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(54) Title: CHITOSAN AND METHODS OF PRODUCING SAME

(57) Abstract: A chitosan, particularly soluble chitosan, having a substantially amorphous structure and a process for manufacturing the chitosan. The chitosan includes a water-soluble chitosan, an acid-soluble chitosan, and/or mixtures thereof, where both the water-soluble and acid-soluble chitosan have the amorphous structure. The amorphous structure of the acid-soluble chitosan allows the acid-soluble chitosan to be soluble in an acidic pH range of 6.5 and below. The amorphous structure of the water-soluble chitosan of the present invention allows the water-soluble chitosan to be soluble in acidic, neutral, and basic pH ranges.



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CHITOSAN AND METHODS OF PRODUCING SAME

This application claims priority from U.S. Provisional Application Serial No. 60/388,694, filed June 14, 2002, the entire content of which is incorporated
5 herein by reference.

BACKGROUND

Chitin is the major component of crustacean shells (such as crab, shrimp, crawfish, krill, and lobster), the bones of squid, and the cell walls of
10 fungi. Chitosan can be prepared from chitin. Chitosan is very similar to chitin. The primary difference between the two structures is the presence of amine groups in chitosan and amide groups in chitin. Chitosan has many industrial uses, including the production of viscosity control agents, adhesives, chromatography carriers, paper-strengthening agents, flocculent agents, food
15 additives, drugs, and cosmetics.

The conventional process for isolating chitin and producing chitosan therefrom involves grinding crustacean shells, which then undergo deproteinization and decalcification processes. Deproteinization and decalcification reactions can be carried out with enzymes, or under acidic or
20 alkaline conditions. The reaction conditions for the deproteinization and decalcification reactions can range from room temperature to 100 degrees Celsius. The resulting product is predominantly chitin.

Chitin molecules are relatively insoluble in water at neutral pH values. This is due in part to the crystalline structure present in the chitin molecule.
25 Chitin can be converted into chitosan in an effort to make a more water-soluble product. This process is accomplished by deacetylating the chitin to produce at least two different chitosan products. The at least two products produced during the deacetylation process include conventional water-soluble chitosan and/or conventional acid-soluble chitosan. Both products retain much of their
30 crystalline structure present in the chitin starting material.

For acid-soluble chitosan, the deacetylation process is usually performed by reacting chitin with an aqueous concentrated sodium hydroxide or potassium

hydroxide solution with heating under either a heterogeneous deacetylation reaction or a homogeneous deacetylation reaction. For these reactions, the typical ratio of base to chitin is within a range of 1:0.33 to 1:0.1 by weight and the typical ratio of chitin to water is within a range of 1:9 to 1:3 by weight. For example, in the heterogeneous deacetylation reaction, the ratio of base to chitin is about 1:0.33 by weight, and the base to water ratio is about 1:1 by weight. The reaction temperatures for the heterogeneous deacetylation reaction are in the range of 100 degrees Celsius to 150 degrees Celsius. Processing times for the heterogeneous deacetylation reaction are typically one hour or longer.

Alternatively, in the homogeneous deacetylation reaction, the ratio of base to chitin is about 1:0.1 by weight, and the base to water ratio is about 1:1.5 by weight. The reaction temperature for the homogeneous deacetylation reaction is about 25 degrees Celsius, with a processing time of about 48 hours or longer.

The resultant chitosan is typically 80% deacetylated. Chitosan having 80% deacetylation or greater is typically referred to as "acid-soluble" chitosan, as this chitosan is most soluble in solutions having a pH below 6.5. The morphology of the acid-soluble chitosan produced by the conventional deacetylation process also includes at least a partial crystalline morphology. Although acid-soluble chitosan is soluble in solutions having a pH below 6.5, the crystalline morphology prevents the acid-soluble chitosan from rapidly and completely solublizing in the solution.

Production of conventional water-soluble chitosan has also been described in the literature. Conventional water-soluble chitosan is typically about 45% to 55% deacetylated. Three different processes have been described for producing water-soluble chitosan. The first includes reacetylating free amino groups of conventional acid-soluble chitosan, as described above, with acetic anhydride (N. Kubota et al., Carbohydrate Res., 324, 268-274, 2000). The second includes soaking chitin in a dilute sodium hydroxide solution (e.g., a 1% aqueous sodium hydroxide solution by weight) at about zero degrees Celsius for at least forty-eight hours (T. Sannan et al., Makromol. Chem., 177, 3589-3600, 1976). The third process involves reacting the chitin with an

enzyme such as lipase (D. H. Kim et al., Korean J. Food Sci. Technol., 31(1), 83-90, 1999).

The conventional water-soluble chitosan, however, retains a significant amount of the crystalline structure of the chitin starting material. The retained
5 crystalline structure makes dissolving the conventional water-soluble chitosan difficult. As a result the uses of both the conventional acid-soluble chitosan and the conventional water-soluble chitosan are limited.

Thus, there is a need for a more efficient manufacturing process for chitosan and for a more soluble chitosan, i.e., one that is soluble in water over a
10 wider range of pH values.

SUMMARY OF THE INVENTION

The present invention provides, besides other things, a chitosan (particularly soluble chitosan) having a substantially amorphous structure and
15 an efficient process for manufacturing the chitosan. The soluble chitosan of the present invention includes a water-soluble chitosan, an acid-soluble chitosan, and mixtures thereof, where both the water-soluble and acid-soluble chitosan have an amorphous structure. It is believed that the amorphous structure of the acid-soluble chitosan allows the acid-soluble chitosan to be soluble in an acidic
20 pH range of 6.5 and below. Surprisingly, it is further believed that the amorphous structure of the water-soluble chitosan of the present invention allows the water-soluble chitosan to be soluble in acidic, neutral, and basic pH ranges.

The substantially amorphous structure of the water-soluble chitosan and
25 acid-soluble chitosan of the present invention includes little to no crystalline morphology. Disruption of the crystalline morphology is believed to be due in part to the processing conditions and operating parameters used in producing the water-soluble chitosan. The processing conditions and operating parameters for the equipment used in the present invention allow for a random
30 deacetylation throughout most of the chitin molecule. Random distribution of the deacetylation is believed to result when the morphology of the chitin molecule is sufficiently altered (i.e., crystalline structure disrupted) to allow the

deacetylation reactants to access a large portion of the acetyl groups of the chitin starting material.

This result is surprising in that in order to achieve this random deacetylation, it is believed that the crystalline structure of the chitin molecule is disrupted without destroying the chitin molecule. The random distribution of the acetyl groups removed sufficiently disrupts the crystalline structure of the chitin so as to produce an amorphous chitosan structure. The amorphous chitosan structure is also maintained when the soluble chitosan products of the present invention are dry. As a result of the amorphous structure, the soluble chitosan of the present invention is soluble in water or other solutions at pH values of 6.5 and below for the acid-soluble chitosan and within a broad pH range for the water-soluble chitosan.

The present invention provides for a method of producing soluble chitosan that includes providing an aqueous-chitin mixture comprising a ratio of a chitin source to water of at least about 1:1.9 by weight. The aqueous-chitin mixture can be subjected to shearing and heating while in the presence of a base at a temperature and for a time effective to deacetylate the chitin and produce soluble chitosan having a substantially amorphous structure. In one embodiment, the chitin-water mixture can be subjected to shearing and heating that occurs substantially simultaneously.

The soluble chitosan of the present invention can include water-soluble chitosan, acid-soluble chitosan and mixtures of both water-soluble and acid-soluble chitosan having the substantially amorphous structure. In one example, the water-soluble chitosan produced according to the present invention can have about thirty (30) to about fifty-five (55) percent available acetyl groups and the substantially amorphous structure. In addition, the acid-soluble chitosan produced according to the present invention can have about twenty (20) percent or less available acetyl groups and the substantially amorphous structure. Chitosan having about twenty (20) to about thirty (30) percent available acetyl groups are also possible, where this chitosan displays characteristics more typical of the acid-soluble chitosan than that of the water-soluble chitosan. The chitosan, however, also has characteristics of the water-soluble chitosan.

Preferably, prior to subjecting the aqueous-chitin mixture to shearing and heating, the aqueous-chitin mixture is first subjected to shearing forces to decrease a particle size of the chitin source and produce sheared chitin. The base is then provided to form an alkaline-chitin mixture that is subsequently
5 subjected to shearing and heating at a temperature and for a time effective to deacetylate the chitin and produce soluble chitosan having a substantially amorphous structure. In one embodiment, the base is added to the chitin-water mixture in an amount to produce a ratio of base to the chitin source of at least about 1:2 by weight. In addition, the time effective to deacetylate the chitin and
10 produce soluble chitosan can be less than about four minutes.

