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Review Article

Fundamental overview and applicability of thaumatin

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ABSTRACT

There are currently worldwide efforts to reduce sugar intake due to various adverse health effects linked with the overconsumption of sugar. Natural sweeteners have been used as an alternative to artificial sweeteners, nutritive sugar for numerous applications. However, their long-term effects on human health remain. Thaumatin is a class of sweet proteins found in West African plants from the arils of Thaumatococcus daniellii. The article reviews the Thaumatin Morphology, Collection time, Extraction, Bio-production, Bio-transformation, Properties like sensory properties, Characteristics of plants ie Sweetness, Composition, Isolation process, Mode of action, Binding site of the receptor, Applications, Uses and overall safety aspects of Thaumatin is known as sweetest compound till the date.

1. Introduction

Thaumatin is a sweet protein isolated from the aril parts of Katemfe fruit i.e. Thaumatococcus daniellii . It is a native plant of West Africa. It is a natural sweetener and a basic protein, with approximately 12 AND 1600 times sweeter than sucrose. After tasting it also gives a cooling sensation and liquorice like a slight taste. It is cultivated on a commercial scale and used as a sweetener, flavor enhancer, and flavor modifier. Thaumatin is stable under the condition of PH 2-10 (aqueous solution). Thaumatin has a shelf life of at least 36 months when stored at cool temperatures. Indicated that this protein is thermoresistant in acidic conditions and neutral and basic conditions.[1] There are various proteins in this plant but the two main are Thaumatin 1 and Thaumatin 2 which are composed of 207 amino acids. Thaumatin I and Thaumatin II differ in amino acid sequences at 46,63,67,76,113 which suggests the protein is 98% identical. These sweet proteins are soluble in water and dilute alcohol. This protein can bind to and activate the sweet taste receptors of human TAS1R2 and TASIR3. According to JECFA and EFSA, thaumatin is safe for use as a sweetener with no ADI specified, which means it can be used according to GMP. However, in the USA it has not been approved as a sweetener but has a GRAS status as a flavor enhancer. There is no data on thaumatin's mutagenic, allergenic, or teratogenic effects.[2]

Figure 1: a: Thaumatococcus daniellii Fruits. **b:** Pictorial representation of the inner part of thaumatococcus daniellii fruit.

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2. Materials and Methods

Rhizomes are how the perennial, rhizomatous plant katemfe propagates itself. Depending on the age and habitat of the plant, the rhizomes produce petioles that are roughly 2 or 2.5 m long. Large, broad, oval, papery, robust, and adaptable leaves that are roughly 45 cm long and 30 cm broad are found at the end of these long petioles. The leaves are spherical, ovate-elliptic, truncate at the base, and acuminate briefly at the apex.[3]

T. daniellii's inflorescence often emerges from the lowest node and can be simple or forked, with spikes measuring 8 to 10 cm in length and bracts, which are often umbricate and measure 3 to 4 cm. At the foot of a swelling petiole, the blooms, which could be as long as the bracts form small spikes toward the ground. Sepals are roughly one centimeter long and broadly linear. Lobes are rectangular and roughly 2.5 to 3 cm long, while corolla tubes are short. On each inflorescence, up to 10–12 purplish-pink flowers may emerge, but typically only 2–3 or 4 of these result in full fruits.[3]

The fruit clusters on the soil's surface, within reach of insects and rodents, and grows on short stalks near the ground. It may also be covered in plant detritus. It has a pyramidal or trigonal shape, and depending on whether it has one, two, or three seeds, it can weigh between 6 and 30 g.[4]. When completely ripe, it changes color from dark green to brown to crimson or bright red. The black, hard seeds reside inside the fruit and are thinly coated in a translucent, sticky gel. When the seeds are dried, they resemble stones more clearly, demonstrating their hardness and imperviousness. It also has an aril, which is a delicate, juicy, fleshy top that holds the delicious ingredients.[5]

3. Collection Time of Thaumatin

The fruits were brought to Covenant University in Ota Ogun State, where they underwent a thorough washing to get rid of any dirt or debris that might have surfaced. This washing process started with tap water and continued with distilled water. After that, it was physically inspected, moved to a biochemistry lab, and kept there at a temperature of -180C. After 422 of the meaty fruits were chosen and weighed, the total weight came to 6752 grams (6.752 kilograms). Every fruit was peeled, frozen, and freeze-dried using a freeze-dryer until the seeds broke off. The brittle aril split from the seeds and the remaining fruits when the freeze-dried fruits were repeatedly struck with the hand. The fragile aril was then pulverized into a powder using a blender.[6]

