AUSTRALIA 669495

PATENTS ACT 1990

PATENT REQUEST : STANDARD PATENT

I/We being the person(s) identified below as the Applicant(s), request the grant of a patent to the person(s) identified below as the Nominated Person(s), for an invention described in the accompanying standard complete specification.

Full application details follow:

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[54] Invention Title:

Water-soluble polysaccharide and a process for producing the same

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a member of the firm of
DAVIES COLLISON CAVE for
and on behalf of the
applicant(s)

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NOTICE OF ENTITLEMENT

We, **Fuji Oil Co., Ltd.**, the applicant/Nominated Person named in the accompanying Patent Request state the following:-

> The Nominated Person is entitled to the grant of the patent because the Nominated Person derives title to the invention from the inventors by assignment.

> The Nominated Person is entitled to claim priority from the basic application listed on the patent request because the Nominated Person made the basic application, and because that application was the first application made in a Convention country in respect of the invention.

DATED this TWENTY FOURTH day of NOVEMBER 1992

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a member of the firm of
DAVIES COLLISON
CAVE for and on behalf
of the applicant(s)

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WATER-SOLUBLE POLYSACCHARIDE AND A PROCESS FOR PRODUCING THE SAME

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(57) Claim

1. A water-soluble polysaccharide wherein its constituent sugar components comprise rhamnose, fucose, arabinose, xylose, galactose, glucose and uronic acid, and a degree of esterification of uronic acid is not more than 50%.

9. A process for preparing a water-soluble polysaccharide according to any one of claims 1 to 8, characterized in that it contains a demethoxylation step of water-soluble hemicellulose.

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COMPLETE SPECIFICATION

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INVENTION TITLE:

Water-soluble polysaccharide and a process for producing the same

The following statement is a full description of this invention, including the best method of performing it known to me/us:-

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to a water-soluble polysaccharide, more particularly, a water-soluble polysaccharide having stable protein particles when exposed to acid, and a process for producing such a polysaccharide.

2. Description of the Related Art

Polysaccharides are widely known, and water-soluble polysaccharides have very wide molecular weight ranges. However, if its molecular weight is too low, its ability to stabilise protein particles when exposed to acid is reduced, and if the molecular weight is too high, the viscosity becomes high, good palatability is not obtained and it is difficult to obtain a high concentration solution.

At the present time, pectin, guar gum, gum arabic, xanthan gum and the like are known food stabilizers. Pectin is extracted from fruit, guar gum is extracted from the cell wall of seeds of the acacias, gum arabic is prepared by refining sap and xanthan gum is prepared by fermentation. However, there are no examples of food stabilizers being prepared from the husks or cell walls of legumes such as soybean.

On the other hand, a polysaccharide derived from a soybean husk, its alkaline decomposition product and its enzymatic decomposition product are know. For example, in JP-A 3-236759, water-soluble polysaccharides are obtained by thermally hydrolyzing a water-insoluble fiber under acidic conditions. However, protein particle dispersions of these polysaccharides are not stable enough.

When pectin is utilized in a food or a beverage containing milk protein such as yoghurt, pectin is utilized as a stabilizer for milk protein under acidic conditions. However, due to its high viscosity, it is glutinous, and it does not have a pleasant taste.

SUMMARY OF THE INVENTION

As described above, polysaccharides having a low viscosity that function to stabilize protein particles under acidic conditions were not previously obtained, and a polysaccharide such as pectin, which has a known protein stabilizing effect, has a high viscosity and glutinous taste.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

One aspect of the present invention is a water-soluble polysaccharide wherein its constituent sugar components comprises rhamnose, fucose, arabinose, xylose, galactose, glucose and uronic acid, and the degree of esterification of uronic acid is not more than 50%.

Further according to the first aspect of the present invention is a water-soluble polysaccharide where in it further contains mannose and fructose as a constituent sugar component.

A second aspect of the present invention is a process for preparing such a polysaccharide characterized in that water-soluble hemicellulose is demethoxylated.

