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Studies of some functional properties of thaumatin, a protein sweetener

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Abstract
Thaumatin, an intensely sweet protein from the fruit of Thaumatococcus danielli Benth., was isolated, purified on Sephadex G50, and some of its functional properties studied. The functional properties studied included protein solubility, emulsifying activity, emulsion stability, foaming capacity and stability and the effect of pH on the sweetness and temperature stability of thaumatin. The effect of thaumatin on food color stability was also investigated. Gel filtration on Sephadex G50 yielded four fractions of which three possessed thaumatin activity. The purified, freeze-dried sample was fluffy, light cream-colored powder. Thaumatin was soluble over a wide range of pH (2≤ pH ≤ 10). The protein maintained sweetness at high temperature over both acidic and basic pH, while it lost sweetness beyond pH 9. Thaumatin resulted in high whippability (140%) and foam stability. The protein also had relatively good emulsion properties, and heating had little effect on its emulsion stability. All these are suitable properties required of any novel protein for use in food formulations.

Introduction

Functional properties of proteins are those physical and chemical properties, which affect the behavior of proteins in food systems during processing, storage, preparation and consumption. These characteristics influence the quality and sensory appeal of foods [1]. Functional properties are most important in determining the usefulness of proteins in food systems. With the increasing number of processed and fabricated foods, there is a growing reliance on the consistent behavior of ingredients in specific formulations.

Thus the usefulness and success of a novel protein or protein- polysaccharide concentrate depend on its specific functional properties when incorporated into various products [2, 3].

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Thaumatin, a protein sweetener reported to be 2,000 times sweeter than sucrose, on a weight basis [4, 5], is found in the arils of *Thaumatococcus danielli* fruit, a West African plant which grows extensively in the cocoa-growing regions of Nigeria. Studies on some physicochemical properties of thaumatin have shown that the protein is very soluble in water, stable at acidic pH, and retains sweetness at 100^0C at pH 3-4 [5, 6]. The application of thaumatin at the moment is limited, with the exception of its application in Japanese food products [6]. Attempts to enhance the utilization of this protein however must be accompanied by the ability to predict its behavior in different food systems. This study was therefore carried out to determine selected functional properties of thaumatin, its sweetness stability at different pH values and temperature; and predict its suitability for use in a wide range of food systems.

Materials and Methods

Thaumatococcus danielli fruits were obtained from Ipetu Ijesha, Osun State, Nigeria.

Preparation of samples

Four kilogram of *T. danielli* fruits were thoroughly washed with distilled water, manually cut open with a scarpel, and the seed were separated from the pulp. The arils of the seed were collected and kept at -20^{0}C in a freezer until needed.

Isolation of Thaumatin

Thaumatin was extracted according to the method of Van der Wel and Loeve (4). About 200g of arils were homogenized in 40ml of distilled water in a Phillips blender (Model 4R 1375). The slurry was stirred with a glass rod for 1h and filtered through a cheese cloth. The extraction procedure was repeated on the residue. The pooled extracts were freeze-dried (Labconco, FIIRO, Oshodi). The freeze-dried extract was divided into four 800mg; batches. Each batch was dissolved in 15ml-distilled water and loaded onto a Sephadex G50 column (2.5x 100cm). Elution was done with distilled water at a flow rate of 72ml/h. Eighteen millilitre fractions were collected per tube. Transmittance was measured at 280nm on a Unicam 8625 UV-visible spectro-photometer. Fractions with sweet taste were pooled and freeze-dried.

Functional Properties of Thaumatin

Protein solubility profile was determined by the method of Sathe and Salunkhe [7]. A 0.05g of freeze-dried sample was dissolved in 100ml of distilled water. Ten-milliliter portions of the solution were adjusted to the desired pH (2-10) with 0.1M NaOH or 0.1M HCl, shaken on a Gallenkamp shaker for 15min, and centrifuged at 10,000 x g for 30min. Protein concentration of the supernatant was determined by the method of Lowry *et al* [8].

Emulsifying activity was determined according to the method of Naczk et al [9]. A 0.05 portion of thaumatin was dissolved in 50ml of distilled water and homogenized in a Phillips (model 4R 1375) blender at high speed for 30s. Fifty milliliter of vegetable oil (Avop brand) was added in batches of 5ml and homogenized for 30s after each addition. After the last addition, homogenization was done for 90s. The emulsion was divided equally into two centrifuge tubes and centrifuged for 5min at 1100xg. The emulsifying activity was calculated by dividing the volume of emulsified layer by the volume of emulsion before centrifugation.

