Optimum Condition for Asparginase Activity in Crude Extract of *Capiscum annum*

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Abstract
In this study asparaginase was extracted from fruit part of *Capiscum annum* then Asparaginase activity was detected and optimised. Optimum conditions for the activity of crude asparaginase were studied. Results showed maximum activity of asparaginase was achieved 140 u/ml when the enzyme was incubated with 200 mM of asparagines at 35 °C for 30 minutes in the presence of 0.05 M of potassium phosphate buffer solution at pH 8.

Keywords: *Capiscum annum*, asparginase, enzyme activity

الظروف المثلى للفعالية الانزيمية للاسبارجينيز في المستخلص الخام لثمرة الفلفل الحار

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الخلاصة:
في هذا البحث تم استخلاص انزيم الاسبارجينيز من ثمره الفلفل الحار وعينت الفعاليه الانزيميه ثم تم دراسة الظروف المثلى للفعالية الانزيميه للاسباجينيز في الميستخلص الخام ،حيث تم الحصول على اعلى فعالية 140 وحدة /مل عند استخدام 200 ملي مولر اسبارجين كمادة اساس وبدرجة حرارة 35مؤية لمدة نصف ساعة باستخدام محلول داريء فوسيفات البوتاسيوم (0.05 مولر) برقم هيدروجيني 8.

Introduction

L-asparaginase (E.C.3.5.1.1) is the enzyme that catalyses the hydrolysis of the amide group of L-asparagine releasing L-aspartate and ammonia. The enzyme plays important roles both in the metabolism of all living organisms as well as in pharmacology [1]. The action of asparaginase plays a major role in the cellular nitrogen metabolism of both prokaryotes and eukaryotes [2]. The two largest and well characterized families of asparaginase include bacterial-type and plant-type [3] and it is broadly distributed among the plants, animals and microorganisms [4]. In *Erwinia carotovora* and *E.coli*,[5] reported that asparaginase have only been produced commercially as a drug in the treatment of acute lymphoblastic leukemia and their main side effects are pancreatitis, diabetes and coagulation abnormalities, while [6] noticed that the discovery of a new asparaginase serologically different but having a similar therapeutic effect is a highly desired; therefore, there is a continuing need to screen newer organisms in order to obtain strains capable of producing new potential source and high yield of asparaginase [7]. L-asparaginase is a therapeutically important protein used in combination with other drugs in the treatment of acute lymphocyte Leukemia (mainly in children, Hodgkin disease, acute myelomonocytic leukemia, chronic lymphocytic leukemia, lymphosarcoma treatment, reticlesarcoma

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and melanosarcoma [8,5]. In Food industry, it was used to determine and eliminate acrylamide from bread using gene technology by degrading asparagines, the precursor of acrylamide. Also, in biosensors it is used for monitoring asparagine levels in mammalian and hybridoma cells [9]. The aim of this study is to extraction of asparaginase enzyme from *Capiscum annum and* determine optimum conditions for asparaginaseactivity from this plant.

Materials and Methods

Extraction of asparaginase

Extraction of asparaginase from plant was made according to [10] by homogenizing 10 g of plant part with three volumes of 0.05M potassium phosphate buffer, pH 8.0 containing 1.5 M sodium chloride, 1 mM PMSF, 1 mM EDTA, and 10% (w/v) glycerol, then centrifuged at 10000 rpm for 20 minutes. Supernatant was regarded as crude enzyme.

Enzyme assay

Asparaginase was assayed according to Nesslerization method based on the conversion of L-asparagine to Ammonia and L-asparatate, as described by [11].

Protein concentration in plant extracts and enzyme concentrates was determined according [12].

Optimization of crude asparaginase activity

Effects of different factors on crude asparaginase activity were studied according to [13].

Effect of substrate concentration

The activity of crude asparaginase was determined by incubation it with different substrate concentrations (50, 100, 150, 200, and 250mM), then asparaginase activity was determined according to [14]. Optimum concentration was stated in the next experiments.