The raw materials utilized in the present invention can be any vegetable, however, unlike pectin, which is extracted from fruit, as a raw material from which hemicellulose having a low galacturonic acidic content is extracted, a husk or cell wall derived from legumes such as soybean is preferable as there is a stable supply of soybeans as a raw material. Furthermore, legumes such as soybean are a source of hemicellulose containing uronic acid, which is a sugar having a carboxyl group that promotes stability.

Furthermore, among legumes, soybeans are preferable as a raw material because soybean is a raw material of tofu (soybean curd) and soybean isolate, okara (soybean curd refuse), which is prepared as a by product when producing tofu. The by product, okara, is deproteinized, defatted and the husk and cell walls, which are sources of water-soluble hemicellulose, are purified to a certain degree.

A process for producing a water-soluble polysaccharide of the present invention is described below. In the process, the most important feature is that the methylated carboxyl group of uronic acid, which is constituent sugar component of water-soluble hemicellulose, is demethoxylated. For demethoxylation, an acid, alkali, or enzyme can be used, and it is preferable to use acid or alkali because it is inexpensive and simple. Alkali is most preferable because of the efficiency. Demethoxylation can be conducted during extraction of a water-soluble hemicellulose, but it is preferable to perform the demethoxylation before or after the said extraction.

In the case that demethoxylation is effected before extraction of a water-soluble hemicellulose, at first, the raw material is demethoxylated as follows. To the raw material, water is added and stirred. The amount of water depends on the water content contained in the raw material, 1 - 8 fold amount is suitable. The pH of this suspension is adjusted to 9 - 14, preferably 11 - 13 by alkali. An example of alkali comprises sodium hydroxide, potassium hydroxide, calcium hydroxide, ammonia water and the like. A stabilizing effect of this treatment is more efficient at a high pH level than at a low pH level. However, the lower the pH level, the higher the coloring. After a pH adjustment is made, the suspension is heated above room temperature, preferably above 50°C. The higher the temperature the greater the effect, however, the coloring is deeper when the temperature is higher.

Next, solid-liquid separation is effected by centrifugation, or press filtration and this separation process can be performed under the alkali conditions as stated above, or protein eluted from raw material by alkali can be recovered by isoelectric precipitation. The latter case is preferable because organic materials present in the liquid phase is low, and the problem involving wastewater treatment is minimized. Unlike a known method involving purification of a polysaccharide by deproteinization carried out at room temperature, in the present process, the presence of protein in solid phase is not critical.

Water-soluble hemicellulose is then extracted from the solid obtained above. A water-soluble hemicellulose can be extracted at any pH range, i.e., alkali, neutral, or acid condition. It is preferable to extract same at the isoelectric point of protein contained in this solid because it is efficient and pure. For example, when using okara, the extraction is conducted at a pH of 2 - 7, preferably at 3 - 6, more preferably at 4 - 5.5. The temperature during said extraction should be above room temperature, preferably above 80°C, more preferably 100 - 130°C. Optionally, water can be added to promote the extraction and stirring efficiency at this heat extraction step.

After extraction, a water-soluble fraction and a water-insoluble fraction are separated by centrifugation or press filtration. The separated water-soluble fraction contains water-soluble hemicellulose. This solution is decolored, deodorized, demineralized, and dried to obtain a water-soluble polysaccharide of the present invention. The stabilizing effect of the water-soluble polysaccharide of the present invention is similar to that obtained without decoloring, deodorizing and demineralizing steps.

Demethoxylation may be effected after extraction of water-soluble hemicellulose. Initially, hemicellulose is extracted and then water to effect demethoxylation is added to the extracted hemicellulose and stirred. The amount of water depends on the water content contained in the raw material, 1 - 5 fold amount is suitable. Preferably, the extraction of hemicellulose is affected at the isoelectric point of protein contained in the raw material similar to the extraction process above. The temperature of this extraction is above room temperature, preferably above 80°C, and particularly 100 - 130°C.

