The Effect of Aspartame on L-asparaginase Activity using *Streptomyces* species

Dimowo C. G.* and Osazuwa N. I.
Department of Microbiology, University of Benin, Benin City, Edo State, Nigeria
* Corresponding author: courage.dimowo@uniben.edu

Abstract: L-asparaginase is the first therapeutic enzyme with antineoplastic properties that has been studied broadly by researchers and scientists far and wide. Aspartame is a methyl ester of aspartic acid and phenylalanine dipeptides. The aim of this research was to ascertain the effect of aspartame on L-asparaginase (enzyme) activities from marine water Isolate. Soil and water samples from lagoon and ocean were collected for the isolation of *Streptomyces* spp. Isolation was carried out by serial dilution and pour plate method. Pure culture of actinomycetes were obtained by using the streak plate method, Identification of isolated marine actinomycetes was carried out according to Bergy's Manual of Determinative Bacteriology. Production of L-asparaginase was carried out in Erlenmeyer flask containing Czapek Dox broth supplemented with L-asparaginase (1g/L), with one used as the control and the other with 0.5 g aspartame in it, as production medium for 7 days at 120 rpm and at 28 ^{0}C. Optical density (growth rate) were determined at 600 nm using the spectrophotometer. Aspartame had highest optical density of 0.3826 ± 0.0015 on day 7 of production and the least optical density of 0.094 ± 0.001 on initial day. L-asparaginase activity with aspartame was 25 μmol/ml/min on day 7 and the control had activity value of 66.6666 ± 0.0524 **.** Conclusively, this study explains that aspartame did not support the production of L-asparaginase.

Key word: Aspartame, L- asparaginase, spectrometrically, production, supplemented

INTRODUCTION

L-asparaginase is the first therapeutic enzyme with antineoplastic properties that has been studied broadly by researchers and scientists globally. Kidd 1953 did a progression of experiments to prove the ability of guinea pig serum as tumor inhibitor. He carried out diverse sets of analysis where he concocted the findings that two sorts of subcutaneously executed lymphosarcoma in mice failed to proliferate once animals were given injections of guinea pig serum, while untreated controlled mice died because of carcinomas. In the second set of experiments, two different types of lymphomas namely mammary carcinoma and fibrosarcoma of mice failed to degenerate when treated with guinea pig serum.

In 1961, yet another breakthrough was achieved when Broome (Broome, 1961) demonstrated L-asparaginase as an antitumor agent in guinea pig serum with substrate specificity. Later on, some other substrate specific L-asparaginases were found to inhibit tumors (Capizzi *et al.*, 1969). McCoy through his *in vitro* experiments proved the centrality of amino acid asparagine for Walker carcinoma 256. Altenbern in 1954 and Broome in 1961 reported the antitumor activity of L-asparaginase in bacteria and yeast, respectively. The L-asparaginase is found in the serum of guinea pig and rodents, but is absent in humans. Two isozymes of L-asparaginase, namely type I and type II, have been identified by Ohnuma (1967). Both type I and type II asparaginases are characterized by enzymatic activity for both L-asparagine and L-glutamine. However, type II asparaginase displays higher specific action against L-asparagine. The type II asparaginase precisely shows antitumor activity and it is utilized as chemotherapeutics in acute Lymphoblastic leukemia (ALL) (Dimowo *et al.*, 2021)

Artificial sweetener aspartame is entirely digested and absorbed as aspartate, phenylalanine, and methanol in the gut. Aspartic acid is tasteless to mildly sour, whereas phenylalanine is bitter, therefore it was impossible to estimate how sweet aspartame would be based on the tastes of its constituent amino acids. About 40 % of the weight of aspartame is aspartic acid.

