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A METHOD OF PREVENTING A DECREASE IN THE SWEETNESS
OF THAUMATIN

DESCRIPTION

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The present invention relates to a method of preventing a decrease in the sweetness of thaumatin.

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Thaumatococcus Danielli Benth. or by expression of the gene encoding thaumatin in a suitable host microorganism, using standard recombinant techniques. When negatively-charged substances, such as fruit juice and anionic surface active agents, are present in aqueous solutions of thaumatin, precipitates are formed. It has previously been found by the present inventors that chitosan is effective in preventing such precipitation, and also that ascorbic acid and chitosan when used together can prevent any unpleasant after-taste resulting from an aqueous solution of

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thaumatin (cf. Japanese Laid-Open Publication
02/174,649).

5 However, the sweetness of thaumatin decreases when an
aqueous solution of thaumatin contains coloring
agents, such as Tartrazine, Sunset Yellow, Ponceau 4R,
lac dyes and cochineal dyes of the anthraquinone
series, red cabbage dyes of the anthocyanin series,
annatto dyes of the carotenoid series, safflower yellow
10 dyes of the flavonoid series, or contains
polysaccharides, such as carrageenan, xanthan gum,
sodium carboxymethylcellulose, sodium alginate, high
methoxyl pectin, locust bean gum or guar gum. Until
now, no method for preventing a decrease in the
15 sweetness of thaumatin in the presence of such
substances has been found.

The present invention relates to a method of preventing
a decrease in the sweetness of thaumatin in the
20 presence of additives, such as coloring agents and
polysaccharides, commonly used in foods, drugs,
cosmetics and related substances.

According to the present invention there is provided a method of preventing a substance capable of so doing from causing a decrease in the sweetness of thaumatin, characterized by adding chitosan, hydrolyzed chitosan, or a mixture thereof, to an aqueous solution containing thaumatin and said substance, in an amount effective to prevent a decrease in the sweetness of thaumatin.

In a second aspect, the invention provides a composition in which a substance capable of so doing, is prevented from causing a decrease in the sweetness of thaumatin, comprising an aqueous solution of said substance, thaumatin, and chitosan, hydrolyzed chitosan or a mixture thereof, in an amount effective to prevent a decrease in the sweetness of thaumatin.

The chitosan used in the present invention may be obtained by deacetylation of chitin found in the shell or exoskeleton or certain Crustaceae, such as crabs and shrimps, with alkali. Hydrolyzed chitosan may be obtained by acid or enzymatic hydrolysis of chitosan. More specifically, acid hydrolysis of chitosan may be performed using an inorganic acid, such as hydrochloric

acid, or an organic acid, such as acetic acid, at a temperature of 100-121°C for 5 to 180 minutes, as disclosed in Japanese Laid Open Publication 01(1989)/128775. Enzymatic hydrolysis of chitosan may be performed, using an enzyme, such as an acid protease, pectinase, cellulase, papain, chitosanase, at pH 3-5 and at 40-60°C for 1-24 hours as disclosed in Japanese laid Open Publications 62/83877 and 63/63388, Japanese Patent Application 121852/88 and Japanese Laid Open Publication 02/231,065. Depending upon the average molecular weight of chitosan to be prepared, the temperature and length of time for which the hydrolysis is carried out may be adjusted accordingly.

It has been found that the average molecular weight (as calculated from the calibration curve obtained by gel permeation chromatography using dextran as a molecular weight standard) of the hydrolyzed chitosan used in the present invention is preferably within the range of 2,000 to 100,000. When the average molecular weight is less than 2,000, no prevention of a decrease in the sweetness of thaumatin is detectable, whilst, when the

molecular weight is more than 100,000, an astringent taste results. In a preferred embodiment, hydrolyzed chitosans of various molecular weight are mixed and the resulting mixture used to prevent a decrease in the sweetness of thaumatin.

There is a difference between chitosan and hydrolyzed chitosan in the amount effective in preventing a decrease in the sweetness of thaumatin. The amount of hydrolyzed chitosan required to produce such an effect is 0.0002-100 parts by weight to 1 part by weight of thaumatin, or, preferably, 0.0025-10 parts by weight to 1 part by weight of thaumatin, although the exact amount is dependent upon the molecular weight of the hydrolyzed chitosan. Thus, if the molecular weight of the hydrolyzed chitosan fraction is low, a correspondingly large amount may be used, without fear of any astringent taste resulting. For chitosan which has not been hydrolyzed, the amount of chitosan required to produce such a effect is 0.0002-100 parts by weight to 1 part by weight of thaumatin, or, preferably, 0.0025-1 part by weight to 1 part by weight of thaumatin.

