Original Research Article

EXTRACTION AND PARTIAL PURIFICATION OF THAUMATIN FROM Arils of *Thaumatococcus daniellii* FRUIT

ABSTRACT: *Thaumatococcus daniellii* is a large flowering herb with rhizomatous rootstock, commonly found mostly in the western part of Nigeria. *Thaumatococcus daniellii* fruit arils that contain the sweetener, are embedded within a gel-like fluid that is richly composed of polysaccharide. The extraction and partial purification of thaumatin (protein) was undertaken to devise simpler means to extract and purify the protein, following the difficulty associated with the extraction due to the presence of the gel that encapsulates the protein. . The research was carried out in department of biochemistry laboratory, covenant university Ota, Nigeria, between 10 August 2015 and 13 November 2018. In attempt to determine the optimal pH and temperature for extraction of crude thaumatin in different media from 4,000mg of the sample. Extraction was carried out at different pH values (2, 3, 4 and 5) in varied temperatures (25^{o}C, 30^{o}C, 40^{o}C, 45^{o}C, 50^{o}C, 55^{o}C, 60^{o}C, 70^{o}C, 80^{o}C and 90^{o}C). Maximum concentration of crude thaumatin extracted in 50ml of distilled water was 13.40mg/ml at pH 3 and temperature of 50^{o}C while 28.17mg/ml at pH 3 and temperature of 55^{o}C was extracted in 50ml of 0.85M of aqueous sodium chloride (dilute sodium chloride). Each extracted crude thaumatin was subjected to centrifugation at 10,000rpm for 1800secs, the decanted extracted crude thaumatin (supernatant) formed were assayed for protein via lowry method. Having pooled each decanted supernatant, the extracted crude thaumatin were precipitated with ammonium sulphate, dialyzed and subjected to column chromatography while the eluent were collected, tested for protein then free dried and stored as partially purified thaumatin. From the above findings, the extraction and partial purification of thaumatin can be optimized in dilute sodium chloride at optimal pH and temperature thereby making protein readily available. Thaumatin a protein with very low calorie is an excellent alternative to other sweetener with high calories especially sucrose, consequently will go a long way to reducing health challenges associated with sucrose such as obesity, hyperglycaemia, dental cares and even diabetes.

Key Word: rhizomatous rootstock, pectinase, supernatant, viscous medium, hyperglycaemia

1. Introduction

Taste sensation is produced when a substance in the mouth interact chemically with taste receptor cells on taste buds in the oral cavity. The gustatory system also known as the sense of taste is the sensory system that is partially responsible for the perception of taste[1]. (Trivedi and Bijal, 2012). Therefore it is a well-known fact that the major determinant of appetite is sense of taste. Our appetite for food to a large extent determines how much food (nutrients) we consume at any given time; which directly or indirectly determines our growth rate, the amount of energy we can acquire to undertake our daily work and ultimately how

healthy we are. It then becomes very obvious, that the palatability of foods and its ability to excite our sense of taste is of great importance in the sustenance of life. Sugars and sugar alcohols exhibit sweetness potency around and below 1 relative to sucrose, and are considered as bulk sweeteners. However, sulphonamides, peptides, and proteins that activate the sweet receptor show over 10 times higher sweetness in comparison to sucrose, being termed intense sweeteners[2,3] (Barclay, *et al*., 2014; *Lindley,* 2012), with an incredibly reduced calorific value. Sugar assumes a significant role in the human eating regimen being widely appropriated in nature and covering a varied range of all total nutrient intake, at least in the developed nations.

Thaumatin, a very low calorie intensely sweet-tasty protein[4] (Raimi *et al*., 2011), obtained from *Thaumatococcus daniellii (T. daniellii)* fruits, within the fruits it is covered by layer of sticky, transparent gel that has a soft, fleshy and juicy cap called an aril which contains three sweet substances (Thaumatin). These arils compose of a gel mainly of polysaccharide origin. These jellies make it very difficult to extract these thaumatin resulting to a low yield during extraction, therefore more scientific effort is needed to increase yield during extraction to make the sweetener readily available as replacement for sugar. The thaumatin are first sweet– tasting proteins that have been found in nature and the crystals are about 2,000-3,000 times sweeter than sucrose and neither allergic nor mutagenic or teratogenic[5] (Yeborah *et al*., 2003). The arils are attached to the seeds which produce the jelly that swells to many folds its weight and houses the thaumatin. The arils which contain the Thaumatin, constitute 4.8% of the fruit while the fleshy part and the seed account for 72.4% and 22.8%, respectively[4] (Raimi *et al*., 2011). It is a natural protein with exciting features, having single 207 amino acids with eight intramolecular disulfide binds, isoelectric points of 11.5–12.5 which is very high unlike other protein and contains no free cysteine residues[6] (Kant, 2005).