The L-aspartic acid and L-phenylalanine are the two amino acids that make up aspartame. It is a white, crystalline, odorless, and intensely sweet powder with the chemical formula $C_{14}H_{18}N_2O_5$ and a molar mass of

294.31 g mol^{-1}. Aspartame has a high melting point between 246-247 °C and a density of 1.347 g/cm_3. It is hydrolyzed in highly acidic and alkaline environments. The relationship between aspartame stability in aqueous solution and pH follows a bell-shaped curve, with maximal stability at pH 4.3. As the pH and temperature fluctuate, aspartame's solubility also does. Aspartame is partially soluble in alcohol in addition to water. In acidic liquids at higher temperatures, aspartame is more soluble (Lang and Uber, 1904) Aspartame dissolves in water with an acidic pH of 3 at a rate of roughly 0.03 g/ml. Since aspartame is a dipeptide, it has several restrictions. It should not be used for baking, cooking, or long-term liquid storage because heating is said to cause it to lose its integrity. Aspartame has a calorific value that is roughly 180–200 times lower than sucrose, aspartame is a synthetic, dipeptide, intense sweetener. In this study, the effect of aspartame in L-asparaginase activity using *Streptomyces* species isolated from marine soil and water was investigated.

MATERIALS AND METHODS

***Isolation of marine actinomycetes for asparaginase production*:** Isolation was carried out by serial dilution and pour plate method. Serial dilution was carried out by taking 25 ml of each sample and mixed with 225 ml of sterile distilled water. The mixture was agitated for 1 h at 1000 rpm using a water bath to enable the dislodge of microorganisms. The suspension was serially diluted by transferring 1ml aliquots to a series of test tubes, each containing 9 ml of sterile distilled water and from respective dilutions, 1 ml sample was transferred to a sterile labeled Petri plate and the molten agar was poured onto it and it was allowed to solidify. The plates were inverted and incubated at 37°C for 3 to 4 days (Dimowo *et al*., 2021).

***Purification of marine actinomycetes/ isolation of pure culture*:** Pure culture of actinomycetes were obtained by using the streak plate method, here a sterile straight wire was used to pick the isolates and inoculated on a fresh Petri dish containing starch casein agar. The pure cultures were streaked carefully in a zigzag pattern. The cultures were further incubated for 3 to 4 days at 37°C (Venil *et al.*, 2009; Asikhia and Dimowo, 2023).

***Production of L- asparaginase*:** Production of L-asparaginase was carried out in Erlenmeyer flask containing Czapek Dox broth supplemented with L-asparagine (1g/L), with one used as the control. The other flask with 0.5 g aspartame in it, as production medium for 7 days at 120 rpm and at 28℃. A 5 % inocullum was used. Enzyme activity was determined at intervals by withdrawing 5 ml of sample. The cell free supernatant was used for estimating the extracellular enzyme activity (Asikhia and Dimowo, 2023).

***Determination of optical density**:* Using sterile pippete, 5 ml of the Samples were withdrawn from the production medium aseptically at different intervals and optical density was determined at 600 nm using the spectrophotometer (LabTech^{TM}) (Dimowo *et al.*, 2021).

***Determination of L-asparaginase activity*:** Enzyme assay was carried out at intervals. 5 ml of the production medium was withdrawn using a sterile pipette and carefully dispensed into a bottle. The sample was then centrifuged at 120,000 rev/min. The supernatant was used for the assay. 0.5 ml enzyme solution was added to 0.9 ml buffer (pH 8.5) and the amino acid L-asparagine. It was incubated at 37℃ for 10 mins, thereafter, 0.5 ml of TCA was added to stop the reaction and precipitate the different protein molecule. The sample was then centrifuged thereafter, 50 µl of supernatant was collected and placed in a clean tube. 2000 µl of distilled water was added to the supernatant with 250 µl of Nessler's Reagent and 250 µl NaOH solutions were added and the solution was left for 15 mins. Using the spectrophotometer (LabTech ^{TM}) the result was read at 600 nm. Ammonia standard curve was prepared. L-asparaginase

Activity was calculated with the formula (Dimowo *et al.*, 2021).

RESULTS

Table 1, explains the optical density of *Streptomyces* species in production of L-asparaginase, using aspartame. The control had an optical density value of 0.1013 ± 0.0005 on initial day and it increased to 0.9324 ± 0.0005 on day 3 and decreased to 0.0380 ± 0.0010 0n day 7. The medium containing aspartame increased from 0.094 ± 0.0010 on initial day to 0.3826 ± 0.0015 on day 5.

Table 2, depicts the effect of aspartame in L-asparaginase activity using *Streptomyces* species. The control medium had the highest enzyme activity on day 5 (71.7272 ± 0.0909 µmol/ min/ml) and the least activity on day 0 (6.0909 ± 0.0909). The medium containing aspartame had the highest L-asparaginase activity of 25.0909 ± 0.0909 µmol/min/ml on day 7 and the least on day 0.

