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(54) **REDUCED MOLECULAR WEIGHT GALACTOMANNANS OXIDIZED BY GALACTOSE OXIDASE**

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(57) **ABSTRACT**

Presented are compositions of reduced molecular weight galactomannans, particularly guar gum, which have been oxidized by the enzyme galactose oxidase. Further, the invention relates to a process for enzymatically reducing the molecular weight of a galactomannan wherein the galactomannan is simultaneously or subsequently oxidized using galactose oxidase, optionally in combination with other enzymes including peroxidases and or catalases. This process enables production of high concentrations of oxidized galactomannans, which have particular use in the paper making industry.

REDUCED MOLECULAR WEIGHT GALACTOMANNANS OXIDIZED BY GALACTOSE OXIDASE

[0001] This application is related to U.S. Provisional patent application Ser. No. 60/222,869, filed Aug. 3, 2000 from which priority is claimed.

FIELD OF THE INVENTION

[0002] This invention relates to reduced molecular weight galactomannans, particularly guar gum, which have been oxidized by galactose oxidase. A particular aspect of the invention additionally relates to a novel process for enzymatically reducing the molecular weight of the galactomannan using mannanase, wherein the galactomannan is simultaneously or subsequently oxidized using galactose oxidase. This preferred aspect of the invention enables the making of novel compositions comprising high concentrations of reduced molecular weight galactomannans which have been enzymatically oxidized by galactose oxidase.

BACKGROUND OF THE INVENTION

[0003] Seed galactomannans, because of their viscous properties, have long found use as thickening agents and binding or colloidal holding agents in a number of fields, including as food additives, commercial lubricants, and paper additives. However, the use of these inherently viscous materials has always been subject to intrinsic limitations because the viscosity of the native galactomannans is too high to permit use of the compounds in any but dilute concentrations. Further, it has traditionally been difficult and or expensive and thus commercially impractical to chemically modify the properties of these compounds because of the need to effectively carry out these reactions at low concentrations. The present invention addresses this need by providing a commercially efficient means to produce high concentrations of chemically modified galactomannans; most particularly, highly concentrated solutions of low molecular weight oxidized guar are provided which exhibit excellent properties of temporary wet strength in papermaking applications.

[0004] Oxidation of galactomannans, particularly when achieved enzymatically using galactose oxidase, is known to introduce aldehyde groups on the galactose residues within the galactomannans. It is known further that aldehyde-containing galactomannans, in aqueous solution, tend to form crosslinks. Frollini et al, Carbohydrate Polymers 27 (1995) pp. 129-135, and C. Burke (ed.) Carbohydrate Biotechnology Protocols, (1999), Humana Press, (N.J.) p. 79. Galactomannan compositions are known to be useful in the papermaking industry. For example, see U.S. Pat. Nos. 5,633,300; 5,502,091; 5,338,407 and 5,318,669.

[0005] Using galactose oxidase to oxidize the galactomannan gums, especially guar gum has been reported. U.S. Pat. No. 3,297,604 (Germino 1967) discloses galactose-containing polysaccharides which are oxidized chemically or enzymatically with galactose oxidase. U.S. Pat. No. 5,554,745 (Chiu 1996) and U.S. Pat. No. 5,700,917 (Chiu 1997) describe an enzymatic oxidation process using a dual-enzyme system (galactose oxidase and catalase) to convert a cationic guar gum to an aldehyde derivative at the C6 position of the galactose side chain in the guar at 1% solids concentration. The guar gum was not enzymatically degraded prior to the enzymatic oxidation. In fact, efforts were made to preserve the molecular weight of such an oxidized cationic gum. Frollini et al (supra) and C. Burke (supra) reported similar enzymatic oxidation of guar gum. However, none of these disclosures report the oxidation of gum hydrolyzates or a solution of such oxidized gum hydrolyzates at a solids concentration higher than about 1%.