After extraction, water-soluble fraction and water-insoluble fraction are separated by centrifugation or press filtration to obtain a solution containing water-soluble hemicellulose as described above, and the water-soluble hemicellulose is then demethoxylated. This step is conducted as follows. At first, the pH of a solution containing water-soluble hemicellulose is adjusted to 9 - 14, preferably 11 - 13 by use of alkali. After adjusting the pH, the solution is heated above room temperature, preferably above 40°C. The higher the temperature and the higher the pH, the greater the effect and the coloring.

After demethoxylation, this solution is dried to obtain a water-soluble polysaccharide of the present invention.

The water-soluble polysaccharide obtained in the process of the present invention contains, as its constituent sugar component, rhamnose, fucose, arabinose, xylose, galactose, glucose and uronic acid and optionally mannose and fructose. The composition is usually 1 - 7 wt% of rhamnose, 2 - 8 wt% of fucose, 15 - 50 wt% of arabinose, 2 - 10 wt% of xylose, 25 - 60 wt% of galactose, not more than 4 wt% of glucose and 10 - 35 wt% of uronic acid, wherein the methyl ester of uronic acid is demethoxylated, and its degree of esterification is not more than 50%, preferably not more than 30%, particularly not more than 20%. The water-soluble polysaccharide containing this composition is suited for stabilization of protein particles.

The water-soluble polysaccharide obtained by the process of the present invention is fractionalized by gel filtration chromatography (G500, TOSOH Corp., eluate: 0.1M phosphate buffer, pH 6.8) to obtain A, B, and C fraction depending on the molecular weight. A fraction, the water-solubel polysaccharide, has a high molecular weight, and has an excellent stabilizing effect of protein particles under acidic conditions. B and C fraction have relatively low molecular weights, and insufficient stabilizing effect of protein particles, but they contribute reducing viscosity and may be contained in a small amount.

The water-soluble polysaccharide of the present invention exhibits an excellent stabilizing effect of protein particles and has a low viscosity by being constituted with this A, B and C fractions. The average molecular weight of A, B and C fractions measured using the following method (B method) are from hundreds of thousands to millions, tens of thousands, and thousands respectively. Sugar compositions of A and B fractions consist of uronic acid, rhamnose, fucose, arabinose, xylose, galactose, and glucose. Especially, A fraction contains mainly uronic acid. C fraction has a low molecular weight component consisting mainly of low molecular weight sugars and proteins.

Protein particles have an improved stabilising effect under acidic conditions when the weight proportion of the A fraction relative to the B and C fractions is increased.

The average molecular weight of a water-soluble polysaccharide of the present invention is from tens of thousands to millions, preferably from 50,000 to 1,000,000 by A method described below. The methyl ester of uronic acid contained therein should be demethoxylated, and its degree of esterification should be not more than 50%, preferably not more than 30%, and most preferably not more than 20%.

In the present invention, the proportion of polysaccharide is determined by the following analytical methods.

Uronic acid is determined by the Blumenkrantz method. Neutral saccharide is determined by the alditol-actetate method.

The average molecular weight of polysaccharide is determined by the following methods.

A method

Standard pullulan (manufactured by Syowa Denko K.K.) is used as a standard substance to measure the viscosity in 0.1M sodium nitrate according to intrinsic viscosity to determine the molecular weight.

B method

Standard pullulan (manufactured by Syowa Denko K.K.) is used as standard substance to prepare a standard curve of retention time of HPLC gel filtration chromatography (Tosoh-G500 OPWLX, eluate: 0.1M phosphate buffer, pH 6.8) and to determine the molecular weight from retention time of a sample from this standard curve.

The specific rotation (25°C) of a polysaccharide of the present invention is not less than 15, preferably from 20 to 70.

The protein content of the polysaccharide of the present invention is not more than 13 wt%, preferably not more than 9 wt%.

The ash content of the polysaccharide of the present invention is from 1 to 12 wt%, preferably from 2 to 8 wt%.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

The invention will be understood more readily with reference to the following examples; these examples are intended to illustrate the invention and do not to limit the scope thereof.

Preparation of water-soluble polysaccharide

Example 1

To okara obtained during the preparation of soybean isolate, an amount of water was added, and sodium hydroxide was added to this suspension to adjust the pH to 12. The suspension was then heated to 60°C for 1 hour, and thereafter centrifuged (5000G, 10 min) to separate the supernatant and the alkali treated okara.