Table 1: Optical density of *Streptomyces* species in L-asparaginase production

Sample	0 (Mean ± SD)	3 (Mean ± SD)	5 (Mean ± SD)	7 (Mean ± SD)
Control	0.1013±0.0005	0.9243±0.0005	0.8563±0.038	0.038±0.001
Aspartame	0.094± 0.001	0.1023± 0.0015	0.3826±0.001	0.019± 0.001

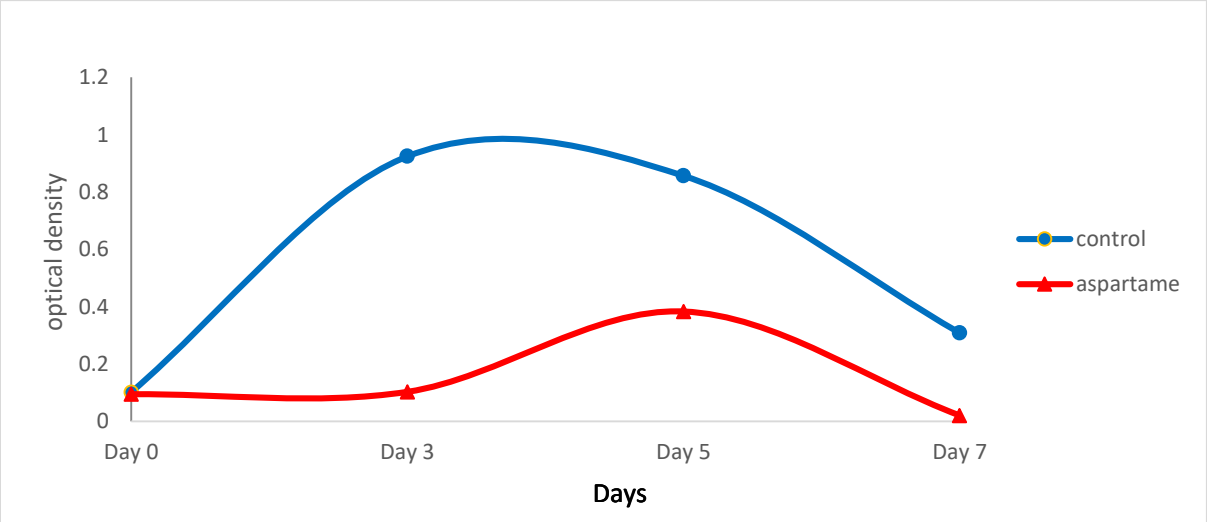

Figure 1: Optical density of *Streptomyces* species grown in CZAPEK Dox broth supplemented with Aspartame. Values are Mean ± SD

Table 2: L-asparaginase activity (µmol/min/ml) of *Streptomyces* species supplemented with Aspartame

	Days			
	0	3	5	7
Sample	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
Control	6.0909± 0.0909	47.7545± 0.0524	71.7272 ± 0.0909	66.6666± 0.0524
Aspartame	6.3636±0.0909	14.7272 ± 0.0525	19.1818 ± 0.0909	25.0909 ± 0.0909