[0006] Use of mannanase to hydrolyze galactomannan gums such as guar gum has been practiced for at least five decades. Whistler described in 1950 (Whistler et al, J. of Chemical Society 72 (1950) 4938-4939) enzyme preparations from germinated guar seeds that caused rapid decrease in viscosity of a guar gum solution. McCleary (Carbohydrate Research, 71 (1979) 205-230) used mannanase to hydrolyze guar gum in order to analyze the fine structure of the gum. Japanese patent Hei 10 [1998]-36403 describes cationized decomposed galactomannans useful in the cosmetic industry. Japanese patent Sho 55 [1980]-27797 describes a method for producing low viscosity guar using mannanase. EPA 0 557627 A1 (1992) discloses a method of hydolyzing guar with mannanase to produce a food grade gum, and U.S. Pat. No. 4,693,982 (Carter 1987) discloses a method of treating solid guar gum particles with hydrolytic enzymes to reduce molecular weight and thereby improve solubility.

SUMMARY OF THE INVENTION

[0007] The present invention provides galactomannan compositions having a reduced molecular weight wherein the galactomannans are enzymatically oxidized by galactose oxidase.

[0008] The preferred galactomannans include guar, locust bean and tara gum, with guar being most preferred. The preferred reduced molecular weight of the guar will range from about 1,000 to about 500,000, while more preferred ranges are from about 10,000 to 400,000, from about 50,000 to about 350,000 and from about 70,000 to about 350,000, and from about 70,000 to about 150,000 daltons.

[0009] The molecular weight of the galactomannans of the invention can be reduced in a number of ways, including with acid treatment, enzymatic treatment and treatment with hydrogen peroxide at high temperature being three preferred methods. One of the most preferred means of reducing the molecular weight of the galactomannan is enzymatically, with mannanase being the most preferred enzyme.

[0010] The reduced molecular weight galactomannans of the invention are enzymatically oxidized by galactose oxidase, which acts to oxidize the C6 carbon of the galactose residues of the galactomannan to yield an aldehyde group. The preferred reduced molecular weight, enzymatically oxidized galactomannan is guar, having a preferred range of oxidation of from about 5% up to about 100% of the C6 carbon atoms of the galactose residues being oxidized. More preferred ranges are wherein the galactose oxidase oxidizes from about 15% to about 70% of the galactose C6 carbon atoms, from about 15% to about 60% of the galactose C6 carbon atoms while the most preferred range of oxidation is from about 30% to about 45% of the C6 galactose carbon atoms being oxidized. Optionally, the enzymatic oxidation using galactose oxidase can be carried out in the presence of one or more additional enzyme activities, with catalase activity and peroxidase activity being most preferred.

[0011] The reduced molecular weight galactomannans of the invention can be in derivatized form, with cationic derivative groups being most preferred. Derivatization of the galactomannan can take place prior to molecular weight reduction or after.

[0012] In a preferred aspect of the invention, the reduced molecular weight, enzymatically oxidized galactomannan is made by a process comprising enzymatic molecular weight reduction using mannanase, wherein the process comprises adding the galactomannan to a prepared solution of mannanase with stirring, and subsequently or simultaneously providing galactose oxidase to oxidize the reduced molecular weight galactomannan. In this method of the invention it is possible to achieve novel compositions comprising enzymatically oxidized galactomannans of reduced molecular weight at high concentrations.

[0013] In this process of the invention the preferred galactomannan is guar, which can be made in oxidized form to a preferred concentration range of from about 1.5% to about 80%. More preferred ranges include from about 1.5% to about 20%, with the most preferred range being from about 2% to about 10%. The preferred reduced molecular weight range of the guar in this process of the invention is about 1,000 to about 500,000, with more preferred ranges include about 10,000 to 400,000, from about 50,000 to about 350,000, from about 70,000 to about 350,000, and from about 70,000 to about 150,000 daltons.

[0014] The process of the invention yields a preferred range of oxidation of guar including from about 5% up to about 100% of the C6 carbon atoms of the galactose residues being oxidized, with a more preferred range of about 15% to about 70%, with an even more preferred range of about 15% to about 60%, while the most preferred range is from about 30% to about 45%. Additionally, in this process of the invention the galactomannan can be in derivatized form.