It is also possible to use chitosan and hydrolyzed chitosan together in the present invention. When the method of the present invention is carried out in an aqueous solution at pH 6 or above, it is preferable to
5 use hydrolyzed chitosan because of its enhanced solubility.

The method of the present invention is usually conducted in an aqueous solution in which thaumatin is
10 present with other additives. There are a number of ways of preparing such a solution: (i) chitosan or hydrolyzed chitosan may be made into an acidic aqueous solution and thaumatin added thereto; (ii) chitosan or hydrolyzed chitosan may be dissolved in an (acidic)
15 aqueous solution of thaumatin followed by addition of other additives thereto; or (i) or (ii) may be added to an aqueous solution of the additives, whereupon the desired liquid preparation is obtained. To obtain a solid preparation, a solution containing the correct
20 amounts of thaumatin and chitosan or hydrolyzed chitosan may be spray-dried or lyophilized, and the resulting powder used as desired.

The method of the present invention may be applied to any of the fields in which thaumatin is used as a sweetening agent, including foods, drugs, cosmetics, feeds, and related uses.

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The present invention is illustrated by way of the following examples.

Example 1.

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I: Preparation of sample solutions.

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pH adjustment of each of the following sample solutions to pH 4 or pH 6 was made using 0.01N or 0.1N hydrochloric acid or sodium hydroxide.

a: Thaumatin solution (0.4% w/v) adjusted to pH 4 or pH 6 was prepared and kept at 4°C.

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b: Chitosan of average molecular weight 1.19×10^6 (hereinafter referred to as "chitosan A") was dissolved in 0.08% (w/v) of acetic acid solution, so as to make

the concentration of chitosan A 0.04% (w/v). Hydrolyzed
chitosan of average molecular weight 5.6×10^4
(hereinafter referred to as "hydrolyzed chitosan B")
was dissolved in 8% (w/v) acetic acid solution as as to
5 make the concentration of hydrolyzed chitosan B 4%
(w/v).

The pH of chitosan A solution of concentration 0.04%
(w/v) was adjusted to pH 4 to 6 followed by dilution
10 with water (adjusted to pH 4 or 6) to prepare chitosan
A solutions of concentration 4×10^{-8} , 4×10^{-7} ,
 4×10^{-6} , 8×10^{-6} , 4×10^{-5} , 1×10^{-4} , 2×10^{-4} ,
 4×10^{-4} , 8×10^{-4} , 8×10^{-3} and 0.04% (w/v). These
solutions were kept at 40°C.

15 The pH of hydrolyzed chitosan B solution of
concentration 4% (w/v) was similarly adjusted to pH 4
or 6, followed by dilution with water (adjusted to pH 4
or 6) to prepare hydrolyzed chitosan B solutions of
20 concentration 0.04, 0.4, 0.8, 2.0 and 4% (w/v). These
solutions were kept at 40°C. Aqueous solutions of
chitosan A of concentration 0.4% (w/v) and higher

concentrations were very highly viscous, and mixing with thaumatin solution was difficult. Accordingly, hydrolyzed chitosan B of low viscosity was used.

5 c: lamda-carrageenan solutions of concentration 0.08% (w/v), adjusted to pH 4, or 0.45% (w/v), adjusted to pH 6 were prepared and kept at 40°C. The amount of the lamda-carrageenan was chosen such that the sweet taste of thaumatin at a final concentration of 0.01%
10 (w/v) at pH 4 and 6 completely disappeared.

II: Mixing of the sample solutions.

Each of the sample solutions, which were adjusted to pH
15 4 or 6 and kept at 40°C, was mixed in accordance with the following procedures.

II-1: Effect of order of mixing of three sample solutions on the sweet taste of thaumatin.

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a: To 50 ml of thaumatin solution was added 50 ml of 0.04% (w/v) chitosan A solution, followed by mixing for

one minute. 100 ml of lamda-carrageenan solution was added and the mixture shaken at 40°C for 5 minutes.