Emulsion stability was determined by the modified method of Naczk *et al* [9]. The sample used for emulsifying activity was heated at 85^{0}C for 15min, cooled and divided evenly into two 50ml centrifuge tubes, and centrifuged (Eagle 5S) at 1100x g for 5min. The emulsion stability was expressed as a percentage of emulsifying activity remaining after heating. Emulsifying activity and stability was also determined as a function of thaumatin concentration using the following distilled water, 0.005%, 0.01% and 0.1%, thaumatin solutions.

Whippability and foam stability were determined by the method of Lin *et al* [10]. Fifty milliliter of 1% thaumatin solution was homogenized in a Phillips blender for 30s. The mixture was immediately transferred into a 250ml-graduated cylinder. The foam volume was measured. Whippability was expressed as percentage of volume increase. Foam stability was expressed as the volume of foam remaining after 360min. All determinations were carried out on duplicate samples.

Thaumatin Stability Tests

Effect of pH on the temperature stability was measured by preparing 100ml of 0.01% thaumatin. Ten-milliliter portions were adjusted to the desired pH (2-11) with 0.1M HCl or 0.1M NaOH. The solutions were heated in a Griffin water bath at 97^{0}C for 20min and allowed to cool, and turbidity was measured at

380nm on a Spectronic 20 spectrophotometer using distilled water as blank.

Effect of thaumatin concentration on the stability of food color and flavor was determined by preparing serial dilutions of 0.1% thaumatin ($2x10^{-4}$ – 1×10^{-3}g/ml). Five milliliters of each solution was added to 0.2ml of 1% FD&C yellow No 5 and 0.05ml of pineapple flavor (Akras) to give final thaumatin solutions of 0.02g/dl – 0.10g/dl. Samples without the pineapple flavor were also prepared. Degree of precipitation was observed visibly and compared with absorbance values measured at 490nm on a Spectronic 20 spectrophotometer [11].

Results and Discussion

The results from Sephadex G50 column chromatography showed four major fractions having thaumatin activity. This was similar to the results of Van der Wel and Loeve [4].

The solubility profile of thaumatin (Fig 1) showed the protein to be maximally soluble (up to 80%) at pH 6. The protein was soluble over the entire range of pH used (2 - 10), but having the least solubility (40%) at pH 10. The poor solubility of thaumatin at alkaline pH probably reflected the overall positive charge on the protein due to the relatively high concentration of basic amino acids- lysine and arginine [12]. Thaumatin has been reported [4] to have an isoelectric point of 12, from studies using starch gel electrofocusing technique. The wide range of solubility of thaumatin suggests its likely use in a wide variety of food systems

Differences in methods of measuring emulsion properties sometimes make direct comparisons with other proteins difficult. The emulsifying activity of 0.01% thaumatin (Table1) was 53.52%. This value was lower than values obtained for 10% soy protein isolate (72.0%) and Chinese rapeseed precipitated protein isolate (56.1%) [13], in which similar procedures were used. The low concentration of thaumatin was to simulate the quantity of thaumatin that would normally be used in food systems. For proteins to form a stable emulsion, the protein molecule migrating to the interface should be able to partially unfold in order to reorient its hydrophobic and hydrophilic patches, the former to entrap oil droplets and the latter to interact with the aqueous phase. This in turn depends very much on the conformational stability of protein [14]. The emulsion stability (Table 1) of thaumatin was high (91.3%). This showed that heating had very little effect on the emulsion. As a function of thaumatin concentration, emulsifying activity (Table2) was highest at 0.01% thaumatin (51.8%), and it dropped to 42.1% at 0.1% thaumatin concentration. Values obtained for all thaumatin solutions were higher than that of distilled water. The stability of the protein however decreased with increase in concentration. In Table 1, the whippability of thaumatin (140%) was lower than the values obtained for 3% dispersion of rapeseed protein isolate (281%) and soy protein (252%) [13, 14]. Thaumatin foam was stable, lasting for over 3h.