To this okara, an equal volume of water was added, and the pH was adjusted to 5 using hydrochloric acid, and the suspension was heated to 120°C for 1 hour in an autoclave to extract water-soluble hemicellulose. After extraction, the suspension was centrifuged (5000G, 10 min) to separate the water-soluble fraction containing mainly water-soluble hemicellulose. The water-soluble fraction was dried by a spraydryer to obtain water-soluble polysaccharide (1).

Example 2

To okara obtained during the preparation of soybean isolate, an amount of water was added, sodium hydroxide was added to this suspension to adjust the pH to 12. And to this suspension, hydrochloric acid was added to adjust the pH to 5. The mixture was then centrifuged (5000G, 10 min) to separate the supernatant and the alkali treated okara.

This okara, to which solubilized protein was resettled, contains most of okara's protein. To this okara, an equal volume of water was added, and heated to 120°C for 1 hour in an autoclave to extract water-soluble hemicellulose. After extraction, this suspension was centrifuged (5000G, 10 min) to separate the water-soluble fraction containing mainly water-soluble hemicellulose, and the water-soluble fraction wad dried by a spraydryer to obtain a water-soluble polysaccharide (2).

Example 3

To okara obtained during the preparation of soybean isolate, an amount of water was added, and hydrochloric acid was added to this suspension to adjust the pH to 5. The suspension was then heated to 120°C for 1 hour in an autoclave, and thereafter centrifuged (5000G, 10 min) to separate a water-soluble fraction containing mainly water-soluble hemicellulose.

To this solution containing water-soluble hemicellulose, sodium hydroxide was added to adjust the pH to 12. The mixture was then heated to 90°C for 30 min. The generated precipitate was removed, and hydrochloric acid was added to neutralized (pH 7). The solution was then dried to obtain water-soluble polysaccharide (3).

Example 4

By using okara, which is a byproduct of tofu preparation, water-soluble polysaccharide (4) was obtained using a method similar to that of Example 1.

Example 5

To the okara obtained during the preparation of soybean isolate, an amount of water was added, and hydrochloric acid was then added to the suspension to adjust the pH to 5. The suspension was then heated to 120°C for 1 hour in an autoclave, and thereafter centrifuged (5000G, 10 min) to separate water-soluble fraction containing mainly water-soluble hemicellulose. The solution was then dried to obtain water-soluble polysaccharide (5).

Example 6

To okara obtained during the preparation of a soybean isolate, an amount of water was added, and sodium hydroxide was added to this suspension to adjust the pH to 12. The suspension was then stirred without heating, and thereafter centrifuged (5000G, 10 min) to separate the supernatant and the alkali treated okara.

To this okara, an equal volume of water was added, and the pH was adjusted to 5 using hydrochloric acid. The suspension was then heated to 120°C for 1 hour in an autoclave to extract water-soluble hemicellulose, and after extraction, the suspension was centrifuged (5000G, 10 min) to separate water-soluble fraction containing mainly water-soluble hemicellulose. The water-soluble fraction was then dried by a spraydryer to obtain water-soluble polysaccharide (6).

The sample of water-soluble polysaccharides (1) - (3), (5) and (6) are prepared in a 10% solution (pH 7), and its infrared spectrum is measured by a spectrometer FT-300 (Horiba Seisakusho). The transmittance thereof is shown in Table 1.

Table 1 Transmittance of infrared spectrum (%)

sample	frequency (cm^{-1})			
	1740	1594	1415	1240
polysaccharide (1)	100.3	92.9	87.8	94.3
(2)	99.6	92.8	87.9	92.6
(3)	98.7	93.1	88.0	93 6
(4)	98.9	93.2	88.1	93.5
(5)	96.6	95.9	89.3	90.0
(6)	97.7	94.6	89.5	90.3