5 b: To 50 ml of thaumatin solution was added 100 ml of lamda-carrageenan solution, followed by mixing for one minute. 50 ml of 0.04% (w/v) chitosan A solution was added and the mixture shaken at 40°C for 5 minutes.

10 c: To 50 ml of 0.04% (w/v) chitosan A solution was added 100 ml of lamda-carrageenan solution, followed by mixing for one minute. 50 ml of thaumatin solution was added and the mixture shaken at 40°C for 5 minutes.

15 The mixed solutions of a, b and c were heated at 80°C (liquid temperature) for 10 minutes and cooled down to 25°C in cold water at 10°C.

20 II-2: Effect of concentration of chitosan A or hydrolyzed chitosan B solution, in which the mixing order of the three sample solutions was the same, on the sweetness of thaumatin.

The mixing method was the same as that in the II-1 a
above. Thus, to 50 ml of thaumatin solution was added
50 ml each of chitosan A or hydrolyzed chitosan B
solution of various concentrations, followed by mixing
5 for one minute. 100 ml of lamda-carrageenan solution
was added and the mixture shaken at 40°C for 5
minutes.

The mixed solutions prepared in accordance with II-1
10 and II-2 above were heated at 80°C (liquid
temperature) for 10 minutes and cooled down to 25°C
in cold water at 10°C.

Control solution was prepared as follows. 50 ml of
15 thaumatin solution, 100 ml of lamda-carrageenan
solution and 50 ml of water (adjusted to pH 4 or 6 and
kept at 40°C) were mixed, the mixture shaken at
40°C for 5 minutes, heated at 80°C (liquid
temperature) for 10 minutes and cooled down to 25°C
20 in cold water at 10°C.

III. Organoleptic test of the sweetness of thaumatin.

a: Preparation of standard sweet solution.

Standard sweet solutions of thaumatin (pH being
5 adjusted to 4 or 6) of concentrations of 0 to 0.01%
(w/v) with a concentration interval of 0.001% (w/v)
were prepared.

b: Organoleptic method.

10 The sweetness of thaumatin solutions of concentration
0.01, 0.009, 0.008, ..., 0.001 and 0% (w/v) were defined
to be 100, 90, 80, ..., 10 and 0, respectively.

15 Test panels each comprising ten males and females were
set up. Instructions were given to the panellers that
they should choose a standard sweetness solution having
the same sweetness as that of each one of the mixed
sample solutions at pH 4 and 6. The results were
collected, subjected to verification by pair test and
20 the sweetness significant at $p < 0.05$ was judged to
be the sweetness of the mixed sample solution.

Table 1

Effect of the Mixing Order of the Sample Solutions on the Sweetness of Thaumatin

Sweetness of Thaumatin (%)

	Final Concn of Chito- san A % (w/v)	Final Concn of Thau- matin % (w/v)	Final Concn of λ -Carrageenan % (w/v)		pH 4		pH 6	
			at		Heating		Heating	
			pH 4	pH 6	Temperature (°C)		Temperature (°C)	
					40	80	40	80
Control Soln	-	0.01	0.04	0.225	0	0	0	0
Sample Soln a	0.01	0.01	0.04	0.225	100	90	100	100
Sample Soln b	0.01	0.01	0.04	0.225	30	30	80	80
Sample Soln c	0.01	0.01	0.04	0.225	30	30	90	90

Sample Soln a: Thaumatin $\rightarrow$ Chitosan A $\rightarrow$ λ -Carrageenan
 " b: Thaumatin $\rightarrow$ λ -Carrageenan $\rightarrow$ Chitosan A
 " c: Chitosan A $\rightarrow$ λ -Carrageenan $\rightarrow$ Thaumatin

Table 2

Effect of Concentrations of Solutions of A or B
(of Sample Solution a with the Same Mixing Order)
on Sweetness of Thaumatin

