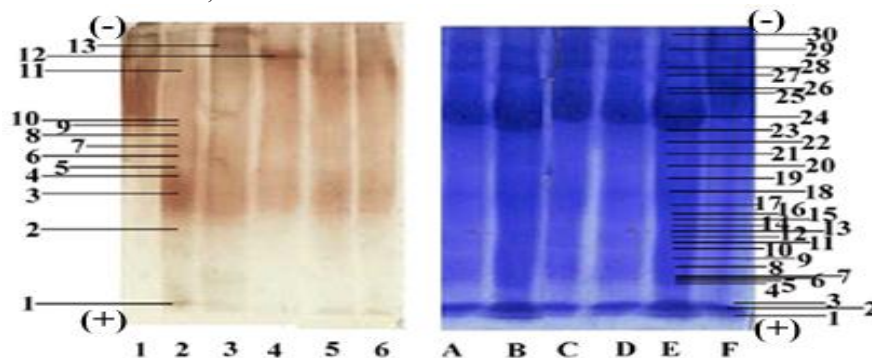


FIGURE 1. Native-PAGE (10% acrylamide) analysis for esterase activity and total protein of germinated *Jatropha curcas* seeds extracts using different extraction buffers.

Lane 1 & F: NaOH; Lane 2 & E: NaCl; Lane 3 & D: Distilled water; Lane 4 & C: Tris-HCl, Lane 5 & B: Citrate buffer and Lane 6 & A: Phosphate buffer.

Several workers have observed specific metabolic changes during germination and plant development. These changes have been correlated with the parallel variation in the types and levels of several enzymes and soluble proteins. The alteration in the zymogram pattern of esterases during development has been reported to be due to differential gene activity [10].

CONCLUSION

The amount of proteins and esterase activity were found to be the highest when extracted with 0.1 M NaCl solution. The esterase activity decreased in the order 0.1 M NaCl > 0.1 M phosphate buffer pH 7.0 > 0.1 M citrate buffer pH 5.0 > Distilled water > 0.1M Tris-HCl buffer pH 8.5 > NaOH. Salts can affect protein conformation to the extent that the anions or cations of the salt could be potential buffer components along with pH of the solution. When the salt concentration is much larger than that of the buffer, the salt becomes the effective buffer in the reaction.

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