Effect of reaction time

Different incubation periods (15, 30, 45, 60, and 90 minutes) were done at 37°C, then enzyme activity was determined.

Effect of buffer pH

pH of the reaction mixture was adjusted to different values range (7.5, 8.0, 8.5 and 9). Then enzyme activity was determined and Optimum pH was stated in the next experiments.

Effect of Temperature

Optimal temperature for crude asparaginase activity was determined by incubation the reaction mixture at different temperatures (25, 30, 35 and 40°C). Then enzyme activity was determined and Optimum temperature for activity was stated in the next experiments.

Results

Optimum conditions for asparaginase activity

Optimum conditions for asparaginase activity were studied after extraction of the enzyme from chilli plant fruit. These conditions include the optimum substrate concentration, reaction time, pH of the reaction mixture, temperature of the reaction. Changing in any of these parameters may affect the enzyme activity [15].

Substrate concentration

Results presented in figure-1 showed that the activity of asparaginase was increased gradually with the increase in L-asparagine concentration. Maximum activity of asparaginase was obtained when the substrate concentration was 200mM, at this concentration,asparaginase activity was 1 U/ml. This concentration of L-asparagine was regarded as the optimum for asparaginasee activity, and was used in the next experiments of optimization. These results were agreed with [16] who found that there is a positive relationship between the enzyme activity and substrate concentration, hence the reaction was increased with the increase of substrate concentration when the asparaginase concentration was constant until the maximum rate was achieved (steady state), then the increase of substrate concentration doesn't affects the rate of reaction and doesn’t significantly affects the formation of the product [17].

At the steady state, there is no any enzyme molecules free to act on extra-substrate molecules. In addition, substrate inhibition will sometimes occur when excessive amounts of substrate are present in the reaction mixture [18]. According to results mentioned in figure-1 asparaginase produced by *Capsicum annuum*was reached the steady state when the asparagine concentration was 200mM.

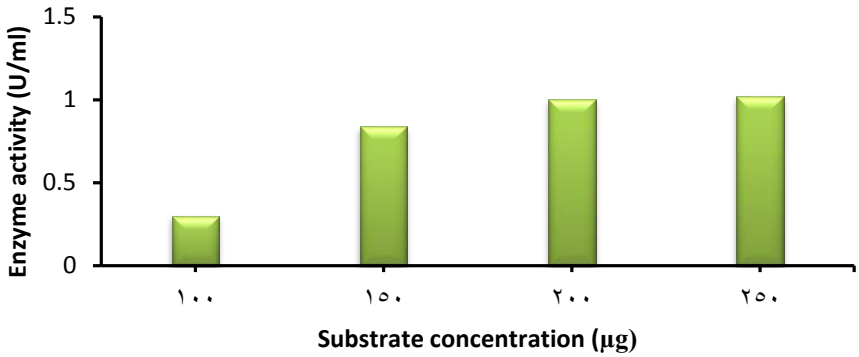

Figure 1- Effects of substrate (asparagine) concentration on the activity of asparaginase extracted from fruits of *Capsicum annuum* at 37°C for 30 minutes, pH=8.

Effect of Reaction time

Results indicated in figure-2 showed that the optimum reaction period was 30 minutes, the enzyme activity was 100 U/ml. According to these results, it has been concluded that 30 minutes of incubation was enough for asparaginase to bind substrate perfectly in reaction mixture reaching maximum enzyme activity. [19] reported that time-scale is an important factor in determining the enzyme activity, and it was preferred to use methods with short time incubation to determine the enzyme activity.