The soluble chitosan of the present invention can also be useful in the preparation of products for the food, pharmaceutical, nutraceutical, filtration, water treatment, and medical industries, among others. For example, the soluble chitosan of the present invention can be useful in fat removal, where an
15 effective amount of the soluble chitosan having a substantially amorphous structure can be provided to bind fat, where the fat molecules are then bound with the soluble chitosan. Compositions formulated for use in a mammal comprising the soluble chitosan having an amorphous structure can also be produced. These compositions can be useful as fat binding compositions, as a
20 food composition, as a pharmaceutical composition, as a cosmetic composition, and/or as a nutraceutical composition.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 is a flow chart generally illustrating one embodiment of the
25 present invention.

FIG. 2 is a plot of chitin particle size distribution as a function of various particle sizes for lobster shells with various moisture contents.

FIG. 3 is a wide-angle X-ray diffraction patterns for commercially produced acid-soluble chitosan and samples of acid-soluble chitosan of the
30 present invention.

FIG. 4 is a wide-angle X-ray diffraction patterns for commercially produced chitin, commercially produced acid-soluble chitosan, a sample of the

chitin source used in the present invention, and the water-soluble chitosan of the present invention.

FIG. 5 is a viscosity versus pH plot for various concentrations (wt./vol.) of the water-soluble chitosan (120 kDa) of the present invention.

5 FIGS. 6A and 6B are wide-angle X-ray diffraction patterns for the water-soluble chitosan of the present invention produced under different processing conditions, and a sample of the chitin source used in the present invention.

10 **DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS**

The present invention provides for, besides other things, the production of a chitosan (particularly soluble chitosan) having a substantially amorphous structure. The soluble chitosan of the present invention includes a water-soluble chitosan, an acid-soluble chitosan, and mixtures thereof, where both the water-
15 soluble and acid-soluble chitosan have an amorphous structure. The acid-soluble chitosan is soluble in an acidic pH range of 6.5 and below. The water-soluble chitosan is soluble in acidic, neutral, and basic pH ranges. Preferably, the water-soluble chitosan is soluble at pH values of about 6 up to and including about 8. More preferably, the water-soluble chitosan is soluble over a broad pH
20 range. Most preferably, water-soluble chitosan is soluble at pH values less than or equal to 6.5 down to 0 and will even be soluble as pH values are raised above 6.5 (even up to 14 for certain embodiments).

Both the water-soluble and the acid-soluble chitosan of the present invention are soluble in water. The acid-soluble chitosan of the present
25 invention is soluble in room temperature (25 degrees Celsius) aqueous solutions having a pH of 6.5 and below. The water-soluble chitosan of the present invention is soluble in room temperature aqueous solutions as described above. A solution as used herein means that the chitosan of the present invention is sufficiently soluble in water so as to produce transparent, translucent, or
30 opalescent compositions. Such "solutions" generally have no or few visible particles or precipitates of either the acid-soluble chitosan or the water-soluble chitosan in the solution, although submicroscopic particles may form to produce

a gel. Thus, as used herein "solution" encompasses gels. With respect to additional physical distinctions between the acid-soluble chitosan and the water-soluble chitosan, it is known that both are soluble in room temperature aqueous solutions having a pH of 6.5 and below. As the pH of the solution increases, there are distinctions that arise between the solubility of the water-soluble and acid-soluble chitosan of the present invention. For example, the water-soluble chitosan of the present invention forms a solution at a pH of 6.5 and below. As the pH is increased above 6.5, the water-soluble chitosan generally stays in solution, although a gel may form. However, the water-soluble chitosan does not precipitate out of solution. In contrast, the acid-soluble chitosan forms a solution at a pH of 6.5 and below, but generally will fall out of solution (e.g., precipitate) at a pH of greater than 6.5. The water-soluble chitosan of the present invention also exhibits a surprisingly high viscosity at a much lower concentration of the water-soluble chitosan as compared to water-soluble chitosan reported in the literature. For example, the water-soluble chitosan of the present invention with the same molecular weight has viscosity values that range from approximately 50 centipoises (cps) at a water-soluble chitosan concentration of 0.5 percent weight to volume to approximately 660 cps for a water-soluble chitosan concentration of 2.0 percent weight to volume. Thus, solutions having high viscosities can be produced with a relatively low concentration of the water-soluble chitosan of the present invention. The water-soluble chitosan of the present invention would be useful in the preparation of products for the food, pharmaceutical, nutraceutical (also referred to as phytochemicals or functional foods), filtration, water treatment, and medical industries, to name only a few. Nutraceuticals include, but are not limited to, those natural, bioactive compounds that have, or are believed to have, health promoting, disease preventing or medicinal properties.

The water-soluble chitosan of the present invention is also particularly useful in pH sensitive applications, such as cosmetic products, and coating of surfaces made of materials that are hydrophobic in nature. In addition, comparable solutions of water-soluble chitosan of the present invention and conventional water-soluble chitosan have surprisingly different viscosities.

These differences in viscosities are believed to be attributed to morphological differences (e.g., amount of crystalline morphology) in the water-soluble chitosan of the present invention and the conventional water-soluble chitosan.

The present invention uses chitin as a starting material in producing
5 soluble chitosan. Chitin is a polymer having a high degree of crystalline structure. The crystalline structure contributes to chitin's water insoluble characteristic. Chitosan produced by conventional processes retains much of the crystalline structure of the chitin starting material. This retained crystalline structure in the chitosan contributes to the difficulties encountered in trying to
10 dissolve conventionally produced chitosan in water.

Attempts have been made to make conventional chitosan having a crystalline structure water-soluble above neutral pH levels. These attempts include producing conventional water-soluble chitosan having at least forty-five (45) percent deacetylation. This percentage of deacetylation helps to reduce,
15 but not eliminate, the amount of crystalline structure in the chitosan molecules. In addition, the distribution of the deacetylation (i.e., the region from which acetyl groups are removed) is concentrated primarily in the non-crystalline regions (i.e., the amorphous regions) of the chitin starting material, as opposed to the crystalline regions of the chitin starting material. One reason for this
20 occurrence is that nothing in the deacetylation process disrupts or changes the crystalline structure of the chitin starting material.

Additional attempts have been made to make conventional chitosan having a crystalline structure water-soluble above neutral pH levels. Theses attempts have included adding additional functional groups to deacetylated
25 chitin. The resulting products are typically referred to as chitosan derivatives.

The methods of the present invention, however, differ in that the deacetylation process of the chitin is controlled to produce soluble chitosan that can include water-soluble chitosan that is soluble across a wide range of pH values, including acidic, neutral, and basic pH environments. Thus, the "water-
30 soluble" chitosan and the "acid-soluble" chitosan of the present invention are "as-produced" from the deacetylation process. They are not chemically

functionalized to form a "chitosan derivative" (although they can be if desired) with desired solubility.

In contrast to the previous attempts to deacetylate the chitin starting material and/or add functional groups to deacetylated chitin to produce chitosan
5 derivatives, the present invention preferably controls the amount and characteristics of chitin deacetylation in at least three significant ways. First, the reaction mixture used in the present invention preferably has a chitin source to water ratio by weight that is substantially higher than is used in conventional methods. That is, conventional methods use much more water and hence dilute
10 solutions. Second, the reaction mixture used in the present invention preferably uses less base relative to the amount of chitin source compared to conventional methods. Finally, the operating parameters and the equipment used in the present invention preferably are significantly different than used in conventional methods. The present invention controls the amount and characteristics of
15 chitin deacetylation in other significant ways, as will be discussed below.

The operating parameters and equipment used in the present invention allow for the production of a soluble chitosan having a substantially amorphous structure. The soluble chitosan of the present invention can include water-soluble chitosan, acid-soluble chitosan, or mixtures thereof, where the water-
20 soluble and acid-soluble chitosans both have a substantially amorphous structure. One distinguishing difference between the acid-soluble chitosan and the water-soluble chitosan is the extent of deacetylation, as will be discussed below. Furthermore, operating parameters and equipment of the present invention are adjustable to allow for more or less of one of the acid-soluble or
25 water-soluble chitosan to be produced, as will be described more fully below.

The substantially amorphous structure of the water-soluble chitosan and acid-soluble chitosan of the present invention includes little to no crystalline morphology. Disruption of the crystalline morphology is due in part to the processing conditions and operating parameters used in producing the water-
30 soluble chitosan. The processing conditions and operating parameters for the equipment used in the present invention allow for a random deacetylation throughout most of the chitin molecule. Random distribution of the

deacetylation results when the morphology of the chitin molecule is sufficiently altered (i.e., crystalline structure disrupted) to allow the deacetylation reactants to access a large portion of the acetyl groups of the chitin starting material.

This result is surprising in that in order to achieve this random
5 deacetylation, it is believed that the crystalline structure of the chitin molecule is disrupted without destroying the chitin molecule. The random distribution of the acetyl groups removed sufficiently disrupts the crystalline structure of the chitin so as to produce an amorphous chitosan structure. The amorphous
10 chitosan structure is also maintained when the soluble chitosan products of the present invention are dry. As a result of their amorphous structure, the soluble chitosan of the present invention is soluble in water or other solutions at pH values of 6.5 and below for the acid-soluble chitosan and within a broad pH range for the water-soluble chitosan.