The temperature stability of thaumatin at various pH values was related to the turbidity of the solution at 380nm (Fig 2). Maximum turbidity was observed at pH 9, indicating the least stability. Stability was however high between pH 2.5 and 5. Korver et al [15] reported maximum heat stability of thaumatin at pH 5, using circular dichroism at 228nm; and attributed this heat resistance to a temperature-dependent, reversible chromophore in thaumatin involving a tyrosine and disulphide chromophore. Also, thaumatin has been reported to contain 8 disulphide bonds, which are known to confer heat stability to proteins [12, 16]. It is therefore possible that at low pH, a suitable environment is created which enhances S-S bond stability, which in turn, enhances stability of native state conformation. Reduction of sweetness has been observed with S-S bond reduction [5].

Figure 3 showed that thaumatin reacted with synthetic food colors to form precipitates. This was however dependent on the concentration of thaumatin used. Similar findings were reported by Higginbotham [17] and Hussein [11]. This behavior was attributed to the high overall positive ionic charge on thaumatin which causes it to associate with suitably spaced and negatively charged sulphonate groups on the colors; thus forming precipitates under certain conditions of temperature, pH and concentration [17]. The study was also carried out to determine the minimum concentration of thaumatin required for precipitation to occur. Formation of precipitate increased with increasing thaumatin concentration. Using visual observation, precipitate was first noticed at 0.04g/dl (Fig 3). Samples in which pineapple flavor was added were more turbid than those without flavor.
This increased turbidity might be due to the formation of an emulsion, since the flavor was an oil-based flavoring agent. It might therefore be expected that at the concentration at which thaumatin is normally applied in beverages ($<1x\ 10^{-3}\%$) [6], precipitation is unlikely to occur, barring the effects of pH and temperature.

Conclusion

The overall functional properties of thaumatin suggest that the protein may be suitable for use in a wide range of foods and beverages, such as soft drinks, where high solubility is required at acidic pH; mayonnaise, soups and sweets, where excellent emulsion properties are required; and products such as ice creams and cakes where good whipping properties are required. Thaumatin is potentially an ideal replacement for sucrose due to its non-cariogenic, non-calorific and flavor-enhancing properties. Thaumatin is also safe for use in foods.

Table 1. Selected Functional Properties of Thaumatin

Emulsion Activity (%)	53.5± 1.5
Emulsion Stability(%)	91.3± 0.1
Whippability(%)	140.0± 0.0

[a] Values are means of duplicate determinations

Table 2. Emulsifying Activity of Thaumatin at Different Concentrations

	Distilled water	0.005 (%)	0.01 (%)	0.1 (%)
Emulsifying Activity (%)	38.5	39.0	51.8	42.1
Emulsion Stability (%)	97.5	98.1	87.1	ND

Table 3. Foam Stability of Thaumatin

Time (min)	0	30	60	90	120	150	180	210	240	270	300	360
Volume (%)	70	66	65	64	62	60	58	58	57	57	56	55