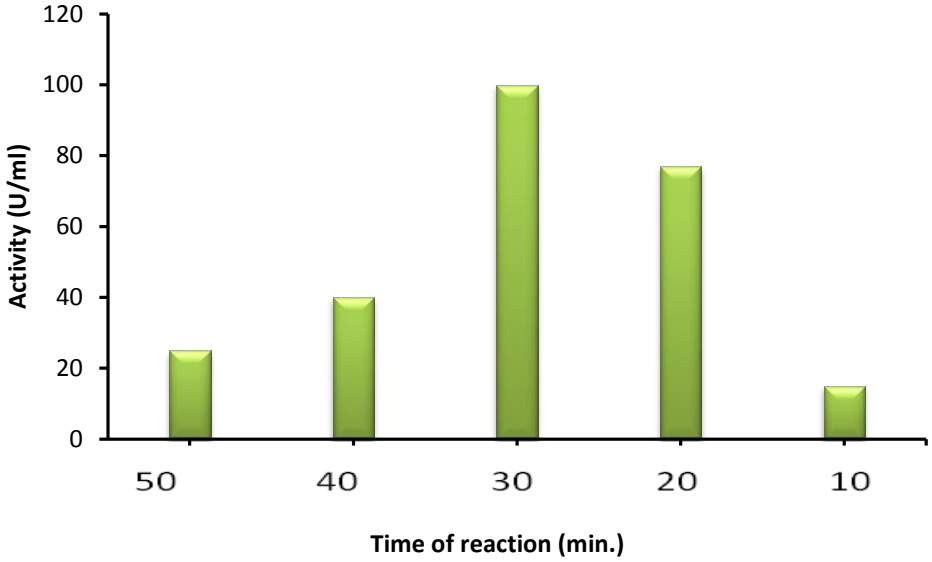

Figure 2- Effect of reaction time on asparaginase activity extracted from fruits of *Capsicum annuum***.**

Effect of buffer pH

Effect of pH on the activity of asparaginase activity produced by *Capsicum annuum* was studied. Result illustrated in figure-3 showed that maximum asparaginase activity was obtained when pH of the reaction mixture was adjusted to 8.0, at this value; the enzyme activity was 117 U/ml. It has been reported that most plant asparaginases have its maximum activity in alkaline pH, and was determined in pH range of 7.5, 8.0 and 8.5 [4]. In general most plants show maximum enzyme activity at or near neutral pH [20].It was reported that asparaginase produced from *E.coli*have its maximum activity in alkaline pH that probably due to the balance between L-aspartic acid and L-aspartate. L-aspartic acid in acidic pH has greater affinity to the active site of the enzyme.Any decrease or increase in hydrogen ions (H+) concentration causes pH changes in the reaction mixture which may lead to drastic changes in three-dimensional structure of protein, resulting in the enzyme denaturation [21]. On the other hand, the effect of pH on enzyme activity resulted from its effect on the ionization state of the substrate [22].

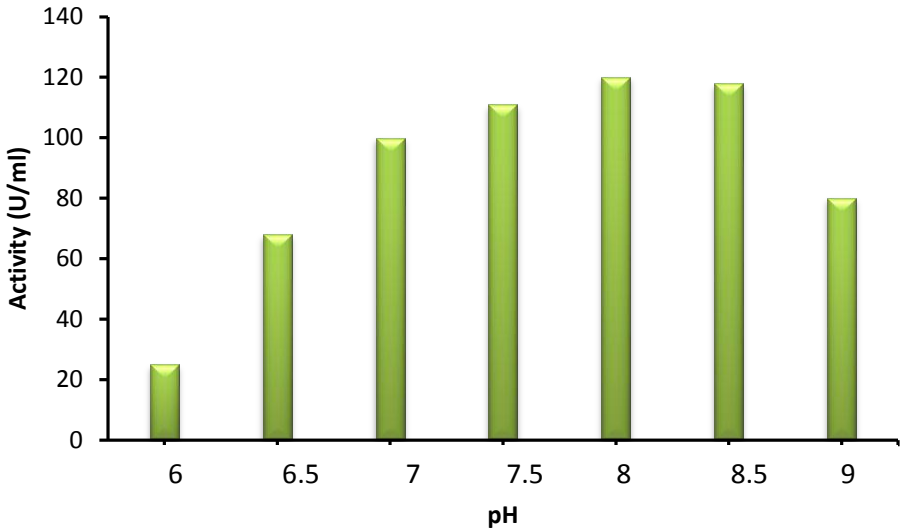

Figure 3- Effect of buffer pH on the activity of asparaginase extracted from fruits of *Capsicum annuum*.