The prevalence of diabetes are largely determined by individuals living with type 2 diabetes mellitus (T2DM), which comprises about 90% of the total number of people living with diabetes. These numbers are characterised by various degrees of relative insulin deficiency

in conjunction with a wide range of insulin resistance. Following the high incidence of sugar related diseases associated with the consumption of sucrose, it has turned into the principal driver for the advancement of sugar alternatives that have the improving properties of sucrose yet with little or no calorie. Therefore the urgent need, to replace part if not total while sustaining the same sensation that is derived from sugar, displayed ab initio from requirement of sucrose reduction particularly in the diets as a way of reducing sugar in the blood and in the management of conditions associated with high blood sugar especially in diabetics. Therefore Thaumatin a protein with very low calorie is an excellent alternative to sweeteners with high calories especially sucrose, and will go a long way to reducing health challenges associated with sucrose such as obesity, hyperglycaemia, dental cares and even diabetes

2. Materials and Methods

2.1 Collection of fruits

The fleshy ripe *Thaumatococcus daniellii* fruits were harvested from *Thaumatococcus daniellii* plant from Otun village bush, Ayetoro local forest in Moba Local Government Area of Ekiti State, South-western Nigeria. The fruits were between the range of 1.4 – 2.1cm long, trigonal or pyramidal in shape, deep red and bright red colour as ripe fruits. The weight of fruits was within the range of 10 to 22g which depend**s** on the number of seeds inside it. The *Thaumatococcus daniellii* Benth fruits were identified by Forest Herbarium, Ibadan 110158. The fruits were transported to Covenant University, Ota Ogun State, where the fruits were thoroughly washed was with tap water followed by distilled water in order to remove any dirt or filth particles present on the surface, physically examined, then transported to biochemistry laboratory and stored at temperature of -18^0C. Four hundred and twenty-two (422) of the fleshy fruits were selected and weighed; the total weight amounted to 6752g (6.752kg). Each of the fruits was peeled, frozen and freeze dried with freeze drier until the arils became brittle. By hitting the freeze dried fruits with hand repeatedly the brittle aril became separated from seeds and the rest of the fruits. Then the brittle aril was ground

using a blender into powdered form. A total weight of 400g (400,000mg) of powdered arils was obtained and stored in an air tight bottle as sample. Forty portions each of 4,000mg of the dried powdered sample were weighed, collected and stored for extraction of crude thaumatin.

2.2 Extraction of Crude Thaumatin in Distilled Water at different pH values in Varied Temperatures

Four portions of 4,000mg of the dried powdered sample were weighed, collected and stored separately. Distilled water was also prepared and stored in two of 1000ml conical flask each. Exactly 4,000mg each of the samples was dissolved in 50ml of distilled water, after thorough shaking for 120secs at pH value of 2, 3, 4 and 5 each; 5mls each from the extracted thaumatin were pipetted into 10 empty test tubes. A total of forty (40) test tubes were collected and each was subjected to different temperature of 25^{o}C, 30^{o}C, 40^{o}C, 45^{o}C, 50^{o}C, 55^{o}C, 60^{o}C, 70^{o}C, 80^{o}C and 90^{o}C with the aid of a water-bath adjuster for 1200secs and thaumatin precipitated. Then at the end, 4ml of the raw crude thaumatin each was pipetted into 4ml micro tubes and centrifuged at 10,000rpm for 1,800secs and later tested orally. After the centrifugation (Uniscope Laboratory Centrifuge Surgifriend Medicals, England) each of the supernatant formed was decanted and stored, while little of it was tested orally the remaining portion each was for assayed for protein.