DETAILED DESCRIPTION OF THE INVENTION

[0015] The present invention provides new compositions comprising low molecular weight galactomannans, particularly guar, that have been oxidized by galactose oxidase. The invention further provides a preferred method of making the oxidized reduced molecular weight galactomannans using mannanase, wherein the method is capable of producing novel compositions comprising oxidized galactomannans at commercially desirable concentrations exceeding 1.5%.

[0016] There are several distinct advantages of the compositions of the inventions. (1) First, these compositions can be made at higher concentrations than can be achieved with galactomannans at their native molecular weight. For guar gum and its derivatives, for example, it is normally difficult to make solutions at much higher than 1% because of high viscosity. Commercially useful gums in solution form can be shipped more conveniently and less expensively at higher concentrations, and are ready to use in solution form. (2) Second, a higher level of enzymatic oxidation at the C6 carbon of galactose can be attained using reduced molecular weight galactomannans because the enzyme oxidation is less inhibited by gel formation. For neutral guar gum at 1%, for example, oxidation beyond 20% is difficult because gel formation limits the oxidation. In contrast, a reduced molecular weight guar at 1% (and higher) can be enzymatically oxidized up to a level of more than 40%. (3) A third advantage is that a liquid gum product with good enzymatic oxidation can be achieved at high concentrations. For example, native guar (molecular weight approximately 2 million) at 1% concentration and 20% oxidation of galactose C6 is a gel, whereas guar at 70,000 molecular weight and 5% concentration is a flowable liquid at up to 35-40% oxidation. (4) And finally, the lower molecular weight of the compositions of the invention provides better wet strength decay in temporary wet strength applications in paper. High initial wet strengths can be obtained with the low molecular weight compositions, but a particular advantage of the low molecular weight composition is that the wet strength is lost more quickly and to a greater extent on contact with water than for the corresponding high molecular weight oxidized gums.

[0017] These compositions can be especially useful, therefore, in a variety of temporary wet strength applications in paper, such as in tissue and towel. For bathroom tissue, for example, good wet strength decay prevents pipes from getting clogged. Other paper uses include situations where it is advantageous to achieve improved dry strength, z-direction tensile, Scott bond, Mullen burst, ring crush, STFI, tensile energy absorption (TEA), fracture toughness, and possibly sizing enhancement. This would include uses in paper coating, liquid packaging board, virgin and recycled linerboard, lightweight coated paper, fine paper, and newsprint. Application of these compositions can be at the wet end or after the wet end, such as at the size press or in a spray application. Other possible uses include cosmetics, oilfield recovery, construction, adhesives, tablet coating, paint, textiles, toys, and removable adhesives.

[0018] The galactomannans of the invention are well-known polysaccharide materials generally derived from seed gums. The commercially important galactomannans are locust bean gum, guar gum and tara gum. Galactomannans are structurally linear polysaccharides based on a backbone of β(1-4)-linked D-mannose residues. Single α-D-galactose residues are linked to the mannose chain by C1 via a glycosidic bond to C6 of mannose. The degree of galactose substitution on the mannose backbone varies depending on the botanical source of galactomannan. In locust bean gum, the average galactose to mannose ratio is 1:4; in tara gum the ratio is approximately 1:3; and for guar, the most preferred galactomannan of the invention, the ratio of galactose to mannose is approximately 1:2.

[0019] Within the context of the present disclosure Applicants intend to include various derivatized forms of galactomannans within the scope of the invention. Derivatives of the galactomannans are very well known in this art, and many are commercially available. Roy L. Whistler and James N. Bemiller, ed., Industrial Gums; Polysaccharides and Their Derivatives (Third Edition), Academic Press, New York, 1993. For example, the most common commercially available guar derivatives include hydroxypropyl, carboxymethyl, carboxymethyl-hydroxypropyl, and 2-hydroxy-3-(trimethylammonium chloride) propyl. Other common derivatives include hydroxyethyl, ethyl, guar gum phosphates, and mixed derivatives including mixed cationic and anionic (amphoteric).