FIG. 1 is a flow chart showing one embodiment of the general process of
15 making the soluble chitosan according to the present invention. An aqueous-chitin mixture is provided for processing, which is a combination of one or more chitin sources and water. In one embodiment, providing the aqueous-chitin mixture includes first mixing a chitin source (e.g., crustacean shells, bones from squid, and/or fungi cell walls) with water to produce the aqueous-
20 chitin mixture.

The ratio of the chitin source to water used in the present invention can have a significant influence on the production of soluble chitosan. The ratio of the chitin source to water for the present invention is significantly higher than is used in producing conventional water-soluble or acid-soluble chitosan. The
25 present invention preferably uses a chitin source to water in a ratio of at least about 1:1.9 by weight. That is, the chitin source to water ratio of at least about 1:1.9 ratio provides for the minimum amount of chitin for the chitin source to water ratio of the present invention. More preferably, the ratio of a chitin source to water is no greater than about 1:0.7. Most preferably, the ratio of a
30 chitin source to water is about 1:1. In contrast, chitin to water ratios for conventional processes include 1:9 to 1:3 chitin to water by weight. Thus, the method of the present invention allows for higher concentrations of solids

(chitin source), which contributes to greater efficiencies in manufacturing the soluble chitosan of the present invention.

Furthermore, the ratios used in the present invention are significant in that they allow for efficient size reduction in the particle size of the chitin source. Size reduction in the particles of the chitin source also creates more surface area that allows for more water to be absorbed by the chitin source. The availability of water in the chitin source during the deacetylation reaction is one important factor in producing the amorphous structure of the soluble chitosan of the present invention.

Referring again to Fig. 1, the aqueous-chitin mixture is subjected to shearing and heating while in the presence of a base. The base in the present invention can include, but is not limited to, hydroxide-containing compounds such as sodium hydroxide, or calcium oxide, or other compounds effective to produce an alkaline solution. The water of the aqueous-chitin mixture and the base form an alkaline solution (e.g., calcium oxide). Hereinafter this mixture of the aqueous-chitin mixture and base may be referred to as the alkaline-chitin mixture. It will be recognized that the use of the phrase alkaline-chitin mixture refers to the aqueous-chitin mixture in the presence of the base.

Preferably, the shearing and heating occur substantially simultaneously during at least a portion of the processing time. The shearing and heating of the alkaline-chitin mixture occurs at a temperature and for a time effective to deacetylate the chitin so as to produce soluble chitosan having a substantially amorphous structure. As discussed, the shearing conditions serve to decrease the particle size of the chitin source. The decreased particle size of the chitin source allows the water and the base to more readily impregnate the chitin. In addition, the heating increases the deacetylation reaction rate so as to produce the soluble chitosan of the present invention in a reduced amount of time.

Preferably, the shearing and heating conditions are applied to the alkaline-chitin mixture simultaneously in a high shear stress apparatus. Preferably, the high shear stress apparatus is a heated screw extruder. In one example, the heated screw extruder can have a steam jacket heated with steam in a range of about

200 KPa (about 135 degrees Celsius) to about 850 KPa (about 180 degrees Celsius) of saturated steam gauge pressure.

The shearing and heating conditions are sufficient to cause the chitin in the alkaline-chitin mixture to undergo a deacetylation reaction. The degree to which the deacetylation reaction occurs can be controlled by adjusting the ratio of the chitin source to water, the ratio of the base to chitin source, and the ratio of the base to water. In addition, the feed-rates, operating temperatures, and shear stresses used with the equipment used in processing the alkaline-chitin mixture also influence the degree to which the chitin source undergoes the deacetylation reaction.

The amount of reagents necessary to produce the water-soluble chitosan of the present invention is far less than is necessary to produce conventional water-soluble chitosan or conventional acid-soluble chitosan. The present invention preferably uses a base to chitin source in a ratio of less than 1:0.33. That is the present invention uses less base relative to the amount of chitin than conventional methods. More preferably, the ratio of base to chitin source is no greater than about 1:0.5. Even more preferably, the ratio of base to chitin source is at least about 1:2 by weight. Even more preferably, the amount of base to chitin source has a range of ratios of about 1:1.25 to about 1:1. A ratio of 1:1 base to chitin is most preferred. In contrast, for the same alkaline solution concentrations as the present invention, methods of producing conventional soluble chitosan from chitin use ratios of a base to chitin source of anywhere from 1:0.33 to 1:0.1 by weight.

In addition, reaction times for conventional methods of producing chitosan from chitin can take one or more hours, whereas the reaction times for the methods of the present invention are typically much less. Preferably, the reaction (i.e., processing) times of the present invention are less than about one hour, more preferably less than about 30 minutes, even more preferably less than about 15 minutes, even more preferably less than about 10 minutes, even more preferably no more than about four minutes, and most preferably no more than about one minute to complete. Thus, the present invention produces soluble chitosan with less liquid volume and reagents (e.g., base) in a shorter

amount of time as compared to traditional methods of producing conventional soluble chitosan from chitin.

In an additional embodiment, one or more additional steps can be included in the process of making the soluble chitosan of the present invention.

- 5 For example, it is preferred that prior to subjecting the alkaline-chitin mixture to shearing and heating, the aqueous-chitin mixture be first subjected to shearing forces to decrease the particle size of the chitin source. This process produces sheared chitin and allows for size reduction in the particle size of the chitin source, as discussed above. This process also allows the sheared chitin source
10 to absorb more water than would be absorbed without the shearing process. The aqueous-chitin mixture can then be made alkaline (the alkaline-chitin mixture) by providing the base to the aqueous-chitin mixture having the sheared chitin. For example, the base can be added directly to the aqueous-chitin mixture. The alkaline-chitin mixture can then be processed under shearing and heating
15 conditions, as discussed.

- In an alternative embodiment, the chitin source can be mixed directly with an alkaline solution to provide the alkaline-chitin mixture. The alkaline-chitin mixture can then be subjected to shearing forces to decrease the particle size of the chitin source so as to produce sheared chitin. The decreased particle
20 size of the chitin source allows the base to more readily impregnate the chitin source. The alkaline-chitin mixture can then be processed under shearing and heating conditions to produce the soluble chitosan, as discussed.

- Chitin sources for the present invention include, but are not limited to, the shells of crustaceans (e.g., lobsters, crabs, or shrimp), bones from squid,
25 insects, and mold. Other chitin sources are also possible. In addition, it is understood that chitin sources can contain other components besides chitin. These other components can include, but are not limited to, naturally occurring chitosan (acid-soluble chitosan), and naturally occurring compounds that include both chitin and/or chitosan. The chitin sources described in the present
30 invention are from the shells of crustaceans that have been cleaned so as to be free of non-chitin containing material, such as internal organs, soft tissues, sand, dirt, etc.

The weight ratios of the chitin source are based on a chitin source that is dry, where dry for a chitin source is defined as crustacean shells having less than about 3%, and preferably less than about 1%, by weight, water in the shell. Alternatively, a wet chitin source, or a combination of wet and dry chitin sources, could be used to provide the alkaline chitin source-water mixture. When a wet chitin source is used, the amount of water present in the wet chitin source can be taken into consideration in determining the ratio of chitin source to water ratio or the base to chitin source ratio. Preferably, deionized and/or distilled water are used in preparing the aqueous chitin source mixture, although the purity of the water is not necessarily important as long as at least a portion of the chitin can be converted to soluble chitosan.

The chitin source can swell (i.e., increase in volume) on the addition of water or an alkaline solution. This swelling process causes pores in the chitin source to open. The opening of the pores allows additional water or alkaline solution to impregnate into the chitin source. The degree of alkaline solution impregnation into the chitin source, however, can be decreased if the chitin on the surface of the chitin source swells to such an extent that the pores into the chitin source are prematurely closed. Swelling can happen rapidly when the temperature of an alkaline-chitin mixture is greater than one hundred (100) degrees Celsius. Swelling can also happen when the ratio of base to water is greater than about 1:1.5 by weight. Preferably, the ratio of base to water is kept at or below 1:1.5 by weight. Alternatively, when the ratio of the base to water is greater than 1:1.5 by weight, the temperature of the alkaline-chitin mixture should be kept as low as possible to prevent swelling of the chitin particles. For example, the temperature of the alkaline-chitin mixture should be kept at or below one hundred (100) degrees Celsius.