2.2 Extraction of Crude Thaumatin in Dilute sodium chloride at different pH values in Varied Temperatures

After the preparation of exactly 0.85M of aqueous sodium chloride, it was stored in two of 1000ml of conical flask. Four portions of 4,000mg of the sample were dissolved in 50ml of 0.85M of aqueous sodium chloride, after thorough shaking for 120seconds at pH value of at different adjusted pH value of 2, 3, 4 and 5 each; 5mls each from the extracted thaumatin were pipetted into 10 empty test tubes. A total of forty (40) test tubes were collected and each was subjected to different temperature of 25^{o}C, 30^{o}C, 40^{o}C, 45^{o}C, 50^{o}C, 55^{o}C, 60^{o}C, 70^{o}C, 80^{o}C and 90^{o}C with the aid of a water-bath adjuster for 1200seconds and thaumatin precipitated. Then at the end, 4ml of extracted crude thaumatin each was pipetted

into 4ml micro tubes and centrifuged (GEN105 UV-VIs) at 10,000rpm for 1800secs. After the centrifugation each supernatant formed was decanted and assayed for protein via Lowry method, while little of it was tested orally.

2.3 Purification of crude

The various supernatant (crude thaumatin) ~~form~~ from the various extractions of crude thaumatin were pooled together in two 1000ml beakers and freeze dried with the aid of a freeze-drier to obtain dried crude thaumatin for partial purification of the protein. The dried crude was dissolved with ammonium sulphate. The protein was transferred into a dialysis bag and both ends of the bag were tied then placed into a 1000ml beaker filled with water with a stirrer placed inside it and finally mounted on a magnetic stirrer. It was stirred for 4 hours, however the water was removed and replace in every 60minutes. Then a dissolved sephadex G-25 was poured into a column with glass wool inside it, while the column was suspended with resort stand. An Agary infusion set filled water and brought close to the column, while allowing the set to continuously dripping water into the column to avoid the gel to cake. Finally the partially purified crude thaumatin is poured into the column while the eluent were collected with sixty 10ml test tubes.

3. RESULT AND DISCUSSION

3.1 Crude Thaumatin Concentrations Extracted in Distilled Water at different pH Values in varied Temperatures

The concentrations of thaumatin in distilled water at varied pH (range; 2.0-5.0) in varied temperature (range; ambient temperature to 90^{o}C) were determined by means of standard curve, as shown in figure 4.7. The calculated values for protein in distilled water are shown in table 4.1, the values varied randomly, between 1.74mg/ml at ambient temperature and 13.40mg/ml concentration of thaumatin at 50^{o}C.