3.1. Extraction

The Thaumatococcus daniellii plant from Otun village bush, Ayetoro local forest, in Moba Local Government Area of Ekiti State, Southwest Nigeria, produced juicy, ripe fruits. When ripe, the fruits ranged in length from 1.4 to 2.1 cm, had a trigonal or pyramidal shape, and were bright crimson or deep red.[6] The fruits ranged in weight from 10 to 22g, contingent upon the quantity of seeds present. The Forest Herbarium, located in Ibadan 110158, recognized the fruits of Thaumatococcus daaniellii (Benth).[7]

3.2. Bioproduction

Natural sweeteners acquired popularity as a result of the public's growing awareness of artificial sweeteners. For example, people prefer thaumatin over sucrose when it comes to culinary products. Nevertheless, thaumatin's availability is restricted during periods of strong demand due to its synthesis from a plant growing in a tropical climate. Furthermore, the availability and quality of the raw material might have a significant impact on the production process. Much research has been conducted concerning the manufacture of the protein using transgenic plants and genetically modified microorganisms to achieve a more constant supply to fulfill demand.[8]

Commercially, proteins can be produced using techniques such as genetic and protein engineering. These proteins are used by the biopharmaceutical industry, enzyme industry, and agricultural industry within the fields of medicine, diagnostics, food, nutrition, etc. In the early 1900s, the microbial fermentation industry came onto the scene through the production of chemicals such as acetone, butanol, and citric acid. The first protein pharmaceutical that was produced through recombinant DNA technology and approved by the FDA was Human Insulin, in 1982. Some examples of other recombinant proteins are albumin, human growth hormone (HGH), and factor VIII.

The market for recombinant proteins has also expanded as a result of improvements in production process technologies. The development of expression methods for mammalian cells, baculoviruses, E. coli, and bioreactors, for instance, has made it easier to produce these proteins. At present, over 400 protein medications developed by recombinant technology have been authorized and distributed globally, with over 1300 more in the process of obtaining permission.

For the manufacturing of recombinant proteins to be both economical and effective, many factors need to be taken into account. Gaining insight into the process and metabolic route involved in the formation of thaumatin can assist increase productivity and yield. Since this protein has a lot of potential to replace sugar substitutes or less safe sweeteners, its production needs to be enhanced and made more commercially viable. Still, a lot of work needs to be done to create cutting-edge techniques that can be used for the creation of these recombinant proteins.[9]

3.3. Biotransformation

The sweet-tasting thaumatin proteins are found in the arils of the African species Thaumatococcus daniellii Bennett; ripe fruits contain between 30 and 55 mg of thaumatin per gram of fresh weight.[10] Thaumatin has a sweetness level that is 3000 times higher than that of sucrose without any calories[11] Because of concerns about sustainability, thaumatin's pleasant qualities entice researchers to look for alternate methods of production. As a result, thaumatin biotransformation research shows encouraging outcomes for upcoming applications. To improve the flavor of fruit and vegetables, thaumatin gene expressions have been generated in rice[12], strawberries, barley, tomatoes, potatoes, cucumber, and pear. Therefore, thaumatin's plant gene expression studies show benefits including reduced toxicity and an increase in affordable earnings. However, thaumatin biotransformation by bacteria and fungi results in significantly faster growth, route control, and high thaumatin production.[13]

For example, because of its well-understood genetics, E. coli is the most commonly utilized bacterium for protein expression. Nevertheless, little total thaumatin has been produced by E. coli thaumatin synthesis. To produce thaumatin proteins synthetic E. coli genes.[14] The results of their study showed a molecular weight that was comparable to the genuine thaumatin. After such investigations succeeded in 2000 in generating roughly 40 mg of pure thaumatin that had the same sweetness properties as the original substance. However, one drawback of E. coli is that its byproducts can be hazardous.[15]

Pichia pastoris is a good example of a commercial thaumatin producer that does not use toxins. Because of this biotransformation research, thaumatin may be used for a variety of food products that will have sustainability aspects in the future.[12]