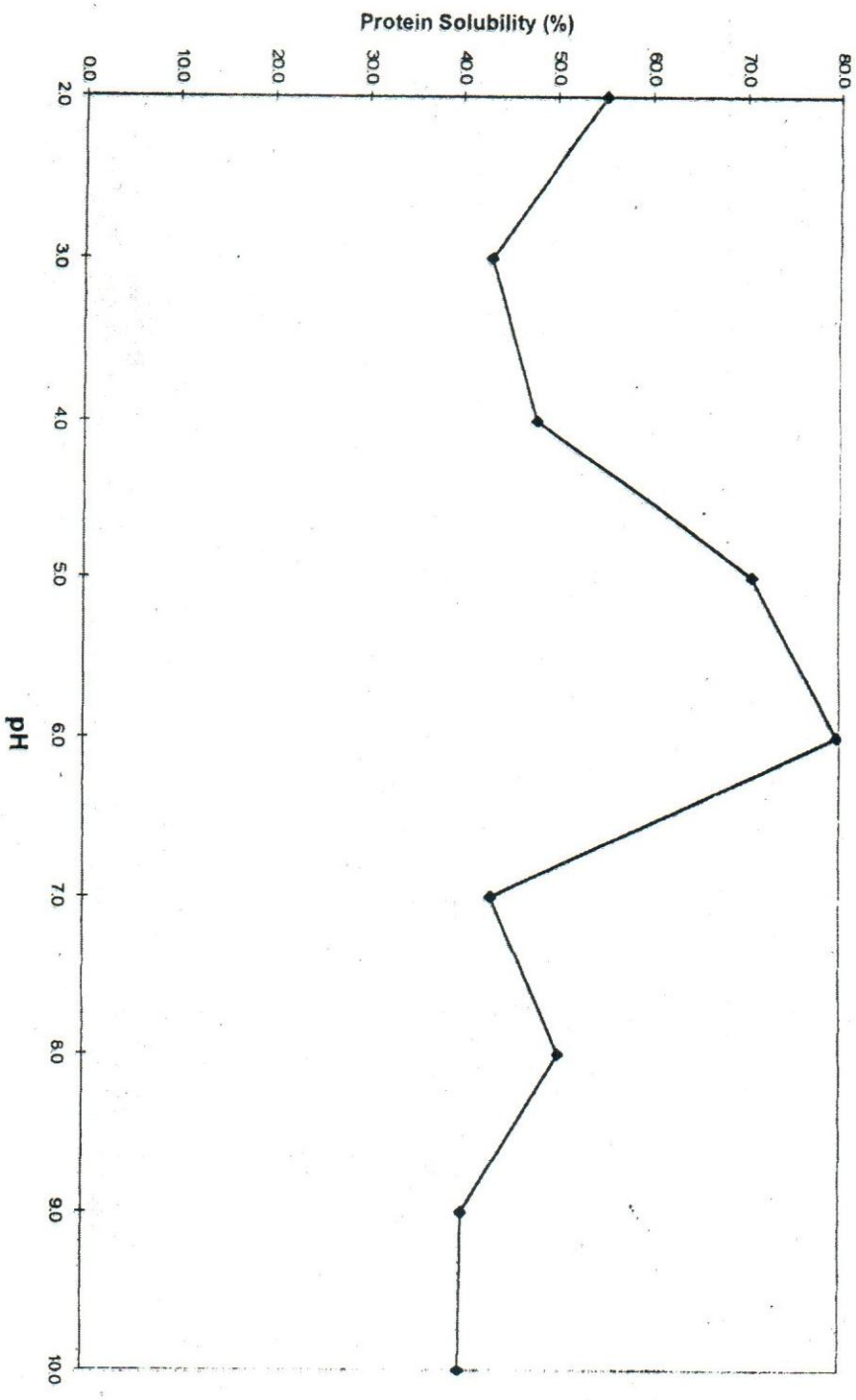

Fig 1: Protein Solubility Profile of Thaumatin

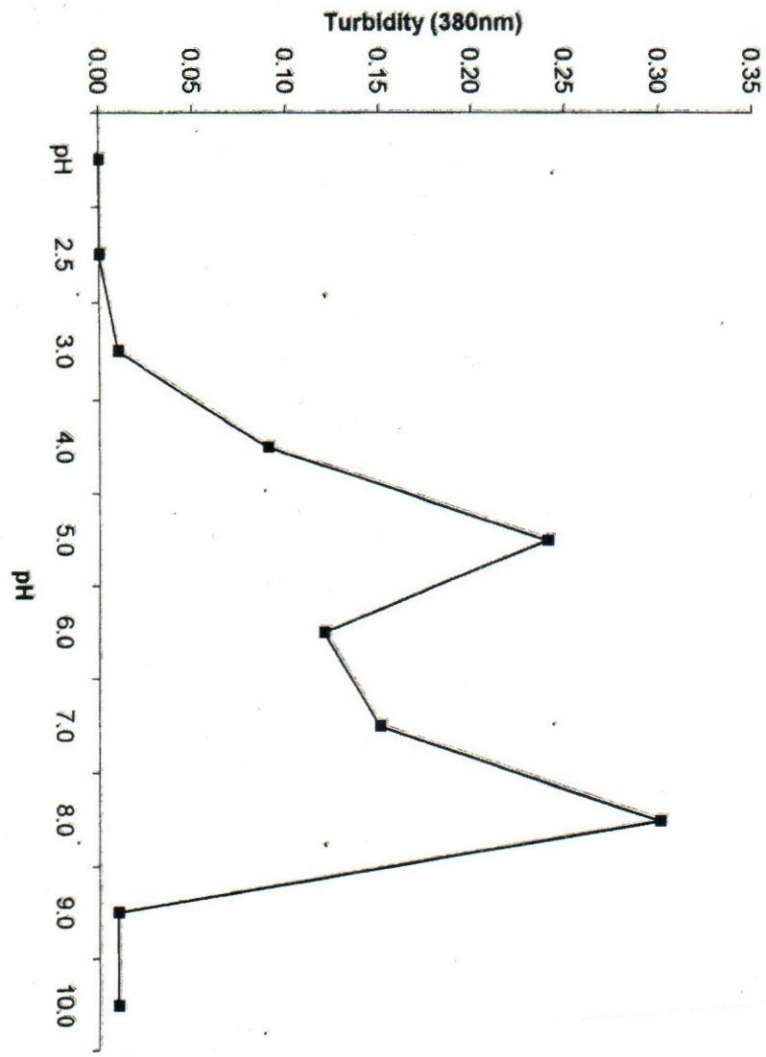

Fig 2: Effect of pH on Temperature Stability of Thaumatin