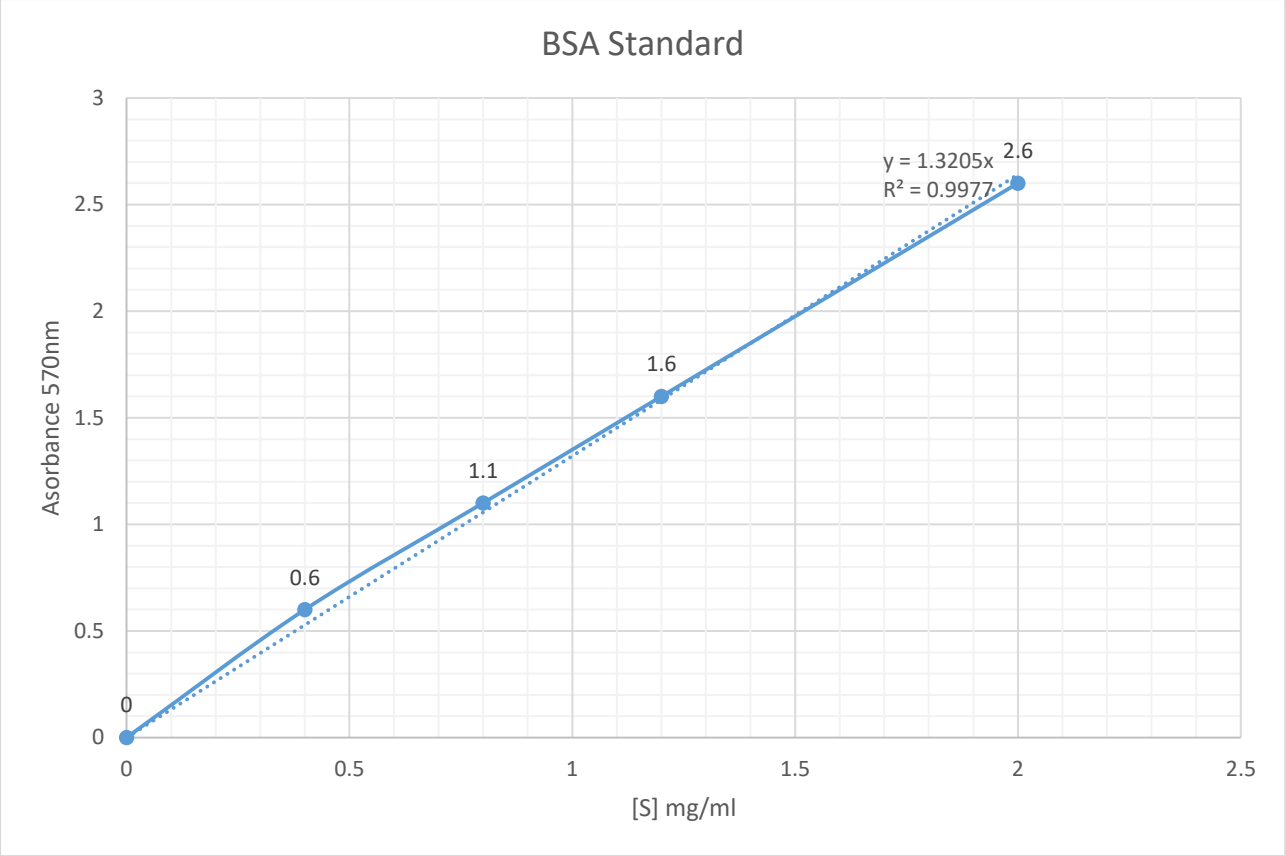

Figure. 1. Lowry Standard Plots for Protein Determination in Distilled Water

Table 1. Crude Thaumatin Concentration Extracted in Distilled Water at pH Values in Varied Temperature

Temp (0^oC)	pH 2.0		pH 3.0		pH 4.0		pH 5.0	
	Y (nm)	X (mg/ml)	Y (nm)	X (mg/ml)	Y (nm)	X (mg/ml)	Y (nm)	X (mg/ml)
25	4.3	3.26	5.0	3.80	2.8	2.12	2.30	1.74
30	5.6	4.24	8.5	6.40	4.4	3.33	3.50	2.65

40	12.8	9.70	14.3	10.80	8.1	6.13	7.30	5.53
45	14.4	11.00	16.4	12.40	9.0	6.80	7.70	5.83
50	16.0	12.10	17.7	13.40	14.7	11.13	12.7	9.61
55	14.3	10.83	15.8	12.00	11.3	8.55	10.4	7.90
60	13.7	10.40	14.7	11.00	10.2	7.72	9.80	7.42
70	12.2	9.20	13.2	10.00	8.7	6.60	7.60	5.75
80	11.8	9.00	12.0	98.00 ?	7.4	5.60	6.80	5.20
90	10.3	7.80	11.7	8.86	7.6	5.76	6.70	5.10

Y (Absorbance) =1.3205X
X= Actual conc.

3.2 Crude Thaumatin Concentrations Extracted in Dilute Sodium Chloride at different pH Values in Varied Temperatures

The concentrations of thaumatin extracted in dilute sodium chloride from the samples at different pH (range; 2.0-5.0) in varied temperature (range; 25 to 90^{o}C) were determined by means of standard as shown in figure 1.0. The calculated values for protein in distilled water are shown in table 2.0, the values varied randomly, between 4.50mg/ml at 25^{o}C and 28.17mg/ml concentration of thaumatin at 55^{o}C. From the result at pH 2, the concentration of thaumatin extracted range from 6.90mg/ml at 25^{o}C to a maximal value of 25mg/ml at 55^{o}C.