4. Properties of Thaumatin

4.1. Sensory properties

The standard description of thaumatin's sweetness potency is that it is roughly 2000 times sweeter than sucrose; however, because of the temporal profile of thaumatin, it is challenging to pinpoint the exact potency.[16] the sweetness potency for thaumatin of approximately 8600 times that of sucrose at 2% sugar equivalent sweetness, approximately 6000 times that of sucrose at 6% sugar equivalency, and approximately 2000 times that of sucrose at 8% sucrose equivalency. This proves that the relative sweetness factor rapidly decreases as concentration increases.[17]

The temporal taste profile of thaumatin is typified by a delayed onset of sweetness perception, an extended period of sweetness increases until the highest level of sweetness is experienced, and a persistent aftertaste that is sweet and licorice-like.[18] Due in large part to the temporal characteristics' incompatibility with common food and drink applications, there has been a dearth of specific sensory data detailing its use at sweetening concentrations.

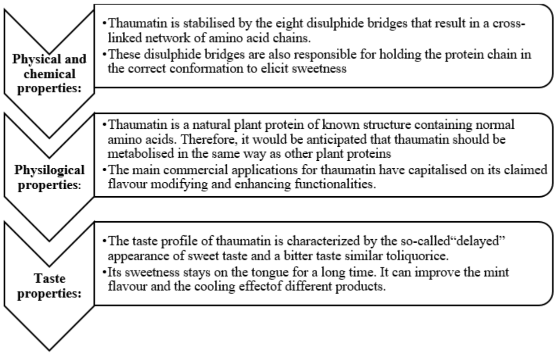

Figure 2: Someproperties of thaumatin.[16 17 19]

5. Characteristics of Plant

Features of the plant known as "katemfe," or Thaumatococcus daniellii (Benn.) Benth. is a member of the Maranthaceae family. It grows in the Central African Republic, Angola, and the hot, humid tropical rainforest and coastal zone of West Africa. Additionally, it has been brought to regions of Singapore and Australia. The forest tree undergrowth is its native home. Although the plant flowers for most of the year, it is most active from July through late October and from January through mid-April when the fruit forms, ripens and matures. It appears to be appropriate for incorporation into systems of agroforestry. Each of the two or three triangular, fleshy pericarps that make up a single fruit, which weighs about 16 g, contains a huge black seed that is encased in a thick, transparent, viscous mucus. Additionally, there is a soft, jelly-like layer beneath each seed.[20]

Leaf juice is used as a sedative and an antidote to venoms, stings, and bites in traditional folk medicine. knife fruits are used as a laxative, while the seeds are utilized as an emetic and for lung issues. The pulp of the katemfe fruit is traditionally used in West Africa to enhance the flavor of sour foods such as bread, fermented palm wine, and acidic fruit sweets, and is generally used to sweeten cuisine. Additionally, katemfe is added to foods like tea, pap (corn porridge), and garri (cake made from fermented manioc flour, typically cooked in palm oil).[21 22]

6. Composition and Specification Characteristic Properties

The THAUMATIN product can contain either individual thaumatin I or thaumatin II proteins or a blend of thaumatin I and thaumatin II proteins blended to the same final total thaumatin content because thaumatin I and II have equal sweetening and taste-modifying characteristics.[16] Notifier conducted a thorough molecular analysis of the allergenic potential of plant-expressed thaumatin I and II for use in food. Based on available data, the company concluded that its thaumatins shouldn't be more allergenic than the commercial thaumatins derived from Thaumatococcus that are currently available for purchase.[23]

7. Sweetness Characteristics

In comparison to sucrose, thaumatin has a very different sweetness time/intensity profile. The sweetness comes on gradually and intensifies to a maximum point, with a long-lasting aftertaste reminiscent of sweet licorice. But unlike some of the other strong sweeteners, it doesn't leave a bitter, metallic aftertaste.