To prevent the premature pore closing on the chitin source, the temperature of the alkaline-chitin mixture is preferably maintained at below one hundred (100) degrees Celsius. More preferably, the alkaline-chitin mixture is maintained at room temperature. Temperatures at which the alkaline-chitin mixture can be maintained also include temperatures above the freezing temperatures, but below one hundred (100) degrees Celsius. Surprisingly, the

alkaline-chitin mixture can be kept for hours, or longer (up to 8 hours) at room temperature without adversely affecting the swelling of the chitin source.

Preferably, the temperature of the alkaline-chitin mixture is also kept at room temperature when the chitin source in the alkaline-chitin mixture is
5 subjected to particle size reduction by the shearing process. During the particle size reduction, the alkaline solution impregnates into the chitin source. Impregnation occurs when the alkaline solution soaks into the chitin source, wetting the chitin source and causing the chains of chitin to expand in volume. Surprisingly, the impregnation of the chitin source with the alkaline solution
10 allows for a more rapid and random deacetylation reaction to take place.

In addition, a non-limiting theory is proposed in which the chitin molecules and alkaline solution form an alkaline-chitin complex at pH values of at least 12. While not wishing to be bound by theory, it is believed that these alkaline-chitin complexes alter the crystalline structures of the chitin molecule
15 so as to lower the glass transition temperature and the melting temperature of the chitin and chitosan molecules. Lowering the glass transition temperature and the melting temperature of the chitin and chitosan molecules allows for the crystalline structure present in the chitin and chitosan molecules to be disrupted to the extent that random deacetylation can take place in the formerly crystalline
20 region. Once this occurs, the molecular structure of the chitosan molecule is disrupted to the extent that recrystallization of the chitosan molecule does not occur. As such, the soluble chitosan (both water-soluble and acid-soluble chitosans) of the present invention has a mostly amorphous morphology.

Preferably, the base can be any hydroxide-containing compound, such as
25 calcium hydroxide, or combinations of hydroxide-containing compounds could be used in providing the alkaline-chitin mixture. In addition, calcium oxide could also be used alone or in combination with the hydroxide compound in providing the alkaline-chitin mixture. Most preferably, sodium hydroxide is used as the base compound for the alkaline-chitin mixture. Generally, the base
30 is used as a catalyst in carrying out the deacetylation of the chitin. Preferably, the chitin adsorbs the alkaline solution during the impregnation of the chitin

source, where the greater the adsorption of alkaline solution the more quickly the deacetylation reaction takes place.

The base can be added to the aqueous chitin source mixture, as previously described. Alternatively, the base can be used to make an alkaline solution that is then mixed with the chitin source, which is typically in a dry state, to provide the alkaline-chitin mixture. The amount of base present in the mixture is such that during heating and shearing chitin in the chitin source undergoes deacetylation so as to produce the soluble chitosan of the present invention.

10 Preferably, the base to water is used in a ratio of at least about 1:4 by weight. Any amount greater than this (e.g., more base than water) can be present in the mixture as long as the resulting chitosan is the water-soluble chitosan of the present invention. More preferably, the base to water is used in a ratio of at least about 1:1.5 by weight. Even more preferably, the base to water is used in a ratio of at least about 1:0.7 by weight. Most preferably, the base to water is used in a ratio of at least about 1:1 by weight. The ratio of base to water for the present invention can also have a range from 1:4 to 1:0.25 by weight. In addition, the base to water ratio for the present invention can be greater than 1:0.25, where a higher ratio of base to water by weight results in a shorter reaction time and/or a lower temperature for the deacetylation reaction.

20 The present invention also provides that the particle size of the chitin source in either the aqueous chitin mixture or the alkaline-chitin mixture be decreased. Preferably, the chitin source is exposed to a high shear stress in a high shear stress apparatus such that the size of the chitin source is decreased.

25 The large ratio of chitin to water in either the aqueous chitin mixture (preferably about 1:1.9 to about 1:0.7 by weight) or the alkaline-chitin mixture (preferably about 1:1.9 to about 1:0.7 by weight) allows for an efficient decrease in the chitin source particle size in the high shear stress apparatus. This is due not only to the structures within the high shear stress apparatus (e.g., the interaction between the screw(s) and apparatus wall), but also due in part to inter-particle grinding of the chitin source moving through the high shear stress apparatus.

30

Preferably, the high shear stress apparatus is effective to decrease the particle size of the chitin source to a size that allows for effective impregnation of the base into the chitin source. As discussed above, more effective impregnation of the base into the chitin source allows the random deacetylation reaction to be completed within the mean residence time in the high shear stress apparatus. More preferably, the high shear stress apparatus is effective to decrease the particle size (i.e., the largest dimension of a particle, e.g., a diameter of a spherical particle) of the chitin source to produce a particle size of five (5) millimeters or smaller in order to complete the reaction within the mean residence time in the high shear stress apparatus. Even more preferably, the high shear stress apparatus is effective to decrease the particle size (i.e., the largest dimension of a particle, e.g., a diameter of a spherical particle) of the chitin source to produce a particle size of three (3) millimeters or smaller in order to complete the reaction within the mean residence time in the high shear stress apparatus. Most preferably, the high shear stress apparatus is effective to decrease the particle size of the chitin source to a particle size of three (3) millimeters or smaller, where the particle sizes are homogenized.

Preferably, the high shear stress apparatus is a mixer, a blender, an agitator, a mill or similar equipment that can decrease and homogenize the particle size of the chitin source. More preferably, the high shear stress apparatus is a screw extruder that can decrease and homogenize the particles of the chitin source. Most preferably, the high shear stress apparatus is a twin-shaft, co-rotating continuous mixer/processor from Readco Manufacturing Inc., (York, PA), where the twin-shafts can be set to rotate at 50 to 250 rotations per minute. Preferably, the mean residence time of the alkaline-chitin mixture in the continuous processor is from 15 seconds to 4 minutes. More preferably, the mean residence time of the alkaline-chitin mixture in the continuous processor is from 15 seconds to 2 minutes. Most preferably, the mean residence time of the alkaline-chitin mixture in the continuous processor is from 15 seconds to 1 minute.

In addition to exposing the chitin source to high shear stress, the alkaline-chitin mixture is preferably subsequently subjected to both shearing

and heating. In a preferred embodiment, the shearing and heating are done substantially simultaneously. In one example, the simultaneous shearing and heating are accomplished in a heated continuous processor. In addition, the shearing and heating are done at a temperature and for a time effective to

5 deacetylate the chitin and produce the soluble chitosan of the present invention.

Preferably, heating and shearing the aqueous chitin source in the presence of base is sufficient to promote the chitin to undergo a sufficient degree of random deacetylation (e.g., to result in at least 50 to 70 percent deacetylation). More preferably, for certain preferred embodiments the aqueous

10 chitin source in the presence of base is heated to a temperature of at least about one hundred (100) degrees Celsius, and even more preferably at least about one hundred seventy (170) degrees Celsius. In an additionally preferred embodiment, the aqueous chitin source in the presence of base is heated to a temperature of at least about one hundred twenty (120) degrees Celsius.

15 During the heating and shearing, the chitin in the alkaline-chitin mixture undergoes a random deacetylation. The deacetylation process results in a random hydrolysis of acetyl groups on the chitin. During this process, acetyl groups are randomly removed and/or added to chitin, and it may result in a random block of acetyl groups on chitin molecules. The present invention can

20 produce water-soluble chitosan, acid-soluble chitosan or mixtures thereof. As previously discussed, water-soluble chitosan and acid-soluble chitosan are defined as a function of the degree of deacetylation of the chitin. It is believed that changes in the glass transition and melting temperature of the chitin caused by complexes formed between the chitin and the alkaline solution allow for

25 disruptions in the altered crystalline structure during processing. The amount of chitin deacetylation can then be accomplished by adjusting reagent concentrations in the chitin source-water mixture along with the operating parameters of the high shear stress apparatus.

To achieve the random deacetylation of the present invention, the

30 crystalline chitin structures normally present in the chitin starting material are believed to be disrupted prior to the deacetylation. Disruption of the chitin and chitosan crystalline structure is believed to begin with the addition of the base

or alkaline solution to the aqueous chitin source water mixture. Under certain conditions it is believed that alkaline-chitin complexes are formed when the pH of the aqueous chitin mixture is pH 12 or above. The formation of these complexes is believed to cause the glass transition temperature and the melting
5 temperature of the chitin and chitosan molecules to be reduced as compared to their normal values.

The heat supplied during the heating and shearing is believed to be sufficient to cause the reaction temperature to be equal to or greater than the reduced glass transition temperature and the melting temperature of the chitin
10 and chitosan molecules. While not wishing to be bound by theory, it is believed that this allows the chains of the chitin and chitosan molecules, including the chains forming the crystalline structure, to relax. In addition to the heat, the high shear stress imposed on the chitin and chitosan molecules is believed to cause a deformation in the crystalline structure. The high shear stress also
15 provides for uniform mixing between the reactants and the chitin molecules. The heating and uniform mixing under high shear stress are believed to be important factors in achieving the random deacetylation of the chitin molecules in the present invention.