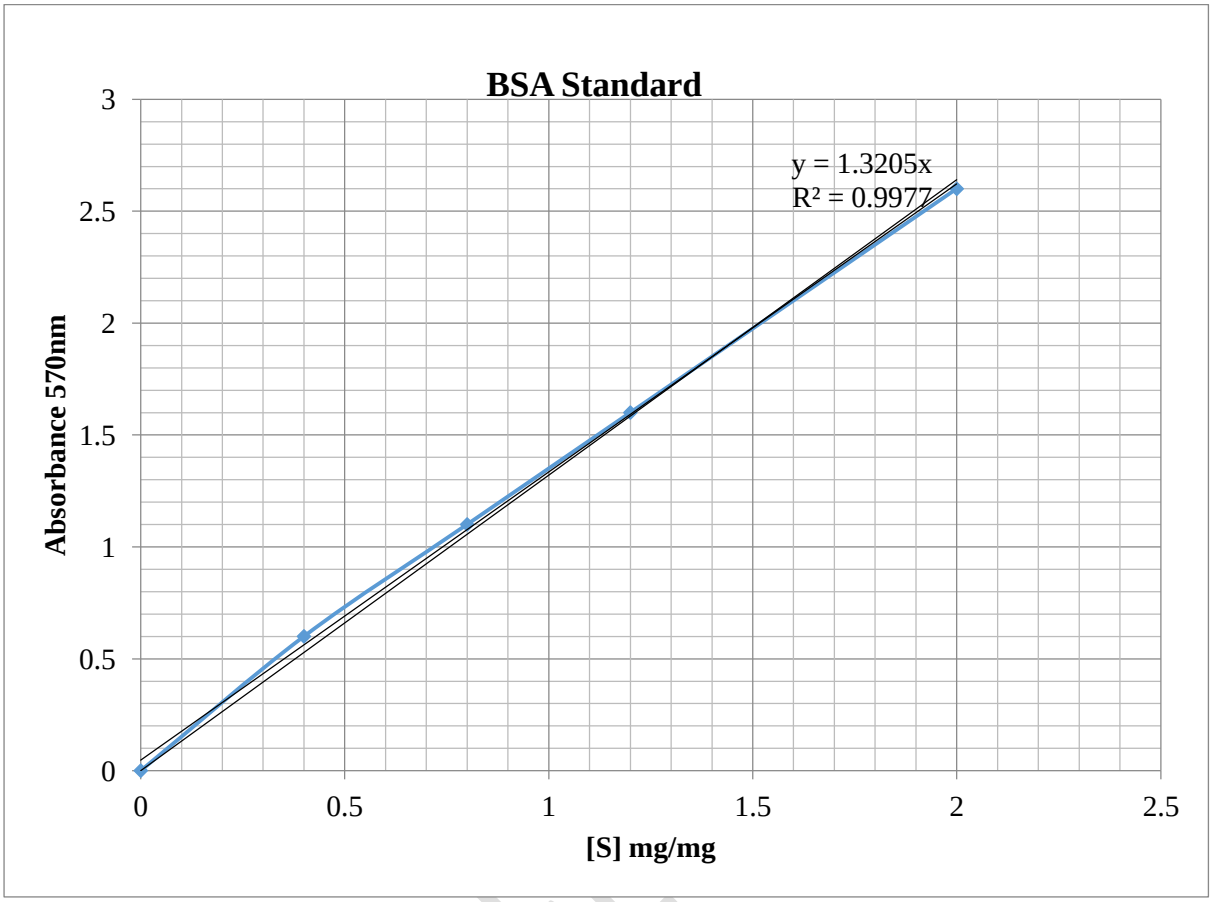

Fig. 2. Lowry Standard Plots for Protein Determination in Dilute Sodium Chloride

Table 2. Crude Thaumatin Concentrations Extracted in Dilute Sodium Chloride at different pH in varied Temperature

Temp. (0^oC)	pH 2.0		pH 3.0		pH 4.0		pH 5.0	
	Y (nm)	X (Mg/ml)	Y (nm)	X (mg/ml)	Y (nm)	X (mg/mg)	Y (nm)	X (mg/ml)
25	8.8	6.70	9.20	7.00	6.90	5.20	5.9	4.50
30	11.2	8.47	13.2	10.00	8.60	6.50	8.0	6.10
40	20.6	15.60	21.8	16.50	18.2	13.8	16.4	12.4
45	23.0	17.40	27.6	21.00	19.8	15.0	17.4	13.2
50	26.2	19.80	29.1	22.00	23.8	18.0	22.4	17.0

55	33.2	25.14	37.2	28.17	28.2	21.35	26.6	20.14
60	21.4	16.20	23.4	17.70	19.8	15.0	20.0	15.0
70	19.1	14.50	21.8	16.50	17.0	12.8	16.8	13.0
80	16.4	12.40	21.5	16.30	15.6	11.8	14.4	11.0
90	16.2	12.30	21.0	16.00	15.0	11.3	14.2	11.0

Y = Absorbance
Absorbance = 1.3205X,
X= Actual Conc.