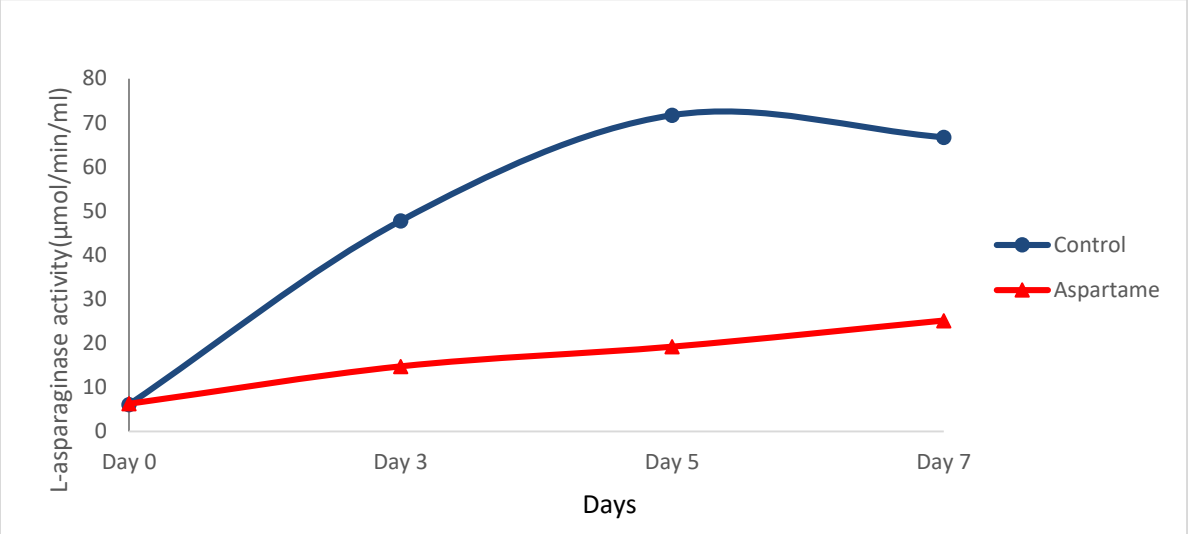

Figure 2: L-asparaginase activity of *Streptomyces* species grown in CZAPEK Dox broth with aspartame. Values are Mean ± SD

DISCUSSION

In this study, aspartame was used to modify CZAPEK Dox broth to determine their impact on L-asparaginase production. Compared with aspartame, L-asparaginase activity was higher in the control. In this study, the growth curve was deduced from the result obtained from the optical density. In the course of production of this enzyme, the growth curve followed a normal growth pattern, with lag phase, exponential phase, stationary phase and death. Level of lag phase seen in aspartame showed that the organism adjusted to the medium and environmental factor. Comparing the different growth levels, day 3 for control had the highest growth level while aspartame had its highest on day 5 (0.382667± 0.001528) where it reached stationary phase and decline on day 7. Both growth rate and death rate are equal in stationary phase, the stationary phase is the time when cells stop growing but are still metabolically active (Dimowo *et al.*, 2021). The specialized proteins produced during the stationary phase are crucial because they ensure the survival of the organisms. The optical density on day 5 had the highest value of 0.3826 ± 0.0015, considering the optical density the aspartame source did not show appreciable growth rate compared to the control.

According to Ahmad *et al.* (2019), during the fermentation process of L-asparagine substrate content, the highest optical density of *Klebsiella* sp. cultures (OD 646 nm = 1.47) was reported at a fermentation time of 24 hrs. As the pH and temperature fluctuate, aspartame's solubility also does. There was a steady, but slow increase in the enzyme activity of aspartame with its peak on day 7 (25.0909 ± 0.0909), while the control which had an exponential increase where it got to its peak on day 5 from there it had a slow and steady decline. This means that aspartame is a source that profers low production of L-asparaginase. Several reports suggest that glucose serves as a best carbon source for l-asparaginase production and a similar effect was observed for L-asparaginase production using *Aspergillus* and *Fusarium* strains (Hosamani and Kaliwal 2011; Baskar and Renganathan 2012).

Prakasham *et al.* (2007) reported that glucose was the best carbon source under

aerobic conditions for the synthesis of L-asparaginase by *Staphylococcus* spp. Venil *et al* (2009) has reported decrease in L-asparaginase production when supplemented with fructose. However, Kenari *et al* (2011) reported that lactose was the best carbon source for L-asparaginase production. This work was aimed at comparing aspartame for the activity of L-asparaginase. Production of L-asparaginase began after 24 hrs of inoculation and reached to maximum levels after 96 hrs of incubation. Maximum biomass production obtained with 96 hrs old culture could be correlated with high levels of L-asparaginase production. L-asparaginase formation has been shown a firm link to the active cell growth. Maximum activity was observed on the seventh day of incubation from the day of inoculation of the culture for the sample containing aspartame.