As previously discussed, because conventional processes are believed
20 not to cause the disruption of the crystal structure of chitin, the deacetylation of the chitin is carried out while the chitin is still in a crystalline state. The acetyl groups are, therefore, not removed randomly from the entire chitin molecule. As a result, the crystalline structure of the chitin molecule remains substantially intact with conventionally produced deacetylated chitosan molecules, as
25 compared to the chitosan molecules produced by the present invention. Because the present invention is believed to cause the disruption of the crystalline structures in the chitin molecules, the deacetylation reaction occurs in regions of the chitin and chitosan molecules that would not normally have been deacetylated. The result is soluble chitosan of the present invention that
30 displays a more homogeneous and random deacetylation than that of conventional deacetylation processes.

While not wishing to be limited by theory, it is believed that the resulting random deacetylation of the chitin molecules under highly viscous conditions interferes with any potential re-crystallization process of the chitin molecule. Surprisingly, the water-soluble chitosan of present invention requires
5 approximately thirty (30) percent of the available acetyl groups (i.e., approximately 70% deacetylation) to interfere with any potential re-crystallization process of the chitosan molecules. This is believed to be due in part to the random distribution of the remaining acetyl groups on the water-soluble chitosan molecule. As a result, the water-soluble chitosan of the present
10 invention has a more highly amorphous structure as compared to the crystalline structure of the chitin source. In addition, the water-soluble chitosan of the present invention also has a more highly amorphous structure as compared to the conventionally produced water-soluble chitosan. The amorphous structure allows the water-soluble chitosan molecules of the present invention to be
15 soluble in water and/or aqueous solution within a broad pH range, as compared to conventionally produced chitosan molecules.

The degree and characteristics of chitin deacetylation resulting from the process of the present invention can be dependent upon processing conditions used and the characteristics of the starting chitin material. For example, under
20 certain conditions the process of the present invention can produce the water-soluble chitosan of the present invention. These conditions can include, but are not limited to, minimizing the stated ratios of base to chitin by weight for the present invention. In addition, the conditions can include, but are not limited to, minimizing the processing time and/or temperature of the alkaline chitin
25 mixture during the heating and shearing.

Alternatively, under other certain conditions the process of the present invention can produce the acid-soluble chitosan of the present invention. These conditions can include maximizing the stated ratios of base to chitin by weight for the present invention. In addition, the conditions can include, but are not
30 limited to, maximizing the processing time and/or temperature of the alkaline chitin mixture during the heating and shearing. Process conditions also exist

where mixtures of both the water-soluble chitosan and the acid-soluble chitosan are produced.

Any number of the reaction conditions can be controlled in an effort to produce more or less of either the water-soluble chitosan or acid-soluble chitosan of the present invention. For example, the ratio of the chitin to water, the ratio of base to water, and/or the ratio of base to chitin by weight can be adjusted to cause more or less deacetylation of the chitin. Also, the operating parameters and the equipment used in the present invention can be adjusted so as to produce more or less of each of the water-soluble and acid-soluble chitosan of the present invention. Examples of operating parameters that can be adjusted include, but are not limited to, the number of screws, screw shape, the screw length and/or the diameter of the screw(s) used in the high shear stress apparatus; the operating temperature of the high shear stress apparatus; and/or the residence time of the alkaline-chitin mixture in the high shear stress apparatus.

In an additional advance over conventional methods of producing chitosan, the present invention also allows for direct conversion of chitin in the chitin source to soluble chitosan, without a deproteinization or a decalcification process. These two later steps are typically required by conventional methods of producing chitosan. Surprisingly, the present invention produces soluble chitosan, and preferably water-soluble chitosan, without the need for separate decalcification and deproteinization steps. This is a significant and preferred advantage over the conventional methods of producing chitosan.

Preferably, the water-soluble chitosan of the present invention has undergone from about forty five (45) percent to about seventy (70) percent deacetylation based on the original amount of acetyl groups in the chitin. Stated in a different way, the water-soluble chitosan of the present invention has from about thirty (30) to about fifty-five (55) percent available acetyl groups based on the original amount of acetyl groups in the chitin, and a substantially amorphous structure. Herein, "available" acetyl groups are acetyl groups that could have potentially undergone, but did not undergo, the deacetylation

reaction. The degree of deacetylation of chitin can be determined by colloid titration method (see K. Toer et al. Anal. Chim. Acta, 83 (1975), 59-65).

Preferably, the acid-soluble chitosan of the present invention has undergone about eighty (80) percent or greater deacetylation of chitin based on the original amount of acetyl groups in the chitin. Stated in a different way, the acid-soluble chitosan of the present invention has about twenty (20) percent or less available acetyl groups based on the original amount of acetyl groups in the chitin and a substantially amorphous structure.

Surprisingly, under preferred operating and reagent conditions, deacetylation of chitin to produce the water-soluble chitosan and/or the acid-soluble chitosan of the present invention occurs in a processing time of no longer than four (4) minutes. Even more surprising is that under particularly preferred operating and reagent conditions, deacetylation of chitin to produce the water-soluble chitosan and/or the acid-soluble chitosan of the present invention occurs in a processing time of no longer than one (1) minute. Even more surprising is that deacetylation of chitin to produce the water-soluble chitosan and/or the acid-soluble chitosan of the present invention occurs in a processing time of no longer than one (1) minute.

As described above, the process of the present invention that provides for deacetylation of at least about forty-five (45) percent to at least about seventy (70) percent of the available acetyl groups on the chitin results in water-soluble chitosan of the present invention. In addition, the process of the present invention that provides for deacetylation of greater than seventy (70) percent of the available acetyl groups on the chitin results in acid-soluble chitin. The percentage of deacetylation produced in the present invention depends in part on the amount of acetyl groups present on the chitin starting material. Therefore, it will be appreciated that the yields of water-soluble and acid-soluble chitosan produced by the present invention will be highly dependent upon the amount of acetyl groups present on the chitin starting material. Given the understanding provided by the present invention, one skilled in the art would be required to adjust one or more of the described ratios, concentrations

and/or operating parameters of the equipment used in the present invention to arrive at the desired water-soluble chitosan of the present invention.

The molecular weight of the resulting soluble chitosan of the present invention is influenced by the chitin source (e.g., crustacean shells, squid, and/or fungi cell walls) used in the process. Preferably, the molecular weight of the soluble chitosan of the present invention is at least 1×10^5 Daltons. In an additional preferred embodiment, the molecular weight of the soluble chitosan of the present invention is no greater than 1×10^7 Daltons. More preferably, the molecular weight of the soluble chitosan of the present invention is in a range from 1×10^5 to 1×10^7 Daltons. When it is desirable, the molecular weight can be decreased by hydrolysis to oligomers during the chitin preparation steps or to increase the reaction time and temperature during the deacetylation reaction step. Molecular weight can be determined by viscometric methods or Gel permeation chromatography. The viscosity can be measured with a Brookfield and Oswald viscometers at various temperatures.

Isolation and purification of the water-soluble chitosan and acid-soluble chitosan of the present invention can be accomplished using any number of techniques. Preferably, the water-soluble chitosan of the present invention can be isolated by adding water to the soluble chitosan mixture resulting from the shearing and heating steps. The water-soluble chitosan is dissolved in the water and the water insoluble fraction is removed by centrifugation. More preferably, the water-soluble chitosan can be further purified by precipitation with a water miscible organic solvent. Most preferably, the water-soluble chitosan is purified by precipitation with ethanol, isopropanol, methanol or acetone. The solvent is added to water-soluble water mixture until precipitation of the water-soluble chitosan occurs. Preferably, the precipitation takes place at room temperature (about 25 degrees Celsius). In an additional preferred embodiment, the water-soluble chitosan is purified by removing base and salts by the use of molecular sieves.

For purifying the acid-soluble chitosan of the present invention, the insoluble fraction isolated during the centrifugation described above is dissolved water at acidic pH. The solution is then centrifuged, where the

insoluble fraction is discarded. The pH of the solution is then adjusted to around 7 and the solution is centrifuged. The resulting precipitate is the acid-soluble chitosan of the present invention. The acid-soluble chitosan of the present invention is purified by repeating the dissolving and precipitating steps
5 three times.

Preferably, the present invention allows for a yield of water-soluble chitosan of greater than 30% of the total weight of all chitin and chitosan (acid-soluble and water-soluble) derived from the chitin source. More preferably, the present invention allows for a yield of water-soluble chitosan of greater than
10 40% of the total weight of all chitin and chitosan (acid-soluble and water-soluble) derived from the chitin source. Even more preferably, the present invention allows for a yield of water-soluble chitosan of greater than 50% of the total weight of all chitin and chitosan (acid-soluble and water-soluble) derived from the chitin source. It is even more preferable that the present invention
15 allows for a yield of water-soluble chitosan of greater than 60% of the total weight of all chitin and chitosan (acid-soluble and water-soluble) derived from the chitin source. Most preferably, the present invention allows for a yield of water-soluble chitosan of greater than 70% of the total weight of all chitin and chitosan (acid-soluble and water-soluble) derived from the chitin source. Under
20 certain conditions, theoretically the yield could reach 100%.