[0020] Also within the context of the present disclosure Applicants intend to include within the scope of the invention galactomannans which have been treated with various

wetting and solubility agents. Many such agents are known in this art. For example, galactomannan products can be mixed with glyoxal or borax to reversibly crosslink the surface of the particles and retard hydration. Glyoxalated guar requires pH of 7 or above to hydrate, while borated guar requires pH below 8 for hydration. These and other treated and coated forms of the basic galactomannans are considered to be within the scope of Applicants' invention.

[0021] For purposes of clarity in describing the present invention, Applicants use the term 'reduced molecular weight' of the various galactomannans to refer to a galactomannan which exists in a form having an average molecular weight which is a fraction of its native molecular weight. For example, for the preferred galactomannans of the invention; guar, locust bean and tara, the reduced molecular weight refers to a value which is approximately one half or less than the native molecular weight. Locust bean (carob) gum has a native molecular weight generally reported to be in the range of about 300,000 to 360,000 daltons. The preferred reduced molecular weight range of the compositions of the invention for locust is about 1,000 up to about 150,000 daltons. The most preferred galactomannan of the invention, guar, is known to have a native molecular weight of approximately 2,000,000 daltons. The preferred reduced molecular weight range of the compositions of the invention for guar is from about 1,000 to about 500,000 daltons. More preferred molecular weight ranges for guar include about 10,000 to about 400,000 and from about 50,000 to about 350,000 and from about 70,000 to about 350,000, and from about 70,000 to about 150,000 daltons. Another preferred galactomannan of the invention is tara gum. Definitive molecular weight ranges for native tara gum have not been reported, but it is believed that the native molecular weight is between the value for native guar and native locust bean. Thus, with respect to tara gum in the instant invention, the term reduced molecular weight would refer generally to a range of about 1000 daltons up to a value representing about one half of tara gum's native molecular weight.

[0022] Within the context of describing molecular weight of galactomannans for the present disclosure, the term molecular weight refers to the weight average. More particularly, the weight average molecular weight refers to a value which is measured by size exclusion chromatography analysis (SEC) using a calibration derived from narrow distribution polyethylene oxide (PEO) and polyethylene glycol (PEG) molecular weight standards.

[0023] Also, it is within the scope of Applicants' invention for an individual galactomannan composition to comprise more than a single botanical species of galactomannan. In some commercial applications for these gums, final characteristics of the compositions can be improved and or tailored to specific purposes by using a mixture of one of more galactomannan. Further, the oxidized reduced molecular weight galactomannan compositions of the invention may comprise additional ingredients, as appropriate and advantageous for the intended purpose of the material. Many additives useful in galactomannan compositions are well known in these arts, including, for example bentonite, alum, starch, cationic polymers, sizing agents, wet strength additives, debonder, defoamers and biocides, any of which might be used to impart additional characteristics for a particular intended purpose of a composition of the invention.

[0024] The molecular weight of the galactomannans can be reduced by a variety of methods, including acid treatment, enzyme treatment, and heating with hydrogen peroxide. These methods are well known in this art. For example, acid treatment and heating with hydrogen peroxide are methods known to reduce the molecular weight of the galactomannans; Roy L. Whistler and James N. Bemiller, ed., Industrial Gums; Polysaccharides and Their Derivatives (Third Addition), Academic Press, New York, 1993; and U.S. Pat. No. 5,480,984. Commercial preparations of reduced molecular weight galactomannans that are made by these methods are also available; for example, Galactosol® 30M1F (Hercules, Inc., Wilmington, Del.).