When compared to TI and TII, which are both slightly less sweet than TII, and Ta, Tb, and Tc, which are even less sweet than TI, the cold aqueous solution of the mixed thaumatins has a sweetness ranging from 5500 times 0.6% sucrose at its threshold sweetness to 1300 times at 13% sucrose. This suggests that increasing basicity confers greater sweetness.[24]

Because thaumatin can cover up the distinct aftertaste of potassium chloride, which is occasionally added to table salt to prevent excessive sodium ion concentrations, it may also be used in goods with lower sodium contents. Thaumatin has an energy value of 4 kcal/g. However, as this sweetener is typically used in modest dosages, it is thought to have little caloric effect on diet. Thaumatin has taste-modifying qualities in addition to being antimicrobial, contraceptive, lowering blood sugar, and cardiovascular system-protecting.[17,25]

7.1. Solubility

While thaumatin is normally used at levels of 0.00005–0.0005% by weight, it is easily soluble in cold water at concentrations of up to 20% (200 mg/ml).Alcohols like ethanol and isopropyl alcohol can be dissolved in up to 90% alcohol by prehydrating in a small amount of water. Of these, 12% solutions can be formed in 60% alcohol. High thaumatin in alcohol concentrations might result in gel formation. In aqueous glycerol, propylene glycol, and polyols like sorbitol (all 60–80% in 40–20% water), solubility is good (at least 10%). Thaumatin is not appropriate in standard aprotic and organic solvents.[17]

8. Isolation of Thaumatin

8.1. Mode of action

Thaumatins have an interaction with the tongue's sweet taste receptors. In contrast to monofunctional sweeteners, thaumatins also modulate additional gustatory receptors.

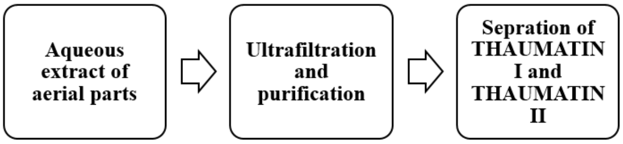

Figure 3: Isolation process.[16,24]

The way that thaumatins affect different taste receptors is context-specific and contingent upon the existence of other flavors. The broad spectrum of industrial applications of thaumatins in flavor modulation can be explained by this pluripotent effect. Apart from its traditional use by African and, more recently, Western and Eastern civilizations, thaumatins have been thoroughly investigated for their ability to affect taste at the molecular level and in both in vivo and ex vivo animal investigations.

According to research conducted on whole animals of different species as well as isolated gustatory cells cultured in vitro, thaumatins have a measurable impact on different types of taste receptors, and these interactions impact taste perception.[16]

8.2. Binding sites of receptors

Model schematic showing the receptor for sweet tastes. The two subunits that make up the sweet taste receptor are T1R2 and T1R3. The two subunits are GPCRs of class C. T1R2 and T1R3 have a large aminoterminal domain (ATD) that consists of a Venus flytrap domain (VFT) joined by a short cysteine-rich domain (CRD) to a helical transmembrane domain (TMD), which is a feature of GPCRs. Most sweeteners attach to one of the huge clefts that separates the two lobes that make up the VFT. G protein-coupled receptors, or GPCRs.[26]

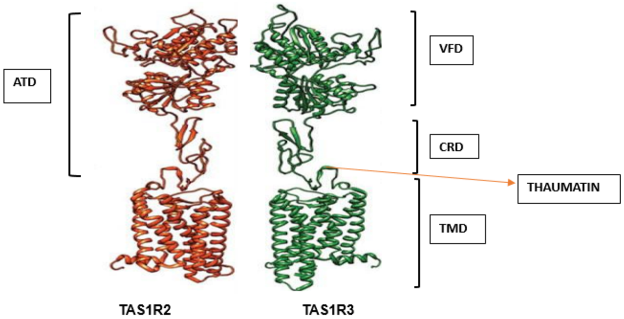

Figure 4: Binding side of sweet taste receptors.

8.3. Application

Thaumatin is employed as a sweetener in drinks and sweets, although its restricted use is due to its flavor being similar to licorice and its delayed sweetness. Thus, thaumatin is most frequently employed in practice as a useful sweetener in combination with other sweets that taste better quickly. Though it has limitations as a sweetener, taumatin is a potent "taste enhancer and can intensify the flavors of peppermint, cinnamon, and wintergreen by up to ten times. The flavor potential characteristics together with the lingering sweet taste can be beneficial for products such as toothpaste, mouthwash, and chewing gum and for enhancing the" flavor of drugs. A flavor enhancer, thaumatin is utilized in the following goods.[24]

