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Exploring a Simple Method of Thaumatin Extraction from *Thaumatococcus daniellii*

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ABSTRACT

Thaumatin is a naturally occurring protein sweetener found in the arils of *Thaumatococcus daniellii* fruits. In spite of its high market value as a safe flavour enhancer and high-intensity sweetener, challenges associated with the extraction and purification process have limited the economic exploitation of *T. daniellii* for thaumatin production in the West and Central Africa. This study examined a simple extraction technique that could be adapted locally. The arils of *T. daniellii* fruits were homogenized (in water), and filtered through a double-folded muslin cloth to obtain a crude protein extract. The extract was precipitated with 80% ammonium sulphate, dialysed and purified by gel filtration (Sephadex G-75). The crude protein extract and proteins from dialysed and gel chromatographic fractions were separately freeze-dried and run through SDS-PAGE. The extract of *T. daniellii* arils contained sweet-tasting proteins (thaumatin) with average molecular weight of about 22 kDa. The proteins were extremely sweet at 20 – 40ºC; the sweetness decreased as temperature increased and became faint at 70ºC. Protein recovery from ammonium sulphate precipitation and gel filtration was 76.14% and 63.0%, respectively. The crude extract was light brown whereas the purified protein was cream coloured. The simple process of homogenisation and filtration through muslin cloth can be adapted for extraction and initial processing of thaumatin in West Africa. Enzymatic hydrolysis of the sticky substances (polysaccharides) in the arils can enhance the extraction process.

Keywords: *Thaumatococcus daniellii*, Thaumatin, Extraction, Filtration, Ammonium sulphate, Gel filtration.

Introduction

Rising prevalence of diseases linked to high sugar intake is a major global health threat. High sugar consumption is associated with higher energy intake and lower diet quality, which tend to increase the risk of obesity, prediabetes, type 2 diabetes, and cardiovascular diseases.[1–3] The use of artificial low calorie sweeteners such as saccharin, aspartame, cyclamate and acesulfame K, thought to reduce the sugar-related problems, have also been linked to severe long-term side effects, including psychological and mental disorders, heart failure, bladder and brain tumours.[4–7] These health concerns drive the increasing interest in naturally occurring sweet and taste modifying proteins isolated from African native plants. The protein sweeteners have the potential to replace sugars and artificial sweeteners by acting as good natural, low calorie sweeteners, and unlike sugars, they do not trigger a demand for insulin.[5,8,9]Among the naturally occurring sweet proteins is thaumatin, a group of intensely sweet-tasting proteins, about 3000 times sweeter than sucrose on weight basis, extracted from fruits of *Thaumatococcus daniellii* (Benn.) Benth, a rhizomatous plant found mainly in the tropical rain forests of West and Central Africa.[9–11] The fruit of *T. daniellii* has black hard seeds, covered by a thin layer of sticky, transparent gel, and a soft, fleshy and juicy cap called aril, which contains the thaumatin.[12] The arils make up about 4.8% of the fruit; [10,13] thaumatin can reach up to 50% of the dry weight of the arils.[14]

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Thaumatin consists of a polypeptide of 207 amino acids with molecular mass of 22.2 kDa, there are five different variants, I, II, a, b, and c.[15]

It is safe and approved for use in many countries as both a flavour enhancer and a high-intensity sweetener [11] Thaumatin is a globally traded commodity with a market value of over $36.21 m in 2018 and has been projected to appreciate at a compound annual growth rate (CAGR) of 2.9% to hit $41.77 m by 2023.[16] The narrow availability zone, mostly in West Africa, which had been a major factor restraining the growth of thaumatin market, led to the development of genetically modified crops and microorganisms as alternative sources for its production.[17,18]

Commercial production of thaumatin can transform West Africa by empowering her to benefit from the growing thaumatin market. Incidentally, challenges associated with its extraction and purification largely limit the economic exploitation of *T. daniellii* in West Africa. Presently, local vendors collect *T. daniellii* fruits from the natural habitat, in an unsustainable manner, and sell them to thaumatin processing companies. [19] This study is a response to the need to develop a simple technique for thaumatin extraction for local use, which can also serve as a preliminary processing step.

Materials and Methods

Crude Thaumatin extract:

Fresh *T. daniellii* fruits were collected from a local cocoa farm in Ekiti State, Southwest Nigeria, between June and September, 2016, and stored at -4ºC. The fruits were washed and cut open to obtain the seeds. The arils (fleshy, juicy cap on the seeds) were carefully harvested and weighed; 200 g was homogenized in distilled water using hex corona mixer grinder (India), and made up to 1000 mL The slurry was stirred with a glass rod for 1 h, and filtered through a double folded muslin cloth to obtain the crude protein extract. Aliquots of the extract were poured into lyophilizing flasks and freeze-dried.