[0025] A particularly preferred method of reducing the molecular weight of galactomannan is accomplished using the enzyme mannanase. Mannanase, which has been well characterized in the art, is known to hydrolyze mannans (mannan endo-1, 4-β-mannosidase E.C. 3.2.1.78) wherein the endo-mannanase randomly cleaves 1,4-β-D mannosidic linkage in mannans. See, for example, European Patent Application 0 557 627A1 or McCleary, Carbohydrate Research 71 (1979), pp. 205-230. Mannanase activity can be provided in the form of purified mannanase enzyme, or alternatively, mannanase activity can be provided by using one of the commercial preparations of hemicellulases or cellulases which are known to contain mannanase activity. Examples of such commercially available preparations include Hemicellulase GM™ sold by Amano; Enzebo™ cellulase CRX sold by Enzyme Development Corp. of N.Y.; and Gamanase 1.OL sold by Novo Nordisk.

[0026] The compositions of Applicants' invention comprise galactomannan having reduced molecular weight wherein the galactomannan is enzymatically oxidized by galactose oxidase. In a well-characterized reaction mechanism, galactose oxidase is known to specifically oxidize the C6 carbon atom of the galactose residues, wherein the alcohol OH group is oxidized to an aldehyde C=O group. Mazur, A. W. ACS Symposium Series, 466 (1991) 99; U.S. Pat. No. 3,297,604 (Germino); and Knowles, P. F. and Ito,N., Perspectives in BioOrganic Chem., Vol.2,207-244, JAI Press LTD (1993). Galactose oxidase may be produced by the fungus *Dactylium dendroides,* recently renamed Fusarium sp., and has been given the E. C. Number 1. 1.3.9. Within the context of the compositions of Applicants' invention, oxidation of the C6 of galactose by galactose oxidase is accomplished on about 5% up to about 100% of the C6 carbon atoms on the galactose residues. More preferred ranges of oxidation are about 15% up to about 70%; about 15% up to about 60%; with a range of about 30% up to about 45% being most preferred.

[0027] The oxidation process can be accomplished using an effective amount of the single enzyme galactose oxidase, however, in a preferred aspect the oxidation reaction can be improved by incorporating a catalase activity and or a peroxidase activity in the galactose oxidase reaction mixture. The presence of either or both of these additional activities can improve the effectiveness of the oxidation reaction, and enables effective oxidation more efficiently and less expensively when commercial quantities of oxidized galactomannan are desired. The increased catalytic activity of galactose oxidase in the presence of a peroxidase and catalase has been shown by Radin, et al., in The Use of Galactose Oxidase_in Lipid Labeling, J. Lipid Res., Vol.

22:536-541, (1981). Applicant has discovered, with respect to the oxidation of galactomannans, that the activity level of galactose oxidase can be increased, i.e., it can be continuously activated, by carrying out the reaction in the presence of a one-electron oxidant such as peroxidase or laccase, together with a hydrogen peroxide remover such as catalase. Galactose oxidase in combination with catalase has been reported in the oxidation of galactomannans, but Applicants have improved this reaction by the addition of a peroxidase activity, wherein surprisingly, the oxidation reaction becomes more economically efficient for large scale commercial applications, even when the three-enzyme system is used.

[0028] This separate invention regarding improving the activity level of galactose oxidase by the addition of a one-electron oxidant to continually activate the galactose oxidase, in the presence additionally of a hydrogen peroxide remover to decompose the hydrogen peroxide which is formed as a coproduct in the oxidation of alcohols, is the subject of a separate commonly-owned and concurrently-filed patent application.

[0029] A preferred embodiment of Applicants' invention is a process for making a composition comprising galactomannan at a concentration of at least about 1.5%, wherein the galactomannan is enzymatically hydrolyzed by mannanase and oxidized by galactose oxidase to yield an aldehyde group on at least about 5% up to about 100% of the C6 carbon atoms of the galactose residues. The process comprises preparing a solution of an effective concentration of mannanase, and slowly adding to that solution, while stirring or otherwise agitating the solution, galactomannan to a concentration of at least about 1.5% up to about 80%. Then, with continued stirring or agitation, an effective amount of galactose oxidase and a source of oxygen are added. Optionally, this last step wherein galactose oxidase is added can be carried out in the presence of one or more additional activity components including a catalase activity and or a peroxidase activity.

