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# L-arginase Based Biosensor for Detection of L-arginine in Juice Samples

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# ABSTRACT

Soybean, glycine max, axes is known to contain L-arginase. The present work is based on development of plant arginase based biosensor for the monitoring of arginine in juice samples. The presence of L-arginine in juice samples has been studied by using immobilization techniques such as gelatin, polyacrylamide gel, agar and calcium alginate beads. The response times and stability of L-arginase with different method of immobilization has been studied. The indicator (Phenol red) was coimmobilized with plant arginase and urease. The detection range of  $10^{-10} - 10^{-1}$  M was studied. These techniques have been utilized for the detection of arginine in juice samples ( $10^{-2} - 10^{-5}$  M) and maximum concentration was found in orange juice ( $10^{-2}$  M).

Keywords: L-arginase, Biosensor, Immobilization, polyacrylamide gel.

# INTRODUCTION

L-arginase is also referred as L-arginine amidinohydrolase (E.C. 3.5.3.1) is found in all five kingdoms of organisms. It catalyzes the hydrolysis of L-arginine into L-ornithine and urea, which is the last step of urea cycle in liver of ureotelic species. Arginase was first discovered by Kossel and Dakin in 1904 in the mammalian liver [1]. Arginase occurs in form of two isozymes that share 60% identity. Arginase I gene located on chromosome 6 is a cytosolic enzyme expressed primarily in liver and to some extend in erythrocytes. Arginase II gene located on chromosome 14 is a mitochondrial enzyme expressed majorly in kidney and minorly in brain, spinal cord, mammary gland and small intestine [2]. Arginase II is found in synthesis of L-ornithine, L-proline and L- glutamate [3]. The enzyme L-arginase is produced by a variety of microorganisms such as *Bacillus licheniformis*, *Bacillus subtilis* KY 3281, *Bacilluscaldovelox* and *Rhodobacter* [4-7]. Cytosol of soybean, *glycine max* is also the potent source for isolation of arginase [8]. Arginase has also been extracted from the root tissues of shade plant ginseng, *Panax ginseng* C.A. Meyer which was stable to heat [9]. Germinated loblolly pine (*Pinustaeda L.*) seeds, *Lycopersiconesculatum* (tomato), cotyledons of *Vignacatjang* and cherry tomatoes, *Lycopersiconesculentum* serve as the great source of arginase [10-13].

With the help of biosensors detection of L-arginine can be done very easily and various arginine biosensors have also been developed. An arginine biosensor was developed by co-immobilization of *Bovine liver* and *Cajanus cajan* tissue acting as a source of arginase [14] and urease respectively on modified stainless steel electrode. Further, arginase and urease were immobilized on the surface of pH electrode by using gelatin membrane which is cross-linked with glutaraldehyde [15]. A potentiometer L-arginine bi-enzyme biosensor was developed based on recombinant human liver arginase I [16].Co-immobilization of arginase and urease allows a conductiometric detection of L-arginine. A highly sensitive biosensor was developed based on cross linking with glutaraldehyde [17]. Use of plants as environmental biosensors has also been described [18]. A potentiometric urea biosensor for clinical purposes has been developed [19]. Novel plant system based biosensor for detecting environmental hazards has been described [20].

# MATERIALS AND METHODS

The enzyme L-arginase was extracted from seeds of plant, soybean, *glycine max*, axes which was collected from different regions within North India. All the chemicals and reagents were of analytical grade. The enzyme was extracted from soybean by homogenizing soybean cotyledons with 50 mM Tris (pH 7.5) containing 1mM MnCl<sub>2</sub> and 10% glycerol. The homogenate was filtered and centrifuged at 10,000 rpm for 30 min to collect supernatant which contained crude extract. Further, the crude enzyme was coimmobilized with phenol red indicator (HiMedia Laboratories Pvt. Ltd., India) along with urease. The detection range of  $10^{-10} - 10^{-1}$  M was studied and response time for color change was observed. Different immobilization techniques for the biosensor fabrication are as follow:

### a) Gelatin Method:

2 gm of gelatin was dissolved in water by heating. After allowing it to cool to 35-40 <sup>0</sup>C, 20 µl enzyme (arginase and urease) were co-immobilized with 10 µl phenol red indicator. Then 2 ml of hardening solution (4ml formaldehyde, 6 ml water and 10 ml ethanol) was added. It was allowed to freeze at -28 <sup>0</sup>C for 4 hr [21]. Gel was warmed to room temperature and cut into square blocks of 1.0 X 1.0 cm. The pieces of gel were taken in different concentration ranging from  $10^{-10} - 10^{-1}$  M of L-arginine and the color change was noted down.

### b) Polyacrylamide Method:

A 10% acrylamide and bis-acrylamide solution (9% acrylamide and 1% bis- acrylamide) was prepared in 0.1 M phosphate buffer (pH 7.0). 10  $\mu$ l of each arginase and urease were co-immobilized with 10  $\mu$ l of each phenol red indicator. 0.5 g of ammonium per sulphate and 50 ml TEMED were added to the above solution. After gentle stirring, the solution was poured into petriplate. After solidification the gel was cut into square blocks of 1.0 X 1.0 cm [22]. The arginine concentrations studied were in the range  $10^{-10} - 10^{-1}$  M and color change was noted.

# c) Agar Method:

The agar solution of 5% concentration was heated to liquify the agar. Gel was allowed to cool to  $45-50^{\circ}$ C and then quickly 20µl enzyme (arginase and urease), 10 µl of phenol red indicator was added. After gentle stirring, the solution was poured into the petriplate and allowed to solidify. 1.0 X 1.0 cm pieces of gel were cut. The pieces of gel were taken in different concentration of L-arginine ( $10^{-10} - 10^{-1}$  M) and the color change from partially orange to dark purple was noted down [23].

### d) Calcium alginate beads:

Sodium alginate (3%) was mixed with 20  $\mu$ l enzyme (10  $\mu$ l arginase and 10  $\mu$ l urease). 10  $\mu$ l phenol red indicator was added. This solution was then poured drop wise through a syringe into a beaker containing 0.075 M chilled CaCl<sub>2</sub> with gentle stirring [24]. After hardening for 5-6 minutes at room temperature, the beads were washed with distilled water for further use. The beads were put into varying concentrations of L-arginine (10<sup>-10</sup>-10<sup>-1</sup> M) and the response time was noted for change in color of beads from partially orange to dark purple.

## Application of the Developed Biosensor in juice samples and storage stability:

Calcium alginate beads, pieces of agar, polyacrylamide gel and gelatin were added into the different juice samples including pineapple juice, orange juice, almond juice and aloevera juice to study the presence of L-arginine. The samples were collected from different areas of Punjab. Response time for color change from partially orange to purple was noted. The detection range of L-arginine levels in all samples was elucidated by relating the response time for change in color of gelatin gel pieces, polyacrylamide gel, agar gel and calcium alginate beads with standard concentration levels from  $10^{-10} - 10^{-1}$  M of L-arginine. To know the storage stability of the biocomponent, gelatin gel pieces, polyacrylamide gel, agar gel and calcium alginate beads were wrapped in whattmann filter paper soaked in CaCl<sub>2</sub> and kept in refrigerator. The activities of the immobilized biocomponent were checked.

## **RESULTS AND DISCUSSION**

# a) Gelatin Method:

Visualization approach was followed for detection of color change in gelatin gel blocks. The figure 1 shows comparison of color of gel blocks before and after the reaction. Detection limit of arginine achieved was  $10^{-10} - 10^{-1}$  M. For concentration level of  $10^{-1}$  M L-arginine, response time detected was 18 seconds and for the concentration level of  $10^{-10}$ -10<sup>-2</sup> M L- arginine, response time detected was in the range of 7-17 seconds (Graph 1). Response time decreased with decrease in concentration of arginine indicating more of NH<sub>4</sub><sup>+</sup> ion produced after hydrolysis.