Type of Chi- to- san	Concn Ratio of A or B to Thau- matin (w/w)	Final Concn of A or B % (w/v)	Final Concn of Thau- matin % (w/v)	Final Concn of λ -Carra- geenan % (w/v) at		Sweetness of Thaumatin (%)			
				pH 4	pH 6	pH 4		pH 6	
						heated		heated	
						at	at	at	80°C
Control Soln	0:1	0	0.01	0.04	0.225	0	0	0	0
A	0.000001:1	1×10^{-3}	0.01	0.04	0.225	0	0	0	0
	0.00001 :1	1×10^{-7}	0.01	0.04	0.225	0	0	0	0
	0.0001 :1	1×10^{-4}	0.01	0.04	0.225	0	0	0	0
	0.0002 :1	2×10^{-5}	0.01	0.04	0.225	20	20	30	40
	0.001 :1	1×10^{-6}	0.01	0.04	0.225	40	30	50	60
	0.0025 :1	0.25×10^{-4}	0.01	0.04	0.225	80	70	90	100
	0.005 :1	0.5×10^{-4}	0.01	0.04	0.225	60	50	100	100
	0.01 :1	1×10^{-4}	0.01	0.04	0.225	60	50	100	100
	0.02 :1	2×10^{-4}	0.01	0.04	0.225	60	50	100	90
	0.1 :1	1×10^{-3}	0.01	0.04	0.225	80	70	100	100
	1 :1	0.01	0.01	0.04	0.225	100	90	100	100
B	1 :1	0.01	0.01	0.04	0.225	90	80	100	90
	10 :1	0.1	0.01	0.04	0.225	80	70	80	70
	20 :1	0.2	0.01	0.04	0.225	50	40	50	40
	50 :1	0.5	0.01	0.04	0.225	30	30	30	40
	100 :1	1.0	0.01	0.04	0.225	20	20	30	40

Note: A - Chitosan A
B - Hydrolyzed Chitosan B

Example 2.

I: Preparation of sample solutions.

5 a: Thaumatococcus solution (the same as that in Example 1).

b: Chitosan or hydrolyzed chitosan solution.

Hydrolyzed chitosan of average molecular weight 1×10^4 (hereinafter referred to as "hydrolyzed chitosan C"), hydrolyzed chitosan of average molecular weight 3.92×10^3 ("chitosan D"), hydrolyzed chitosan of average molecular weight 1×10^3 ("chitosan E") and chitosan A and hydrolyzed chitosan B used in Example 1 were used. Solutions adjusted to pH 4 or 6 containing 4×10^{-3} , 0.04 and 0.4% (w/v) of chitosan or hydrolyzed chitosan were prepared.

c: λ -carrageenan solution (the same as that in Example 1).

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2: Mixing of the sample solutions (the same as that in method II-a of Example 1).

5 3: Organoleptic test of the sweetness of thaumatin (the same as that in Example 1).

Table 3.
Effect of Degree of Decomposition of Chitosan
on Sweetness of Thaumatin

	Ratio of Concn of A, B, C, D or E to Thau- matin (w/w)	Final Concn of A, B, C, D, or E % (w/v)	Final Concn of Thau- matin % (w/v)	Final Concn of λ -Carrageenan % (w/v) at		Sweetness of Thaumatin (%) at			
				pH 4	pH 6	pH 4		pH 6	
						heated		heated	
						at	at	at	at
						40°C	80°C	40°C	80°C
Con- trol Soln	0:1	0	0.01	0.04	0.225	0	0	0	0
A	0.1:1	1×10^{-3}	0.01	0.04	0.225	80	70	100	100
	1:1	0.01	0.01	0.04	0.225	100	90	100	100
A and B (1:1)	10:1	0.1	0.01	0.04	0.225	90	80	100	100
B	0.1:1	1×10^{-3}	0.01	0.04	0.225	80	70	90	90
	1:1	0.01	0.01	0.04	0.225	90	80	100	90
	10:1	0.1	0.01	0.04	0.225	80	70	80	70
C	0.1:1	1×10^{-3}	0.01	0.04	0.225	70	60	80	70
	1:1	0.01	0.01	0.04	0.225	80	70	90	80
	10:1	0.1	0.01	0.04	0.225	70	60	80	70
D	0.1:1	1×10^{-3}	0.01	0.04	0.225	40	40	70	70
	1:1	0.01	0.01	0.04	0.225	40	40	70	70
	10:1	0.1	0.01	0.04	0.225	30	30	60	60
E	0.1:1	1×10^{-3}	0.01	0.04	0.225	0	0	0	0
	1:1	0.01	0.01	0.04	0.225	0	0	0	0
	10:1	0.1	0.01	0.04	0.225	0	0	0	0

Notes: A - Chitosan A
B - Hydrolyzed chitosan B
C - Hydrolyzed chitosan C
D - Hydrolyzed chitosan D
E - Hydrolyzed chitosan E

Example 3.