The soluble chitosan of the present invention (water-soluble chitosan, acid-soluble chitosan, or mixtures thereof) can be used in the preparation of products for the food, pharmaceutical, nutraceutical, cosmetics, filtration, water treatment, and medical industries, to name only a few. For example, the soluble
25 chitosan of the present invention can be used in removing fat from foods, or taken by a mammal to remove ingested fat. In removing fat, an effective amount of the soluble chitosan of the present invention having a substantially amorphous structure is provided to bind fat. The soluble chitosan of the present invention then binds the fat molecules when they are encountered. In addition,
30 the soluble chitosan of the present invention could also be used as a stabilizer, a freshness preserver, and/or a thickener in food products.

In one example a fat binding composition can be formulated for use in a mammal. The water soluble chitosan of the present invention can also be included in a pharmaceutical composition, and/or a food composition. The fat binding composition, the pharmaceutical composition and/or the food composition, can include any number of additives for increasing the effectiveness of the fat binding abilities of the soluble chitosan, including, but not limited to, a pharmaceutically acceptable carrier. The fat binding composition, the pharmaceutical composition, and/or the food composition can further be formulated for oral administration to the mammal. The food composition can also include a food grade acceptable carrier, as are known.

Other uses for the soluble chitosan (water-soluble and/or acid-soluble chitosan) of the present invention can include, but are not limited to, uses in antifungal and antitumour applications. In addition, the soluble chitosan of the present invention can be used in, but are not limited to, medicine where the soluble chitosan of the present invention might find uses in artificial skin, blood vessels, surgical sutures, haemostatics, wound healing agents, antitumor agents, immunity promoter, anti-cholesterol agents, artificial kidneys, super-filter members, detoxicate absorbents, and/or as carriers for enzymes, antigens, antibodies.

The soluble chitosan of the present invention can also be useful in hair care products, such as hair styling products and/or hair conditioners. The soluble chitosan of the present invention can also as a color fixer, adhesive, and/or stabilizer used in products for the plastics industry, textile industry, dyeing industry, and/or color film industry. The soluble chitosan of the present invention can also be useful in the adsorption of heavy metal ions and organic compounds. In agriculture, the soluble chitosan of the present invention can also be useful in seed coatings and in plant antibacterial products.

Objects and advantages of this invention are further illustrated by the following examples, but the particular materials and amounts thereof recited in these examples, as well as other conditions and details, should not be construed to unduly limit this invention.

Examples

The chitin source for the following examples was shells from langostino lobsters (*Pleuroncodes planipes*).

5 Example 1: Reduction of Particle Size of Chitin Source

Various amounts of water were added to one kilogram of dry langostino lobster shells. Each of these resulting aqueous-chitin mixtures was fed into a continuous processor (Readco Manufacturing Inc., York, PA) having a twin two-inch screw rotating at 120 rotations per minute and operating at room
10 temperature. Room temperature is defined as about 25 degrees Celsius. The aqueous-chitin mixtures were fed into the continuous processor at a rate of 60 grams of the mixture per minute. Residence time of the aqueous chitin mixtures in the continuous processor was no longer than one minute. Screen sieves were used to analyze the resulting particle size of the shells in the aqueous chitin
15 mixture. The resultant particle size distribution is shown in FIG. 2, where the percentage values associated with the plot symbols indicate the percentage of moisture (i.e., water) added to the shells.

Example 2: Impregnation of Reagents into Chitin Source

20 Sodium hydroxide pellets were added to an aqueous chitin mixture (1:1 chitin source to water ratio by weight) at room temperature to provide the alkaline-chitin mixture. The sodium hydroxide to chitin source weight ratio ranged from approximately 1:2 to approximately 1:0.5. The alkaline-chitin mixture was fed into the continuous processor of example 1 at a feed rate of 80
25 grams of the mixture per minute. Residence time of the alkaline-chitin mixture in the continuous processor was no longer than one minute. The processing was carried out at room temperature.

After processing the alkaline-chitin mixture, the samples were added to water. The water-soluble fractions and the solid fractions were then separated
30 by centrifugation. The water-soluble chitosan fraction of the samples was precipitated by the addition of ethanol at room temperature. The precipitate was filtered and dried. The residual solid fractions were then added to water again and dissolved by addition of acid to pH less than 6.5. At this pH, acid-soluble

chitosan became soluble and the residual water insoluble chitin was removed by centrifuge. The chitosan solution was then precipitated by first adjusting the pH to above 6.5. The precipitate was recovered by centrifugation and dried. Each fraction was then dried and weighed.

5

Example 3: Production of Soluble Chitosan

The alkaline-chitin mixture having a 1:1 sodium hydroxide to chitin source weight ratio from Example 2, above, was fed at a rate of 60 grams of the mixture per minute into the continuous processor of example 1, where the processor was heated with either 515 KPa or 655 KPa of steam pressure. Residence time of the alkaline-chitin mixture in the continuous processor was no longer than one minute. The combined yield of water-soluble chitosan and acid-soluble chitosan after one pass through the continuous processor under the two heated conditions was analyzed as described in Example 2, above. The results are shown in Table 1.

10
15

Table 1. Percent Yield of Chitosan (Acid-soluble and Water-Soluble) at 515 KPa or 655 KPa of steam pressure in the steam jacket.

Number of Passes	515 KPa of Steam Pressure	655 KPa of Steam Pressure
First pass	75	80
Second pass	70	94
Third pass	76	94

20

The percent yield of water-soluble chitosan and acid-soluble chitosan was determined as previously described. The chitosan yields also included a percentage of insoluble chitin. The insoluble chitin is essentially not soluble in aqueous solutions at any pH value.

25

Example 4: Effect of Feed Rate on the Conversion of Chitin to Acid-Soluble Chitosan, Water-Soluble Chitosan and Insoluble Chitin

One kilogram of water was added to one kilogram of dry Langostino lobster shell at room temperature. The aqueous chitin mixture was then fed into
5 the continuous processor of example 1 at 60 grams of the mixture per minute at room temperature to decrease the resulting chitin particles to an average of 35-mesh size.

One kilogram of sodium hydroxide pellets was then added to the processed aqueous chitin mixture to produce an alkaline-chitin mixture having a
10 1:1:1 ratio of sodium hydroxide to chitin source to water by weight. The alkaline-chitin mixture was fed into the continuous processor at room temperature to assure a uniform mixing.

The alkaline-chitin mixture was then fed at various feed rates into the continuous processor of example 1 heated with 655 KPa of steam pressure.
15 Residence time of the alkaline-chitin mixture in the continuous processor was no longer than four minutes. A yield of insoluble chitin, acid-soluble chitosan and water-soluble chitosan for each of the tested feed rates was determined. The results indicated a mixture of the acid-soluble chitosan, water-soluble chitosan, and insoluble chitin. As discussed above, this mixture can be expected due to
20 the nature of the chitin starting material and the processing conditions. One skilled in the art will recognize that due to the variability in the chitin starting materials (e.g., the amount of acetyl groups present on the chitin source, etc.) there can also be variability in the production of both the water-soluble chitosan, acid-soluble chitosan of the present invention.

25

Example 5: Effect of Moisture Contents and Chitin: Sodium Hydroxide Ratio on the Yields of Water-Soluble Chitosan, Acid-Soluble Chitosan, and Insoluble Chitin

Various amounts of water were added to one kilogram of dry Langostino
30 lobster shell to make the aqueous chitin mixtures at room temperature. Sodium hydroxide pellets were then added to the aqueous chitin mixtures to make alkaline-chitin mixtures having sodium hydroxide to chitin source ratios of

either 0.8:1 or 1:1 by weight. The alkaline-chitin mixtures were fed into the continuous processor of example 1 at room temperature. The alkaline-chitin mixtures were then fed at a rate of 60 grams of the mixture per minute into the continuous processor of example 1 heated with steam pressure of 655 KPa.

- 5 Residence time of the alkaline-chitin mixtures in the continuous processor was no longer than one minute.

Table 2. Effect of Moisture Content and Chitin:Sodium Hydroxide Ratio on the Yield of Water-Soluble Chitosan, Acid-Soluble Chitosan and Insoluble Chitin after One Pass in the Continuous Processor Heated with Steam Pressure of 655 KPa.