[0030] In another aspect of Applicants' process, the reduction of molecular weight of the galactomannan using mannanase can be carried out simultaneously with the oxidation of the galactomannan. In this aspect, the process comprises preparing a solution comprising effective amounts of mannanase and galactose oxidase, and a source of oxygen, and slowing adding to this solution, with continued stirring or other agitation, galactomannan to a concentration of at least about 1.5% up to about 80%. In this aspect also, optionally, the oxidation using galactose oxidase can be carried out in the presence of one or more additional activities including a catalase activity and or a peroxidase activity.

[0031] One important consideration in the process of the invention will be the form in which the galactomannan is added to the mannanase solution. Galactomannans exist in a number of solid, particulate and slurry forms, well known in this art. A preferred technique for the present invention is that galactomannan is added to the mannanase solution in the form of particles. This method allows for putting the galactomannan into solution in a highly concentrated form without rapid viscosity increase that will impair the molecular weight reduction, and without the production of visible "grits" in the final solution product. Galactomannan particle size should be selected carefully. For example, using guar, if the guar particles are too fine, the viscosity of the water-soluble gum will increase so rapidly that the dispersion and solubilization of the gum at high concentration will be virtually impossible and impractical at industrial production scale. If the guar particles are too coarse, the final product will be heterogeneous and have visible large particles ("grits"). The preferred particle size range for a guar gum, for example, is between about 40 to about 250 mesh, more preferably between about 60 to about 200 mesh, and most preferably between about 80 to about 200 mesh (75-180 μm). Given these parameters, the preferred particle sizes of other galactomannans are easily determined empirically, depending upon the desired final characteristics of the intended solution and the properties of the starting galactomannan. Coarsely ground gum or the dehulled seeds from which the gum is obtained, e.g., guar splits, can also be used, but mechanical homogenization may be needed to eliminate visible particles in the finished product.

[0032] Another important aspect of Applicants' process is the step of adding the galactomannan to the mannanase solution, instead of adding the mannanase to the galactomannan, as is traditionally done. By adding the galactomannan into the enzyme-containing solution, while stirring or otherwise agitating, the enzyme is able to effectively degrade the galactomannan as the galactomannan is added while continually lowering its viscosity. This aspect of the process allows for hydrolyzing guar, for example, up to a high concentration in solution without problematic lumping or difficulty of mixing due to the otherwise rapid viscosity build-up that is traditionally experienced in the process of solubilizing galactomannans. If one tries to disperse the galactomannan into solution without controlling the particle size and or having the mannanase present in solution prior to addition of the galactomannan, it will be very difficult and impractical to make even a 1.5% galactomannan solution at large scale. Applicants have discovered that if proper consideration is not given to particle size and manner of addition of the galactomannan to the mannanase solution, it will not be possible or practical to carry out the enzymatic oxidation reaction at high concentrations of galactomannan. One skilled in the art could resort to making low molecular weight low viscosity gum hydrolyzates at normal solids concentration (0.5-1.5%), then using spray-drying or alcohol precipitation methods to obtain powered hydrolyzates before re-dissolving it at higher concentration, but such processes are cumbersome and expensive for commercial production.

[0033] In Applicants' process any galactomannan can be used. Locust bean, tara and guar gums are preferred; with guar gum being the most preferred. As discussed earlier, the galactomannan can be in native form, or it can be used in derivatized form, and or additionally treated to alter the wettability and solubility aspects of the gum. If the starting galactomannan has been treated or coated to improve its wettability or solubility characteristics, the form of the galactomannan which is added to the mannanase solution will be adjusted accordingly, which adjustments are easily determined empirically within the parameters of the invention. For example, if guar is selected as the galactomannan to be reduced and oxidized and the starting guar particles are in a form coated with borate, the particle size and rate of addition of the guar will be less critical. The rate of addition of the galactomannan to the mannanase and the form of stirring or agitation of the solution while the galactomannan

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