I: Preparation of sample solutions.

5 a: Thaumatin solution: (the same as that in Example 1).

b: Chitosan solution:

Solutions adjusted to pH 4 or 6 containing 4×10^{-4} and
0.04% (w/v) of chitosan A were prepared.

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c: Solutions of coloring agent or polysaccharide:

Solutions (adjusted to pH 4 or 6) containing 0.4% (w/v)
of xanthan gum, carboxymethylcellulose sodium, sodium
alginate or hydroxymethyl pectin; containing 0.01%

15 (w/v) of Tartrazine pigment; or containing 0.4% (w/v)
of lac dye, cochineal dye, red cabbage dye, annatto dye
and safflower dye were prepared and kept at 40°C.

III: Mixing of the sample solutions.

Each of the sample solutions, adjusted to pH 4 or 5
and kept at 40°C, was mixed in accordance with the
5 following procedures.

To 50 ml of thaumatin solution was added 50 ml each of
chitosan A solution of various concentrations, followed
by mixing for one minute.

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100 ml of polysaccharide solution or coloring agent
solution was added and the mixture shaken for 5
minutes, heated at 80°C (liquid temperature) for
10 minutes and cooled down to 25°C in cold water at
15 10°C.

Control solutions were prepared as follows. 50 ml of
thaumatin solution, 100 ml of solution of coloring
agent or polysaccharide and 50 ml of water (adjusted to
20 pH 4 or 6 and kept at 40°C) were mixed, the mixture
shaken at 40°C for 5 minutes, heated at 80°C

(liquid temperature) for 10 minutes and cooled down to 25°C in cold water at 10°C.

5 3: Organoleptic test of the sweetness of thaumatin was conducted in the same manner as in Example 1.

Table 4.

Effect of Polysaccharides and Coloring Agents
on Sweetness of Thaumatin

Sweetness of Thaumatin (%)													
Ratio of Chitosan A to Thaumatin Concns (w/w) heated at	pH = 4						pH = 6						°C
	0:1		0.01:1		1:1		0:1		0.01:1		1:1		
	40	80	40	80	40	80	40	80	40	80	40	80	
(Polysaccharides)													
Xanthan gum	0	0	70	60	100	100	10	0	90	100	100	100	
CMC-Na	0	0	60	50	100	90	10	0	70	80	100	100	
Na alginate	10	0	90	80	100	100	20	10	100	100	100	100	
High methoxylpectin	0	0	60	50	100	90	10	0	70	80	100	90	
(Coloring agents)													
Tartrazine	30	20	80	70	100	90	20	10	80	70	100	90	
Cochineal dye	50	50	70	70	90	80	40	40	90	90	90	90	
Lac dye	10	0	70	60	90	80	10	0	70	70	90	80	
Red cabbage dye	30	30	90	100	80	80	20	10	70	70	80	100	
Annatto dye	10	10	50	60	80	70	10	10	30	40	40	50	
Safflower dye	50	60	80	90	70	80	30	20	70	80	40	50	

(Notes) Final concentrations of the samples:

Thaumatococcus: 0.01%(w/v)
Chitosan A: 0.2%(w/v)
Polysaccharide: 0.2% (w/v)
Tartrazine: 0.01% (w/v)
Cochineal dye; lac dye; red cabbage dye; annatto dye;
and safflowerdye: 0.2% (w/v) each

Example 4.

I: Preparation of sample solutions.

- 5 a: Thaumatococcus solution (the same as that in Example 1 except that the solution was allowed to stand at room temperature prior to use).
- 10 b: Solution of chitosan or hydrolyzed chitosan:
A solution adjusted to pH 4 or 6 containing 0.04% (w/v) of chitosan A or hydrolyzed chitosan C and a 1:1 (by weight) mixture of chitosan A and hydrolyzed chitosan C were prepared and allowed to stand at room temperature.
- 15 c: lamda-carrageenan solution: (the same as that in Example 1 except that the solution was allowed to stand at room temperature prior to use).

II: Methods of mixing and drying of the sample solutions.

Each of the sample solutions, adjusted to pH 4 or 6 and
5 allowed to stand at room temperature, was mixed in
accordance with the following procedures.

To 500 ml of thaumatin solution was added 500 ml of
chitosan or/and hydrolyzed chitosan solution, followed
10 by mixing for 2 minutes.