Ratio of NaOH:Chitin	Moisture Content	Yield of Water-Soluble Chitosan	Yield of Acid-Soluble Chitosan	Yield of Insoluble Chitin
1:1	35%	22.53%	20.33%	57.10%
1:1	40%	22.87%	41.10%	36.03%
1:1	40%	12.95%	72.00%	15.10%
1:1	40%	20.20%	40.20%	39.60%
1:1	40%	21.70%	22.40%	55.90%
1:1	45%	61.90%	29.60%	8.50%
1:1	45%	32.80%	53.70%	13.40%
1:1	45%	32.70%	56.10%	11.30%
1:1	45%	53.10%	20.90%	25.90%
1:1	45%	56.00%	24.40%	19.60%
1:1.25	45%	55.20%	24.50%	20.20%
1:1.25	45%	29.30%	26.70%	43.90%
1:1.25	45%	43.00%	11.60%	45.50%

Example 6: A Process that Mixed Sodium Hydroxide Without Impregnation

- One kilogram of water was added to one kilogram of dry Langostino lobster shell at room temperature. The aqueous chitin mixture was fed into the

continuous processor at 60 grams of the mixture per minute to decrease the chitin particle size to an average of 35 mesh.

One kilogram of sodium hydroxide pellets was then added to the aqueous chitin mixture to produce an alkaline-chitin mixture having a 1:1:1 water to sodium hydroxide to chitin ratio by weight. The alkaline-chitin mixture was then fed at a 60 g per minute rate into the continuous processor of example 1 heated with 655 KPa of steam pressure. Residence time of the alkaline chitin source-water mixture in the continuous processor was no longer than one minute. Yields were less than 30 percent for each of acid-soluble chitosan and water-soluble chitosan.

Example 7: Wide Angle X-Ray Diffraction Patterns of Water-Soluble Chitosan and Acid-Soluble Chitosan

The presence of crystalline structure in acid-soluble chitosan and water-soluble chitin and chitin was measured through wide angle X-ray diffraction (WAXD). WAXD was measured on a Siemens D5000 powder diffractometer.

Fig. 3 shows X-ray diffraction patterns obtained for samples of commercially produced acid-soluble chitosan (Aldrich, #44886-9) and three samples of acid-soluble chitosan of the present invention. Fig. 3 includes a first WAXRD pattern 400, a second WAXRD pattern 410, a third WAXRD pattern 420, and a fourth WAXRD pattern 430. The first WAXRD pattern 400 is from the commercially produced acid-soluble chitosan (Aldrich, #44886-9). The first WAXRD pattern 400 shows a crystalline peak 440 at 2-Theta of approximately 20° for the commercially produced acid-soluble chitosan.

The second WAXRD pattern 410, the third WAXRD pattern 420, and the fourth WAXRD pattern 430 are examples of acid-soluble chitosan produced according to the present invention. The second WAXRD pattern 410 is from acid-soluble chitosan of the present invention prepared by washing in water and then drying the acid-soluble chitosan before measuring on the powder diffractometer. The third WAXRD pattern 420 and the fourth WAXRD pattern 430 represent different lots of acid-soluble chitosan of the present invention. Both samples used for the third WAXRD pattern 420 and the fourth WAXRD

pattern 430 were first dissolved in 0.1 molar hydrochloric acid at room temperature. The acid-soluble chitosan was then precipitated with sodium hydroxide. The precipitated acid-soluble chitosan was then separated by centrifugation and dried. The samples of the dried acid-soluble chitosan were
5 then measured on the powder diffractometer.

The WAXRD patterns 410, 420, and 430 all illustrate the amorphous nature of the acid-soluble chitosan of the present invention, as there are no discernable crystalline peaks in the patterns. Peaks 450 and 460 on WAXRD pattern 420 are from sodium chloride present in the test sample.

10 Fig. 4 shows X-ray diffraction patterns obtained for samples of commercially produced chitin (Aldrich, #41795-5), commercially produced acid-soluble chitosan (Aldrich, #44886-9), a sample of the chitin source used in the present invention, and the water-soluble chitosan of the present invention. Fig. 4 includes a first WAXRD pattern 466, a second WAXRD pattern 470, a
15 third WAXRD pattern 474, and a fourth WAXRD pattern 478. The first WAXRD pattern 466 is from the commercially produced chitin (Aldrich, #41795-5). The second WAXRD pattern 470 is from the commercially produced acid-soluble chitosan (Aldrich, #44886-9). The third WAXRD pattern 474 is from a sample of the chitin source used in the present invention.
20 The first WAXRD pattern 466, the second WAXRD pattern 470, and the third WAXRD pattern 474 all show a crystalline peak at or about 480 at 2-Theta of approximately 19°.

The fourth WAXRD pattern 474 is an example of water-soluble chitosan produced according to the present invention. The fourth WAXRD pattern 474
25 is from water-soluble chitosan of the present invention prepared by adding the water-soluble chitosan in water and precipitating by the addition of ethanol at room temperature. The precipitate was filtered and dried before measuring on the powder diffractometer. The fourth WAXRD pattern 474 provides an illustration of the amorphous nature of the water-soluble chitosan of the present
30 invention, as there are no discernable crystalline peaks in the patterns.

Example 8: Viscosity of Water-Soluble Chitosan

The water-soluble chitosan of the present invention also exhibits a surprisingly high viscosity as compared to water-soluble chitosan reported in the literature. Figure 5 provides viscosity versus PH plot for the water-soluble chitosan of the present invention. The water-soluble chitosan tested had a
5 molecular weight of approximately 120 kDa, and a degree of deacetylation of approximately 59 percent. Solutions having a 0.5%, 1.0%, and 2.0% concentration of water-soluble chitosan, as determined from the weight of the water-soluble chitosan in the volume of solvent, were prepared at various pH
10 values. The viscosity of the solutions was tested at room temperature with a Brookfield viscometer with spindle No. 31 at 6 rpm.

Figure 5 illustrates the viscosity versus pH plot for the water-soluble chitosan of the present invention. These data demonstrate the surprisingly high viscosity values obtained for the water-soluble chitosan of the present invention,
15 as compared to conventional water-soluble chitosan reported in the literature. For example, the viscosity of conventional water-soluble chitosan having different molecular weights is reported by Kim *et al.* (Physicochemical and Sensory Properties of Water Soluble Chitosan, Korean J. of Food Sci. & Tech., Vol. 31, No. 1, p.83-90, 1990). The water-soluble chitosan of Kim was
20 produced through a process of enzymatic hydrolysis (e.g., lipase). Kim reports that for a 5% weight/vol. solution of water-soluble chitosan (pH= 7.0) having a molecular weight of 100 or more kDa, the viscosity is 21 ± 5.29 cps at room temperature

In contrast, the present invention provides the water-soluble chitosan
25 solution described above that displays a significantly higher viscosity at a much lower concentration of the water-soluble chitosan. For example, the water-soluble chitosan described above has viscosity values that range from approximately 53 cps at a water-soluble chitosan concentration of 0.5% (pH equal to about 7.0) to approximately 660 cps for a water-soluble chitosan
30 concentration of 2.0% (pH equal to about 7.0). Thus, solutions having high viscosities can be produced with a relatively low concentration of the water-soluble chitosan of the present invention.

In addition, Fig. 5 also demonstrates a rapid increase in the viscosity of higher concentration solutions of the water-soluble chitosan. As Fig. 5 shows, for the 2% concentration of water-soluble chitosan, as determined from the weight of the water-soluble chitosan in the volume of solvent, the viscosity value of the solution increase much more rapidly as compared to the 1% and 0.5% concentrations of water-soluble chitosan. It is during this increase in pH that the higher concentration solutions of the water-soluble chitosan form a gel. In addition, for the 1% concentration solution of water-soluble chitosan, it has been observed that, relative the viscosity at pH 9.0, the viscosity of the solution decreases at pH values of 11 and higher. It is believed that this decrease in viscosity will also occur for other concentrations (e.g., 0.5%, 2%, etc.) of the water-soluble chitosan solution at or above pH values of 11.

Example 9: Effect of Shear and Temperature on the Crystalline Structure of the Soluble Chitosan of the Present Invention

As discussed above, the heat and the high shear stress used in the present invention are believed to cause a deformation in the crystalline structure of the chitin and chitosan molecules. For example, the heat supplied during the heating and shearing is believed to be sufficient to cause the reaction temperature to be equal to or greater than the reduced glass transition temperature and the melting temperature of the chitin and chitosan molecules. While not wishing to be bound by theory, it is believed that this allows the chains of the chitin and chitosan molecules, including the chains forming the crystalline structure, to relax.

The effects of shear stress and temperature on the crystalline structure in the water-soluble chitosan of the present invention was measured through wide angle X-ray diffraction (WAXD). WAXD was measured on a Siemens D5000 powder diffractometer.

Fig. 6A shows X-ray diffraction patterns obtained for two samples of the water-soluble chitosan of the present invention and a sample of the chitin source used in the present invention. Fig. 6A includes a first WAXRD pattern 600, a second WAXRD pattern 610, and a third WAXRD pattern 620. The samples of

the water-soluble chitosan of the present invention were produced under the same conditions, except that the sample of the first WAXRD pattern 600 was subjected to a higher shearing stress during processing than the sample of the second WAXRD pattern 610.