Graph 1: Performance of biosensor with L-arginine standards  $(10^{\text{-1}}\text{-}10^{\text{-10}}\,M)~$  using different matrix



# Figure 1: A. Comparison of color of gelatin blocks (Before and after the reaction) B. Fruit juice sample

### b) Polyacrylamide Method

The figure 2 shows the comparison of color of polyacrylamide gel pieces before and after the reaction. The same detection limit of  $10^{-10} - 10^{-1}$  M was achieved. For L-arginine concentration  $10^{-1}$  M, the response time detected was 17 seconds. For the concentration range  $10^{-10}-10^{-2}$  M L-arginine response time detected was in the range of 6-14 seconds (Graph 1). In this method also due to increased concentration of NH<sub>4</sub><sup>+</sup> ions produced after the reaction, the response time decreased with decrease in arginine concentration.



Figure 2: A. Comparison of color of Polyacrylamide blocks (Before and after the reaction) B. Fruit juice sample

# c).Agar Method:

Detection limit of L-arginine achieved was  $10^{-10} - 10^{-1}$  M. For concentration level of  $10^{-1}$  M arginine, response time detected was 15 seconds and for the concentration level of  $10^{-10}-10^{-2}$  M L-arginine response time detected was in the range of 6-14 (Graph 1). With decrease in concentration of arginine, the response time for color change also decreased. Response time for concentration level of  $10^{-1}$  M was approximately 15 seconds which was higher as compared to calcium alginate beads by 1 second. Color change was observed as shown in figure 3.



# Figure 3: A. Comparison of color of agar blocks (Before and after the reaction) B. Fruit juice sample

### d). Calcium Alginate beads:

Similar visual color change was observed for calcium alginate beads. The figure 4 shows comparison of color of beads before and after the reaction. Detection limit of L-arginine achieved was  $10^{-10} - 10^{-1}$  M. The response time for concentration level of  $10^{-1}$  M L-arginine was 14 seconds and for the concentration level of  $10^{-10}$ - $10^{-2}$  M L- arginine, response time detected was in the range of 5-13 seconds (Graph 1). The response time decreased with decrease in concentration indicating more of NH<sub>4</sub><sup>+</sup> ions produced.



# Figure 4: A. Comparison of color of Calcium alginate bead (Before and after the reaction) B. Fruit juice sample

### e) Application of the Developed Biosensor in juice samples and storage stability:

Various samples including pineapple juice, orange juice, almond juice and aloevera juice from different areas of Punjab were studied for the presence of L-arginine. All these samples were tested using the above listed immobilization methods. The concentrations of L- arginine, found in different fruit juices such as orange juice, pine apple juice, almond juice and aloevera juice was  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ , and  $10^{-5}$  respectively. Arginine level was found highest in orange juice ( $10^{-2}$ ) out of all the juice samples. The biocomponent was found to be active at 4  $^{0}$ C temperature. Biocomponent in immobilized gelatin gel pieces, agar gel and polyacrylamide gel were found to be stable for less than 1 month and calcium alginate beads for more than 2 months.

#### CONCLUSION

Biosensor was developed for the detection of arginine in commonly found juice samples. The developed biosensor was able to detect arginine levels from  $10^{-10}$ - $10^{-1}$  M. Calcium alginate bead method was found to be the most promising out of all the methods followed in terms of storage stability. The observable response time was 14 seconds in calcium alginate beads method which was lowest as compared to the other methods. In comparison with the developed arginine biosensor, arginase concentration of  $10^{-10}$  M was detected while the earlier biosensor could detect arginine level only upto  $10^{-5}$  M [15]. Thus, the developed plant based biosensor is novel, diagnostic, very rapid, easy to use, inexpensive and portable. The biosensor is capable of detection of very low levels of arginine.

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