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Determination of ammonium sulphate saturation (%) for Thaumatin precipitation

One gram (1 g) of freeze-dried crude extract (thaumatin) was dissolved in 10 mL of distilled water and 1 mL each was dispensed into 10 Eppendorf tubes. Calculated amount of ammonium sulphate was added to each tube to obtain specific percentage saturation (10 – 100%). The tubes were chilled on ice for 20 min and centrifuged at 13000 rpm for 15 min. Supernatants were decanted and the protein content was determine using Lowry's method. [20]

Dialysis

The crude thaumatin extract was precipitated with ammonium sulphate (80% saturation) and dissolved in 10 mL of distilled water. The dissolved precipitate was poured into a dialysis bag knotted at one end. The bag was then knotted at the other end close to the solution and placed into 1000 mL conical flask filled with distil water for diffusion of ammonium sulphate into the water. The whole set up was placed on a magnetic stirrer inside the refrigerator and the water changed at interval of one hour three times. The dialysed thaumatin was freeze-dried.

Gel chromatography

Six grams (6 g) of Sephadex G-75 was dissolved in 100ml distil water, boiled and left over night for gel swelling. A chromatography column (40cm ×2.5cm) was loaded with the gel. 5 mL of dialysed thaumatin was introduced into the gel and eluted with degassed distilled water. Fractions of 3ml at a flow rate of 4 minutes 59 seconds were collected in tubes and absorbance was measured at 280 nm using GENESYS 10S UV-VIS spectrophotometer. Fractions with sweet taste were pooled together and freeze dried. A protein standard curve was plotted and the protein concentration was determined.

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE):

The proteins were subjected to electrophoresis. The resolving gel contained 30% acrylamide bisacylamide, 10% ammonium persulfate (APS), tris Cl buffer (pH 8.8,) and tetramethyl-ethylenediamine. The resolving gel was loaded into the gel plate, isopropanol was added to get an even surface and also to remove bobbles. The gel was allowed to polymerize for one hour. Isopropanol was decanted, rinsed with distilled water and filter paper was used to remove the remaining isopropanol. The stacking gel containing 30% acrylamide bisacylamide, 10% ammonium persulfate (APS), tris Cl buffer (pH 8.8) and tetramethylethylenediamine was poured into the already polymerized gel, well comb was inserted and the gel allowed to polymerize for about two hours. Samples and protein marker were added to the wells and the electrophoresis unit was run at 80V electricity for about three hours.[21]

Gel staining

After electrophoresis, the gel was removed from the electrophoresis unit and placed in coomassie blue for 1 h, and then removed and placed into acetic acid/methanol solution for protein band staining.

Results and Discussion

SDS-PAGE of the dialyzed sample and gel filtration gave a single large protein band of closely related protein. There was 76.14% protein precipitation at 100% saturation of ammonium sulphate (Figure 1). The lyophilized crude extract was light brown in colour whereas the purified extract was cream colour (Figure 2).

Thaumatin consists of five intensely sweet forms, with two major components (thaumatin I and II) and three minor components (thaumatin a, b and c). Aqueous extracts of *Thaumatococcus daniellii* arils consists primarily of the thaumatins, but, in addition, contain a number of other proteins that are present at much lower levels. [22]

The protein concentration decreased as the percentage concentration of ammonium sulphate increased. The maximum saturation was attained at 80% ammonium sulphate concentration. However, as the salt concentration increased, a point of maximum protein solubility (80%) was reached. After this point, further increase in salt concentration leads to a decrease in the number of water molecules available to solubilize the protein.[23]

Ammonium sulphate has been widely used in protein precipitation due to its high solubility, relative low-cost, lack of toxicity to most enzymes and its stabilizing effect on some enzymes. Reproducible results can be obtained through precipitation provided the protein concentration, temperature and pH are kept constant. This method allows for easy separation of ammonium sulphate from proteins using dialysis method.

SDS-PAGE electrophoresis of the crude extract yielded four bands of low molecular weight proteins whereas the dialysed and gel filtration each gave two bands of closely related proteins. The molecular weight of the proteins was within the range of 15kDA and 25kDA (about 22kDA), which indicates the presence of thaumatin. The gel filtration chromatography profile showed that proteins with intense sweet taste eluted quickly (between fractions 3 and 23), leaving behind brownish impurities. The protein recovery from the gel filtration process was 63.0%. The cream colour of sweet proteins collected from gel filtration column indicates the removal of impurities via the purification process. The intense sweetness of thaumatin was still intact in spite of the colour change.