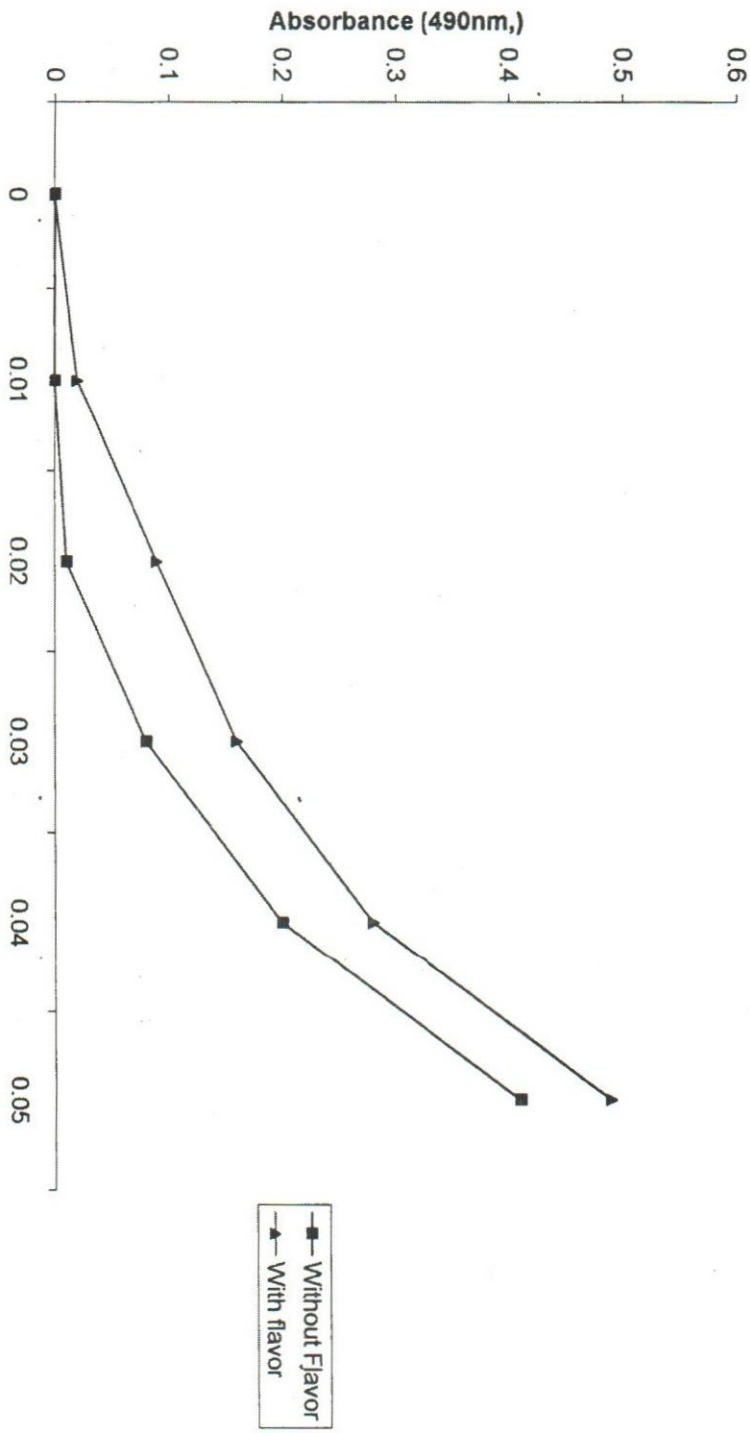

Fig 3: Effect of Thaumatin on Food Color Stabilitty

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