Table no 1 showed that at different pH values, minimal and maximal crude thaumatin were extracted at temperature of 25^{o}C and 50^{o}C respectively. At pH 2, crude thaumatin recorded at these temperatures were 3.26mg/ml and 12.10mg/ml, at pH 3, concentration of 3.80mg/ml and 13.40mg/ml were recorded, and 2.12mg/ml and 14.70mg/ml were recorded while 1.74mg/ml and 9.61mg/ml were recorded at pH 5. From the same table it was also observed that maximum concentration (14.70mg/ml) of crude thaumatin was recorded at pH 3. Therefore the optimal pH and temperature of extraction in distilled water were at pH 3 and 55^{o}C respectively.

However, in Table no 2 showed that at different pH values, minimal and maximal crude thaumatin were extracted at temperature of 25^{o}C and 55^{o}C respectively. At pH 2, crude thaumatin recorded at these temperatures were 6.70mg/ml and 25.14mg/ml, at pH 3, concentration of 7.00mg/ml and 28.17mg/ml were recorded, and 5.20mg/ml and 21.35mg/ml were recorded while 4.50mg/ml and 20.14mg/ml were recorded at pH 5. From the table also it was observe that maximum concentration (28.17mg/ml) of crude thaumatin was recorded at pH 3. Therefore the optimal pH and temperature of extraction in dilute sodium chloride were at pH 3 and 55^{o}C respectively.

The lower yield in the concentration of crude thaumatin recorded in distilled water was as a result of the gel that encapsulates the sweet smelling substance (thaumatin) which possesses remarkable water-absorption properties. Therefore when the gel is in contact with water, it swells many times thereby absorbing up to ten (10) times of its own weight of water[5] (Yeborah *et al*., 2003) and consequently causes problem in the extraction, since the gel absorbs both sweetener and distilled water (extractant), while in dilute sodium chloride, much higher concentration of thaumatin crude were recorded, suggestive of the water absorbing capacity of the gel was remarkably inhibited[8] (Higginbotham, 1976), making the gel to absorb very small amount of the sweetener and the dilute sodium chloride (extractant), therefore increasing the efficacy and ease crude thaumatin extraction substantially and markedly. It was also observed that at these pH values in varied temperatures, below 70^{o}C of extraction, the sweetness of the protein was fully retained when tested orally, as opined

by[7] Gibbs *et al*., (1996) and[8] Lord, (2007). However, at 70^{o}C and above this temperature there was slight loss of sweetness which can be attributed to heat breakage or denaturation of disulphide bridges of the protein[9] (Higginbotham, 1979). Thaumatin when subjected to boiling in deionised water, thaumatins show very little loss of sweetness even after several hours[9] (Higginbotham, 1979; [10]Shallenberger, 1993).

3.3 **COLLECTION OF PURIFIED THAUMATIN BY CHROMATOGRAPHY**

From the figure 3.0 shown below, it can be seen that large volume of thaumatin are eluted in or just after the void volume of thaumatin as they passed through the column at the same speed as the flow of buffer. The protein concentration eluted at each point varies between 35mg/ml and 0.3mg/ml. All through the elution curve it has shown a tortuous curve.