1. **Japanese product:** First, it was noted that talicin improved the flavor of peppermint; several useful uses have been established. When Talin is administered at concentrations below its sweet taste threshold, usually at 5×10–5% Tal. in, flavor enhancement is observed. Drinks, soft drinks, fruit juices, milk drinks, ice candies, sherbets, ice creams, jellies, custard, chewing gum, wafers, soft candies, cream, pickles, cheese, sauces, and dried fruit are all mentioned as having lower costs and better aromas.[24]
2. **Chewing gum:** When talin is used at concentrations between 50 and 150 parts per million, the flavor of gum—particularly peppermint and spearmint varieties—as well as coffee and fruit flavors like lemon, plum, and strawberries are intensified and sustained.[24]
3. **Coffee:** Instant coffee granules (produced by re-drying strong coffee solution) gain ten to fifteen percent more strength when a very low amount of Talin is added.[24]
4. **Soft drinks:** Because of its taste, talin can only be used in carbonated drinks at a maximum of 10–15 ppm of sweetness, depending on the drink's acidity and kind. Reducing the sugar content of a syrup drink (which is often more than 145% sucrose) not only lowers the calorie and cariogenicity of the beverage but also lessens stickiness and enhances flavor perception. This also permits a 10-15% decrease in the concentration of citric acid utilized.[24]
5. **Flavor essences and oils:** Talin's solubility in aqueous alcohol makes it possible to add it directly to flavor essence or oil, giving chewing gum a handy way to get it in. The quality and durability of the aromas are improved by adding Talin directly to the flavor essences in a 50% aqueous alcohol solution.
6. **Medicine and oral care products:** Talin's strong sweetness, non-cariogenicity, and "taste/aroma enhancement property" point to potential uses in enhancing the palatability of medications, especially those containing strong, unpleasant-tasting chemicals that are difficult to cover up with sugar or glucose syrups alone. When strong flavors are present and intense sweetness is needed, the level of usage can be as high as 0.05% in liquid formulations when flavor enhancement and taste masking are the primary requirements.

8.4. USES:

Table 1: Different uses of thaumatin.

Sr. no	Uses	Description	Reference
1.	Flavors	In addition to having unique synergistic effects with distinct individual components, the usage of thaumatin at low levels (usually less than 2 ppm at use level) in flavor formulation would considerably boost the instant impact and longevity of perception.	[27,28]
2.	Flavours enhancers	Even though thaumatin has a flavor-enhancing effect on its own, research has shown that it works very well in concert with monosodium glutamate (MSG), enabling an 80% reduction in MSG without significantly reducing the effect of flavor improvement.	[27,28]
3.	Chocolate	The combination of properties that thaumatin has allows it to significantly alter the flavor of chocolate. First, it is possible to increase the perceived fat content and improve the creamy quality of dairy products, which results in a smoother mouthfeel.	[28]
4.	Animal feed and pet food	The application of thaumatin in animal feed and pet food has been studied quite a bit. However, it seems that thaumatin has an uncommon quality in pet food ingredients—it can be enticing to both cats and dogs. Pets have been demonstrated to prefer foods containing thaumatin and to be able to detect doses as low as parts per billion.	[28]

9. Thaumatin Overall Safety

The safety of thaumatin has been proven for animals and humans. It does not cause tooth decay and can be used by diabetics. Between 1975 and 1985, a minimum of eighteen studies were performed without indicating any safety concerns. It has been marketed in Japan since 1979 and has been approved in the United States by the Flavor Extract Manufacturers Association for use in chewing gum. In Canada, thaumatin is permitted in animal feeds, chewing gum, flavor preparations, breath fresheners, salt substitutes, and salt substitutes.[29] In 1983, the use of thaumatin was approved by Great Britain for dietary products, drinks, pharmaceutical products, and foods (excluding those intended for babies). The majority of sales have been for animal feed (63.6%) followed by use as a flavor-enhancing agent (15.3%) and sweetener of food and beverages (10.8%). Thaumatin is considered by consumers as a novel type of food additive with new properties.[30]

10. Conclusion

The thorough analysis leads to the conclusion that Thaumatococcus Daneilli is a potentially significant sweetest protein with a broad range of applications and uses. According to the current research, Thaumatin has various applications as a Low-calorie sweetener, flavor enhancer, and flavor modifier consisting of two sweet proteins I and II Which can be replaced in place of sugar. This plant has potential properties that should be further studied. It can be used to extract active substances for the production of various medications, and the management of various artificial sweeteners.

11. Source of Funding

None.

12. Conflict of Interest

None.

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