II-1: lamda-carrageenan solution (100 ml) was added to
100 ml of mixed solution of thaumatin and chitosan
and/or hydrolyzed chitosan, the mixture shaken at
15 40°C for 5 minutes, heated at 80° (liquid
temperature) for 10 minutes and cooled down to 25°C
in cold water at 10°C.

II-2: A mixed solution (1,000 ml) of thaumatin and chitosan was:

- 5 (i) spray-dried using an atomizer having an inlet temperature of 160°C, outlet temperature of 80°C and a revolution speed of 25,000 rpm; or
(ii) lyophilized in vacuo (0.15 Torr) at -50°C for 48 hours.

10 The resulting dried product (0.04 g) was dissolved in water at room temperature to a final volume of 100 ml. To this was added 100 ml of lamda-carrageenan solution and the mixture shaken at 40°C for 5 minutes, heated at 80°C (liquid temperature) for 10 minutes and cooled down to 25°C in cold water at 10°C.

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III: Organoleptic test of the sweetness of thaumatin was conducted in the same manner as that in Example 1.

Table 5.

Effect of Drying Method for the Mixed Solution
of Thaumatin/Chitosan and/or Hydrolyzed Chitosan
on Sweetness of Thaumatin

Sweetness of Thaumatin (%)									
Form of Mixt of Thauma- tin/Chi- tosan and (or) Hydrol. Chitosan	Final Concn of Chi- tosan A %(w/v)	Final Concn of Hydro- lyzed Chito- san C %(w/v)	Final Concn of Thau- ma- tin %(w/v)	Final Concn of lamda- Carrageenan % (w/v)		pH 4 heated at		pH 6 heated at	
				at		40		40	
				pH 4	pH 6	80		80°C	
Control Soln	-	-	0.01	0.04	0.225	0	0	0	0
Mixed Soln.	0.01	-	0.01	0.04	0.225	100	90	100	100
	0.005	0.005	0.01	0.04	0.225	90	80	100	90
	-	0.01	0.01	0.04	0.225	80	70	90	80
Spray- dried Prod.	0.01	-	0.01	0.04	0.225	100	100	100	100
	0.005	0.005	0.01	0.04	0.225	100	90	100	100
	-	0.01	0.01	0.04	0.225	90	80	100	90
Lyophi- lized Prod.	0.01	-	0.01	0.04	0.225	100	90	100	100
	0.005	0.005	0.01	0.04	0.225	90	80	100	90
	-	0.01	0.01	0.04	0.225	80	70	90	80

Formulations

Formulation 1.

5 To 97 parts of water was added 1 part of glacial acetic
acid and, after mixing, 1 part of chitosan A was added
and the mixture stirred at room temperature until
dissolved. 1 part thaumatin was added and the solution
10 stirred until dissolved. The resulting solution was
spray-dried in an atomizer as described above having
inlet and outlet temperatures of 160°C and
80°C, respectively (hereinafter the spray-dried
product will be referred to as "dried
15 thaumatin-chitosan").

Dried thaumatin-chitosan A (0.04 part) was dissolved in
36.75 parts of water at room temperature, 1 part of
lamda-carrageenan was added and dissolved, and the
mixture was stirred with 42 parts of calcium secondary

phosphate, 18 parts of glycerol, 1.2 parts of sodium
laurylsulfate, 0.01 part of butyl p-hydroxybenzoate and
1 part of peppermint fragrance. The resulting
compositions were deaerated in vacuo and used to fill a
5 container, to give a tooth paste having good sweetness.

Formulation 2.

80.45 parts of corn, 12 parts of soybean cake, 5 parts
10 of fish powder, 1.5 parts of calcium phosphate, 0.5
parts of calcium carbonate, 0.5 parts of salt and 0.05
parts of dried thaumatin-chitosan A were mixed, the
mixture kneaded with 20 parts of 1% xanthan gum
solution, granulated using a granulator and dried by
15 heating to give a granular feed having good sweetness
and suitable as feed for young pigs.

Formulation 3.

0.04 parts glacial acetic acid was mixed with 87.09
parts of water, 0.02 part of hydrolyzed chitosan B was
5 dissolved therein at room temperature, 0.03 part of
thaumatin was similarly dissolved therein, and the
mixture heated at 80°C for 10 minutes, after adding
0.4 parts of sodium L-glutamate, 0.02 parts of sodium
5'-inosinate, 1 part of lamda-carrageenan and 0.2 parts
10 of carotene dye, the mixture was cooled to 60°C, 1
part of salt, 10 parts of brewed vinegar and 0.2 parts
of spices were added and the solution mixed until
dissolved, followed by cooling to give an oil-free
dressing having sweetness and a good taste.