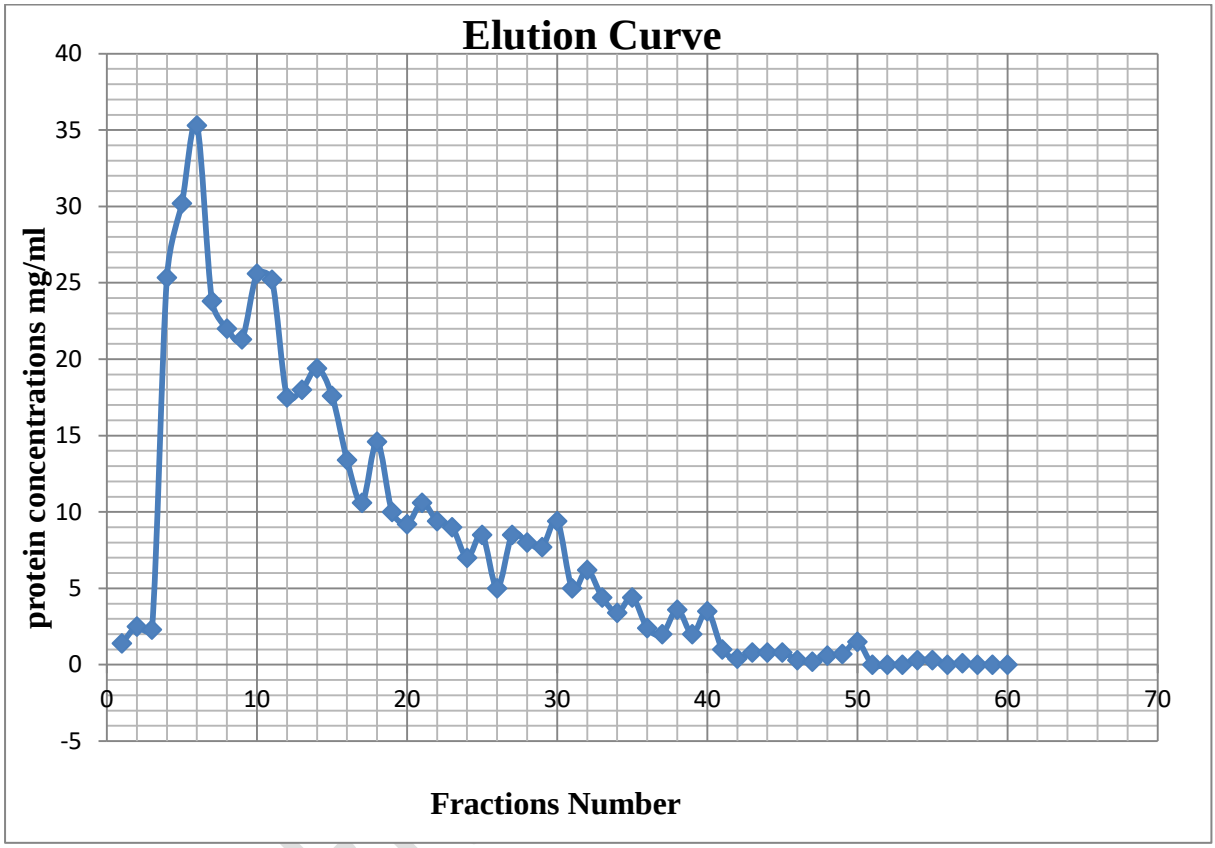

Figure ~~no 2~~ 3: Thuamatin elution profile.

The supernatants formed from various extraction of crude thaumatin was firstly freeze dried in order to precipitate the protein with ammonium sulphate ($(NH_4)_2SO_4$)[11] (Wingfield, 2001). Separation of remaining ammonium sulphate as well as smaller molecules were achieved via dialysis. Further purification were achieved by the use of Sephadex G-25 that acted as buffer exchange, removing small molecules and further desalting of the sample even faster. By these processes, the concentration of contaminants within the thaumatin sample was drastically reduced to acceptable/negligible level. Then having poured the partially purified thaumatin in a column chromatography to separate the large pool of the protein to relatively

smaller pools housing much of protein of interest (**sentence to correct**). Test tubes were set and the eluent collected with sixty (60) test tubes and the absorbance of the thaumatin in each test tube taken. It was observed that large volume of thaumatin molecules eluted in or just after the void volume of thaumatin as they pass through the column at the same speed as the flow of buffer. Then at the end of the process the samples are subjected to centrifugation while the resultant supernatants (clear brown liquor) was freeze dried with freeze-drier to yield a light, fluffy buff-coloured product, collected as partially purified thaumatin and stored in the refrigerator accordingly.

V. Conclusion

Thaumatin an excellent alternative sweetener can be viewed to play crucial role as safe for different food item, medicinal, soft drinks etcetera in time to come. The extraction of the intensely sweet juice called thaumatin, is usually very difficult by pressing or via other means in order to extract the sweetener. This is because the aril that houses thaumatin is encapsulated with gel mainly of polysaccharide origin, and these jellied mass is responsible for difficult in extraction. However by inhibiting the water absorbing capacity of the gel that encapsulate the sweetener with dilute aqueous sodium chloride at pH 3 and temperature of 55^{o}C, higher concentration of crude thaumatin was recorded. ~~Hrnce~~ Hence the crude thaumatin was precipitated, dialyzed and subjected to column chromatography and eluent collected as partially purified thaumatin, then freeze dried and stored accordingly. Thaumatin, a sweetener with very low calorie, possesses high potential to ameliorate complications in diabetics unlike carbohydrate related sweeteners that trigger high demand for insulin. Consequently, the extraction and application of thaumatin could be a valuable alternative to high calories sweeteners for diabetics.

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