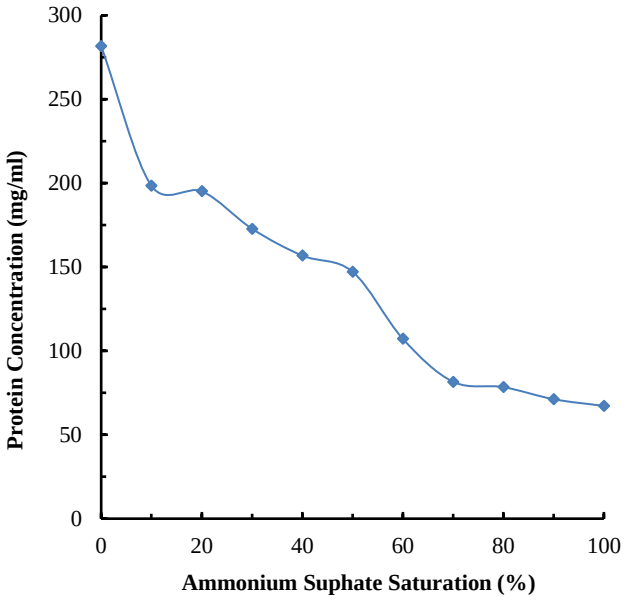

Figure 1: Protein content of supernatant after ammonium sulphate precipitation

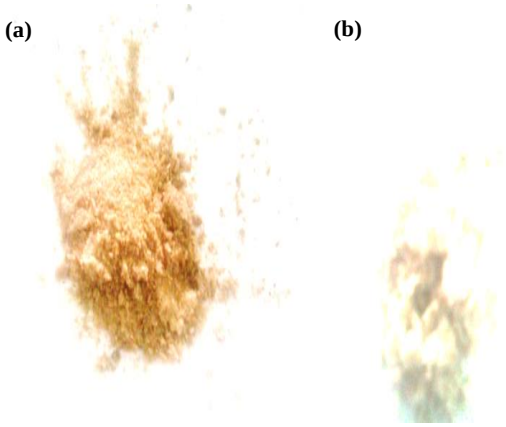

Figure 2: (a) Crude thaumatin extract; (b) Purified thaumatin (after column gel filtration).

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Table 1: Thermo-stability of Thaumatin

Temperature (ºC)	Intensity of Sweet Taste
20	+++
30	+++
40	+++
50	++
60	++
70	+
80	+
90	+
100	+

Key: +++ = intensely Sweet; ++ = very Sweet; + = Faintly Sweet.

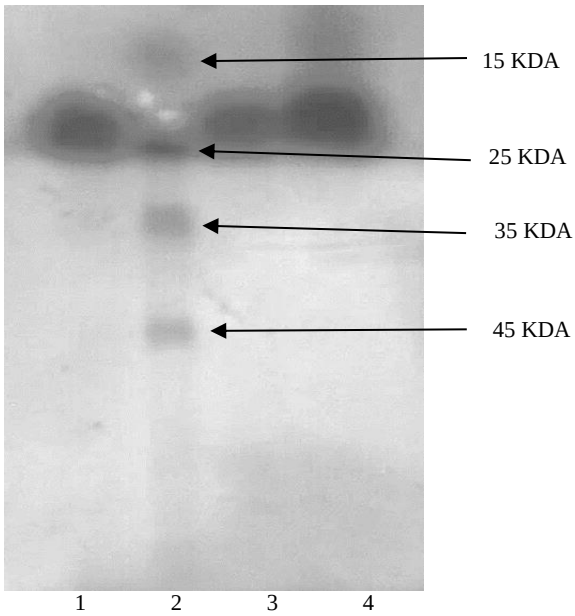

Figure 3: SDS-PAGE of protein standards and thaumatin (1 =Purified thaumatin after Gel filtration; 2 = Protein standard; 3 = Dialysed thaumatin; 4 = Crude thaumatin)

Conclusion

The simple process of homogenisation and filtration through muslin cloth can be adapted for extraction and initial processing of thaumatin in West Africa. This study also showed that ammonium sulphate precipitation and gel filtration are simple procedures for the purification of thaumatin. Enzymatic hydrolysis of the sticky substances (polysaccharides) in the arils can enhance the extraction process.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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