CLAIMS

1. A method of preventing a substance capable of so
5 doing from causing a decrease in the sweetness of
thaumatin, characterized by adding chitosan, hydrolyzed
chitosan, or a mixture thereof, to an aqueous solution
containing thaumatin and said substance, in an amount
effective to prevent a decrease in the sweetness of
10 thaumatin.

2. A method as claimed in claim 1, wherein said amount
of chitosan, hydrolyzed chitosan, or mixture thereof,
is 0.0002 - 100 parts by weight to 1 part by weight of
15 thaumatin.

3. A method as claimed in claims 1 or 2, wherein said
amount of chitosan is 0.0025 - 1 parts by weight to 1
part by weight of thaumatin.

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4. A method as claimed in claims 1 or 2, wherein said
amount of hydrolyzed chitosan is 0.0025 - 10 parts by
weight to 1 part by weight of thaumatin.



5. A method as claimed in any of claims 1, 2 or 4, wherein the average molecular weight of the hydrolyzed chitosan, as measured by gel permeation chromatography, is 2000 - 100,000.

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6. A method as claimed in any of claims 1, 2, 4 or 5, wherein the hydrolyzed chitosan comprises a mixture of hydrolyzed chitosans of various molecular weights.

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7. A method as claimed in any of claims 1-6, wherein chitosan is added to the aqueous solution.

8. A method as claimed in any of claims 1-6, wherein hydrolysed chitosan is added to the aqueous solution.

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9. A method as claimed in any of claims 1-6, wherein a mixture of chitosan and hydrolysed chitosan is added to the aqueous solution.

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10. A method as claimed in any of claims 1-9, wherein the substance, present in the aqueous solution, which can decrease the sweetness of thaumatin is a coloring agent and/or a polysaccharide.



11. A method as claimed in any of claims 1-10, wherein
said colouring agent is selected from the group
consisting of Tartrazine, Sunset Yellow, Ponceau 4R,
lac dyes and cochineal dyes of the anthraquinone
5 series, red cabbage dyes of the anthocyanin series,
annatto dyes of the carotenoid series, safflower yellow
dyes of the flavonoid series, and mixtures thereof.

12. A method as claimed in any of claims 1-11, wherein
10 said polysaccharide is selected from the group
consisting of carrageenan, xanthan gum, sodium
carboxymethylcellulose, sodium alginate, high methoxyl
pectin, locust bean gum, guar gum, and mixtures
thereof.

13. A method as claimed in any of claims 1-12, wherein
15 the aqueous solution is substantially free of ascorbic
acid.

14. A composition in which a substance capable of so
20 doing, is prevented from causing a decrease in the
sweetness of thaumatin, comprising an aqueous solution
of said substance, thaumatin, and chitosan, hydrolyzed



chitosan or a mixture thereof, in an amount effective to prevent a decrease in the sweetness of thaumatin.

5 15. A composition as claimed in claim 14, wherein said amount of chitosan, hydrolyzed chitosan, or mixture thereof, is 0.0002 - 100 parts by weight to 1 part by weight of thaumatin.

10 16. A composition as claimed in claim 14 or claim 15, wherein said amount of chitosan is 0.0025 - 1 parts by weight to 1 part by weight of thaumatin.

15 17. A composition as claimed in claim 14 or claim 15, wherein said amount of hydrolyzed chitosan is 0.0025 - 10 parts by weight to 1 part by weight of thaumatin.

20 18. A composition as claimed in any of claims 14, 15 or 17, wherein the average molecular weight of hydrolyzed chitosan, as measured by gel permeation chromatography, is 2000 - 100,000.



19. A composition as claimed in any of claims 14, 15
17 or 18, wherein the hydrolyzed chitosan comprises a
mixture of hydrolyzed chitosans of various molecular
weights.

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20. A composition as claimed in any of claims 14-19,
wherein the substance which can decrease the sweetness
of thaumatin is a coloring agent and/or a
polysaccharide.

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21. A composition substantially as hereinbefore
described in any of examples 1-4 and formulations 1-3.