Comparing the water-soluble polysaccharide (1) - (3) obtained using a process of the present invention with water-soluble polysaccharide (5) and (6) obtained by heat extraction without an alkali treatment or heat extraction after an alkali treatment at room temperature, absorbance at 1240, 1420, 1600 and 1740 cm^{-1} are different. That is, in polysaccharide (5) obtained without an alkali treatment and polysaccharide (6) obtained using an alkali treatment at room temperature, absorbance at 1240 and 1740 cm^{-1} is high, which indicates the presence of methyl ester. While in polysaccharides (1) - (3) obtained using process of the present invention, absorbance is not detected. Conversely absorbance at

1420 and 1600 cm^{-1} is considered carboxylic group absorbance, and (1) - (3) are higher than that of (5) and (6). These results suggest that the methyl esterificated carboxylic group of uronic acid is demethoxylated by an alkali treatment when heated.

Composition and sugar composition of water-soluble polysaccharide (1), (2) and (5) were measured, and the results are shown in Table 2 and Table 3.

Table 2

component	(1)	(2)	(3)
water	4.4	3.6	5.1
protein	2.4	7.4	5.4
ash	6.4	9.3	5.3
polysaccharides	86.8	79.7	84.2
specific rotation (25°C)	+39.9	+27.3	+37.4

Table 3

sugar	(1)	(2)	(3)
uronic acid	20.1	18.7	19.4
rhamnose	4.1	4.4	2.1
fucose	3.5	3.7	3.9
arabinose	21.6	21.9	23.1
xylose	4.3	4.0	5.8
galactose	45.2	47.1	43.4
glucose	1.2	1.2	2.3
degree of esterification of uronic acid	14.5	18.9	63.6

These results indicate that the methyl esterificated carboxylic group of uronic acid is demethoxylated by an alkali treatment when heated.

The degree of esterification is determined using a method for measuring the degree of esterification of pectin. Namely, using a sample before and after demethoxylation by alkali, the data was obtained from the following equation.

Degree of Esterification (DE) = $V_2/(V_1 + V_2) \times 100$ wherein V_1 represents amount of 0.1N NaOH (ml) when the uronic acid sample is made free by using isopropyl alcohol containing hydrochloric acid, and washed by isopropyl alcohol to prepare a sample not containing hydrochloric acid, and this sample is titrated by NaOH using phenolphthalein as an indicator; V_2 represents amount of 0.1N NaOH (ml) when 0.5N NaOH is added to the above titrated sample solution, then, an amount of HCl in the same volume as that of NaOH used in demethoxylation is added, and this sample is titrated in the same way as V_1.

<u>Effect on protein particles stabilization</u>

Acid milk beverages are prepared using water-soluble polysaccharide (1) - (6) and pectin used as a protein stabilizer for acid milk beverage presently. And the condition and viscosity of the acid milk beverage are measured after 7 days of preparation.

Acid milk beverages were prepared according to the following method.

(i) Preparation of yogurt

Skim milk was added to water (21%), heated and stirred, and sterilized at 95°C. After cooling, as a starter, commercially available plain yogurt was injected into the mixture, and fermented in a thermostatic chamber at 38°C. After grinding the fermented yogurt to homogenize the curd, the mixture was cooled to 10 - 15°C.

(ii) Preparation of stabilizer solution

2% solution of water-soluble polysaccharide was heated while stirring (80°C, 10 min), and cooled to 25°C.

(iii) Preparation of acid milk beverage

Sugar was added to the water to prepare a sugar solution. Into this solution, a stabilizer solution and yogurt were added, and the pH of this mixture was adjusted by the addition of lactic acid and sodium citrate. This mixture was then homogenized by a homogenizer (150 kg/cm^2), bottled, and stored in a refrigerator for one week. The percentage of the components were 20% stabilizer solution, 40% yogurt, 7% sugar, and 33% water. The results are shown in Table 4.