Under such conditions, it becomes a competitive inhibitor. In alkaline pH, the balance is shifted toward the asparatate, which is less affinity to the active site enabling; in this case, there is favorable balance for the connection with the substrate L-asparagine [23,24]. [25] reported that the optimum pH of polyphenol oxidase from *Umbu-caja*(*Spondia* spp.) was 7.0 while [13] found that pH 8.0 was the optimum for polyphenol oxidase from *Irvingia gabonnensis*.

Effect of temperature

In order to determine the optimum reaction temperature for the activity of asparaginase extracted from *Capiscum annum*, different temperatures (25, 30, 35, and 40°C) were used for this purpose. It was reported that this range of temperature was the most suitable for enzyme activity [19].

Results illustrated in figure-4 showed that the maximum activity of asparaginase was obtained when the temperature of the reaction mixture was 35°C. At this temperature, enzyme activity was increased to 140 U/ml. In general, it was found that asparaginase from most organisms have its maximum activity at 37°C [26] While the increase or decrease in the incubation temperature above or below the optimum temperature cause a decrease in enzyme activity.

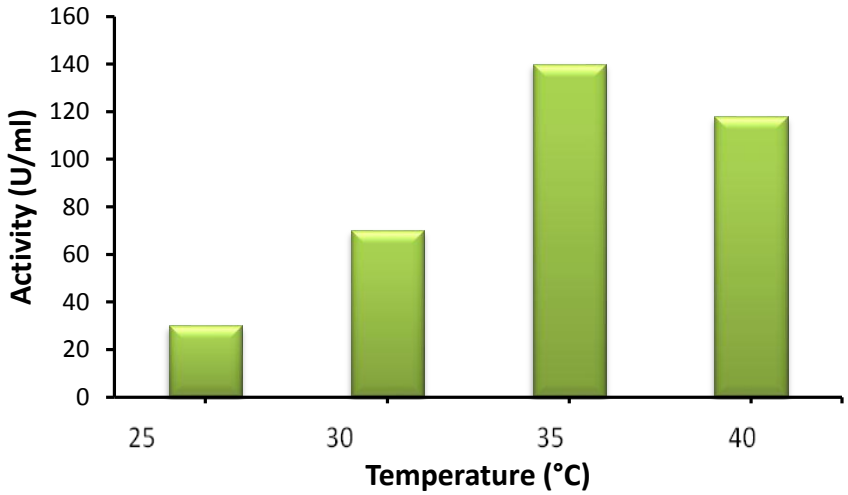

Figure 4-Effect of temperature on the activity of asparaginase extracted from fruits of Capsicum *annuum*.

These variations in the activity of asparaginase proved that the suitable temperature for asparaginase reaction to have maximum activity was 37°C, while the activity was decreased when the temperature was above optimum or below the temperature.

[27] found that the temperature at which polyphenol oxidase from *Thymus logicaulis* showed highest activity was in range of 25-30°C and then decreased at temperature above 40°C. While peroxidase was highly active at 40°C and cost its activity at higher temperature [28,29].The effect of incubation time on the crude extract of chitosanase activity from pepper leaves and Opuntia peels were studied up to 180 min. It was indicated that chitosanase activity from pepper leaves increased as the reaction time increased up to 180 minutes at 40°C. There is a linear relationship between chitosanase activity and reaction time. In the same manner chitoasanase from opntia peels increased with increasing the reaction time up to 120 minutes at 40°C [30].

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