The nitrogen source is consider the secondary energy source after carbon sources and play a vital role in the growth of organisms and enzyme production. In microorganisms, amino acids, nucleic acids, proteins, and cell wall components are metabolized by nitrogen (both organic and inorganic forms). Comparative analysis was carried out by inoculating the culture in the media in order to obtain increased growth and production of the enzyme. Results have shown that higher enzyme activity was observed in L- asparaginase composition as compared to aspartame. The L-asparaginase production was induced by the addition of L-asparagine. The synthesis of L-asparaginase over time is a crucial factor in determining its potency. The highest peak activity of the enzyme production (25.0909 µmol/min/ml) was attained after 7 days of fermentation. The culture supernatant showed that the enzyme activity started with an initial productivity of 6.3636 µmol/min/ml on the day zero (0) and ended with a productivity of (25 µmol/min/ml) on day 7.

CONCLUSION

This study showed that marine bacteria may have a high yield and excellent substrate specificity to L-asparaginase. It also showed that the source aspartame did not favour the production of L-asparaginase.

REFERENCES

Ahmad, Z., Hussain, R., Riaz M. and Ghaffar, A. (2019). Mitigation of toxic effects caused by tartrazine in wistar rats through oral administration of melon seed oil. *Pakistan Journal of Agricultural Science,* 56(2):435 -442.

Altenbern, R. A. and Housewright, R. D. (1954). Stereospecific asparaginases in smooth *Brucella abortus* strain 19. *Archives of Biochemistry and Biophysics,* 49: 130–137.

Asikhia, C. B. and Dimowo C.G. (2023). Comparative studies on sucrose and glucose sugars on L-asparaginase production, using *Streptomyces* species. *FUW Trends in Science and Technology Journal,* 8 (3): 19 -22.

Baskar, G. and Renganathan, S. (2012). Optimization of l-asparaginase production by *Aspergillus terreus* using response surface methodology and artificial neural network-linked genetic algorithm. *Asia-Pacific Journal* of *Chemical Engineering*, **7**(2):212–220.

Broome, J. D. 1961. Evidence that the L-asparaginase activity of guinea pig serum is responsible for its anti-lymphoma effects. *Nature,* 191: 1114-1115.

Capizzi, R. L, Peterson, R., Cooney, D. A., Creasey, W. A. and Handschu, R. E. (1969). L-asparaginase therapy of acute leukemia-biochemical and clinical observations. *Proceedings of the American Association* for *Cancer Research,* 10:12-15.

Capizzi, R. L, Peterson, R., Cooney, D. A., Creasey, W. A. and Handschu, R. E. (1969). L-asparaginase therapy of acute

leukemia-biochemical and clinical observations. *Proceedings of the American Association* for *Cancer Research*, 10:12-15.

Dimowo, C. G., Omonigho, S. E., Oshoma, C. E. and Oyedoh, O. P. (2021). Evaluating the concept of reducing sugar (Glucose) and growth in l-asparaginase production using some selected marine actinomycetes. *African Scientist*, 22: 106-110.

Hosamani, R. and Kaliwal, B. (2011). L-Asparaginase an anti-tumor agent production by *Fusarium equiseti* using solid state fermentation. *International Journal of Drug Discovery*, 3:88–99.

Kenari, S., Alemzadeh, I. and Maghsodi, V. (2011). Production of L-asparaginase from *Escherichia coli* ATCC 11303: Optimization by response surface methodology. *Food* and *Bioproducts Processing*, 89: 315-321.

Kidd, J. G. (1953). Regression of transplanted lymphomas induced in vivo by means of normal guinea pig serum. I. course of transplanted cancers of various kinds in mice and rats given guinea pig serum, horse serum or rabbit serum. *Journal of Experimental Medicine*, 98: 565–582.

Lang, S. and Uber. (1904). Deamination in the animal body. *Contributions to Chemical Physiology*, 190: 321-345.

Ohnuma, T., Bergel, F. and Bray, R. C. (1967). Enzymes in cancer asparaginase from chicken liver. *Biochemical Journal*, 103: 238–245.

Prakasham, R. S., Rao, C., Lakshmi, G. S. and Sarma, P.N. (2007). L-asparaginase production by isolated *Staphylococcus* sp. -6A: Design of experiment considering interaction effect for process parameter optimization. *Journal* of *Applied Microbiology*, 102: 1382-1391.

Venil, C. K., Nanthakumar, K., Karthikeyan, K. and Lakshmana, P. (2009). Production of L-asparaginase by *Serratia marcescens* SB08: Optimization by response surface methodology. *Iranian Journal of Biotechnology*, 7: 10-18.