Table 4

stabilizer	pH of acid milk beverage	condition after 7 days	viscosity of after 7 days(cps)
(1)	4.5	stabilized	11
	4.2	stabilized	10
	4.0	stabilized	10
(2)	4.5	stabilized	12
	4.2	stabilized	10
	4.0	stabilized	11
(3)	4.5	stabilized	12
	4.2	stabilized	11
	4.0	stabilized	11
(4)	4.5	agglutinated	82
	4.2	stabilized	11
	4.0	stabilized	11
(5)	4.5	agglutinated	100
	4.2	agglutinated	75
	4.0	agglutinated	20
(6)	4.5	agglutinated	120
	4.2	agglutinated	80
	4.0	agglutinated	15
pectin	4.5	stabilized	30
	4.2	stabilized	26
	4.0	stabilized	26

From these results, it is apparent that the acid milk beverage is stabilized in the range above pH 4.0 using water-soluble polysaccharide of the present invention, and the acid milk beverage containing water-soluble polysaccharide of the present invention has low viscosity and a good taste.

Although the preferred form of the present invention has been described, it is understood that modifications will be apparent to those skilled in the art, without departing from the spirit of the invention, and thus the scope of the invention is to be determined solely by the following claims.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A water-soluble polysaccharide wherein its constituent sugar components comprise rhamnose, fucose, arabinose, xylose, galactose, glucose and uronic acid, and a degree of esterification of uronic acid is not more than 50%.

2. A water-soluble polysaccharide according to claim 1, wherein said water-soluble polysaccharide further comprises mannose and fructose as a constituent sugar component.

3. A water-soluble polysaccharide according to claim 1 or claim 2, wherein a proportion of its constituent sugar components are 1 - 7 wt% rhamnose, 2 - 8 wt% fucose, 15 - 50 wt% arabinose, 2 - 10 wt% xylose, 25 - 60 wt% galactose, not more than 4 wt% glucose and 10 - 35 wt% mixture of uronic acid and uronic acid methyl ester.

4. A water-soluble polysaccharide according to anyone of claims 1 to 3, wherein the molecular weight is from 50,000 to 1,000, 000.

5. A water-soluble polysaccharide according to any one of claims 1 to 4, wherein the degree of esterification of uronic acid is not more than 30%.

6. A water-soluble polysaccharide according to any one of claims 1 to 4, wherein the degree of esterification of uronic acid is not more than 20%.

7. A water-soluble polysaccharide according to any one of claims 1 to 6, wherein it is prepared from vegetables.

8. A water-soluble polysaccharide according to any one of claims 1 to 6, wherein it is prepared from legumes.

9. A process for preparing a water-soluble polysaccharide according to any one of claims 1 to 8, characterized in that it contains a demethoxylation step of water-soluble hemicellulose.

10. A process according to claim 9, wherein the demethoxylation step is conducted before or after extraction of the water-soluble hemicellulose.

11. A process according to claim 9 or 10, wherein the demethoxylation step comprises adding alkali to a solution of water-soluble hemicellulose to effect a pH of 9 - 14, and heating this mixture above 40°C.

12. A process according to any one of claims 9 to 11, wherein the water-soluble hemicellulose is obtained by heat extracting a raw material and separating a water-soluble fraction.

13. A process according to claim 12, wherein heat extraction step is carried out at a pH of form 3 to 6 and a temperature of from 80°C to 130°C.

14. A process according to claim 12 or claim 13, wherein a raw material from which a water-soluble hemicellulose is extracted is vegetable.

15. A process according to claim 12 or claim 13, wherein a raw material from which a water-soluble hemicellulose is extracted is legumes.

16. A process according to claim 12 or claim 13, wherein a raw material from which a water-soluble hemicellulose is extracted is soybean.

17. An acid milk beverage containing a water-soluble polysaccharide according to any one of claims 1 to 8 as a stabilizer for protein.

18. A water-soluble polysaccharide, a processing for making same and/or a

product incorporating same substantially as hereinbefore described with reference to the Examples.

Dated this 11th day of July, 1995

Fuji Oil Co., Ltd.
by DAVIES COLLISON CAVE
Patent Attorneys for the Applicant(s)

ABSTRACT OF THE DISCLOSURE

There is disclosed a water-soluble polysaccharide composed of rhamnose, fucose, arabinose, xylose, galactose, glucose and uronic acid, and a degree of esterification of uronic acid is not more than 50%. This water-soluble polysaccharide is useful as a protein stabilizer. A process for preparing the same and food containing the same is also disclosed.