5 The first WAXRD pattern 600 is from a first sample of the water-soluble chitosan produced under a first shearing condition according to the present invention. The second WAXRD pattern 610 is from a second sample of the water-soluble chitosan produced under a second shearing condition according to the present invention. The shearing conditions for the water-soluble chitosan of
10 the first WAXRD pattern 600, the first shearing conditions, were higher as compared to the second shearing conditions for the water-soluble chitosan of the second WAXRD pattern 610.

 The first WAXRD pattern 600 provides an illustration of the effect of higher relative shear stress during production of the water-soluble chitosan of
15 the present invention, as there are no discernable crystalline peaks in the first WAXRD pattern 600. In contrast, there is a slight discernable crystalline peak in the second WAXRD pattern 610 for the second shearing condition at or about 640 at 2-Theta of approximately 20°. The third WAXRD pattern 620 is from a sample of the chitin source used in the present invention. The third WAXRD
20 pattern 620 shows a crystalline peak at or about 644 at 2-Theta of approximately 19°.

 Fig. 6B shows X-ray diffraction patterns obtained for four samples of the water-soluble chitosan of the present invention and a sample of the chitin source used in the present invention. Fig. 6B includes a first WAXRD pattern
25 650, a second WAXRD pattern 660, a third WAXRD pattern 670, a fourth WAXRD pattern 680, and a fifth WAXRD pattern 690. The samples of the water-soluble chitosan of the present invention were produced under the same conditions, except that the sample of the first WAXRD pattern 650 was subjected to a higher shearing stress during processing than the sample of the
30 second WAXRD pattern 660, and steam pressure used to process the sample of the first WAXRD pattern 650 and the sample of the second WAXRD pattern 660 (both 65.5 KPa of saturated steam gauge pressure) were different than the

steam pressure used to process the sample of the third WAXRD pattern 670 (51.7 KPa of saturated steam gauge pressure) and fourth WAXRD pattern 680 (37.9 KPa of saturated steam gauge pressure).

5 The first WAXRD pattern 650 is from a first sample of the water-soluble chitosan produced under a first shearing condition according to the present invention. The second WAXRD pattern 610 is from a second sample of the water-soluble chitosan produced under a second shearing condition according to the present invention. The shearing conditions for the water-soluble chitosan of the first WAXRD pattern 650, the first shearing conditions, were higher as
10 compared to the second shearing conditions for the water-soluble chitosan of the second WAXRD pattern 660. In addition, both samples for the first WAXRD pattern 650 and the second WAXRD pattern 660 were processed with a steam pressure of 65.5 KPa of saturated steam gauge pressure.

The third WAXRD pattern 670 is from a third sample of the water-soluble chitosan produced under the second shearing condition, but at a steam
15 pressure of 51.7 KPa of saturated steam gauge pressure (i.e., at a lower temperature relative the first and second samples). The fourth WAXRD pattern 680 is from a fourth sample of the water-soluble chitosan produced under the second shearing condition, but at a steam pressure of 37.9 KPa of saturated
20 steam gauge pressure (i.e., at a lower temperature relative the first, second, and third samples).

The second, third and fourth WAXRD patterns, 660, 670, and 680 provides an illustration of the effect of temperature during production of the water-soluble chitosan of the present invention. As the WAXRD patterns 660,
25 670, and 680 indicate, as the processing temperature of the water-soluble chitosan increases, the relative amount of crystallinity in the water-soluble chitosan decreases. Note that there is a more discernable crystalline peak 694 in the fourth WAXRD pattern 680 (at 2-Theta of approximately 20°) as compared to the crystalline peak 696 in the second WAXRD pattern 660 (at 2-Theta of
30 approximately 20°). The fifth WAXRD pattern 690 is from a sample of the chitin source used in the present invention. The fifth WAXRD pattern 690 shows a crystalline peak at or about 698 at 2-Theta of approximately 19°.

The complete disclosures of the patents, patent documents, and publications cited herein are incorporated by reference in their entirety as if each were individually incorporated. Various modifications and alterations to
5 this invention will become apparent to those skilled in the art without departing from the scope and spirit of this invention. It should be understood that this invention is not intended to be unduly limited by the illustrative embodiments and examples set forth herein and that such examples and embodiments are presented by way of example only with the scope of the invention intended to
10 be limited only by the claims set forth herein as follows.

What is claimed is:

1. A method of producing soluble chitosan, comprising:
providing an aqueous-chitin mixture comprising a ratio of a chitin
5 source to water of at least about 1:1.9 by weight; and
subjecting the aqueous-chitin mixture to shearing and heating while in
the presence of a base at a temperature and for a time effective to deacetylate
the chitin and produce soluble chitosan having a substantially amorphous
structure.
10
2. The method of claim 1, wherein subjecting the chitin-water mixture to
shearing and heating occurs substantially simultaneously.
3. The method of claim 1, wherein providing the aqueous-chitin mixture
15 includes providing an aqueous-chitin mixture comprising a ratio of a chitin
source to water of about 1:1.9 to about 1:0.7 by weight.
4. The method of claim 1, wherein subjecting the aqueous-chitin mixture to
shearing and heating produces a water-soluble chitosan having a substantially
20 amorphous structure.
5. The method of claim 4, wherein the water-soluble chitosan has about
thirty (30) percent to about fifty-five (55) percent available acetyl groups.
- 25 6. The method of claim 1, wherein subjecting the aqueous-chitin mixture to
shearing and heating produces an acid-soluble chitosan having a substantially
amorphous structure.
7. The method of claim 6, wherein the acid-soluble chitosan has about
30 twenty (20) percent or less available acetyl groups.

8. The method of claim 6, wherein subjecting the aqueous-chitin mixture to shearing and heating produces a mixture of the acid-soluble chitosan and a water-soluble chitosan each having the substantially amorphous structure.
- 5 9. The method of claim 1, comprising subjecting the aqueous-chitin mixture to shearing forces to decrease a particle size of the chitin source and produce sheared chitin prior to subjecting the aqueous-chitin mixture to shearing and heating; and
providing the base to the aqueous-chitin mixture having the sheared
10 chitin.
10. The method of claim 9, wherein subjecting the aqueous-chitin mixture to shearing and heating produces a water-soluble chitosan.
- 15 11. The method of claim 10, wherein the water-soluble chitosan has about thirty (30) percent to about fifty-five (55) percent available acetyl groups.
12. The method of claim 9, wherein subjecting the aqueous-chitin mixture to shearing and heating produces an acid-soluble chitosan.
20
13. The method of claim 12, wherein the acid-soluble chitosan has about twenty (20) percent or less available acetyl groups.
14. The method of claim 12, wherein subjecting the aqueous-chitin mixture
25 to shearing and heating includes producing a mixture of the acid-soluble chitosan and a water-soluble chitosan each having the substantially amorphous structure.
15. The method of claim 9, wherein subjecting the aqueous-chitin mixture to
30 shearing forces produces a particle size of the chitin source of five (5) millimeters or smaller.

16. The method of claim 15, wherein subjecting the aqueous-chitin mixture to shearing forces includes maintaining a temperature of the aqueous-chitin mixture at or below one hundred 100 degrees Celsius.
- 5 17. The method of claim 1, wherein providing the aqueous-chitin mixture includes using an alkaline solution in providing the aqueous-chitin mixture.
18. The method of claim 1, including adding the base to the chitin-water mixture in an amount to produce a ratio of base to the chitin source of less than
10 about 1:0.33 by weight.
19. The method of claim 1, wherein heating includes heating at a temperature of at least 100 degrees Celsius.
- 15 20. The method of claim 1, wherein shearing includes producing a particle size of the chitin source of five (5) millimeters or smaller.
21. The method of claim 1, wherein the time effective to deacetylate the chitin and produce soluble chitosan is less than about four minutes.
20
22. The method of claim 1, including adding functional groups to the soluble chitosan to produce chitosan derivatives.
23. A soluble chitosan prepared in accordance with the method of claim 1.
25
24. A soluble chitosan produced by a process comprising:
providing an aqueous-chitin mixture comprising a ratio of a chitin source to water of at least about 1:1.9 by weight; and
subjecting the aqueous-chitin mixture to shearing and heating while in
30 the presence of a base at a temperature and for a time effective to deacetylate the chitin and produce soluble chitosan having a substantially amorphous structure.

25. The soluble chitosan of claim 24, wherein subjecting the chitin-water mixture to shearing and heating occurs substantially simultaneously.
- 5 26. The soluble chitosan of claim 24, wherein providing the aqueous-chitin mixture includes providing the aqueous-chitin mixture comprising the ratio of the chitin source to water is about 1.1:9 to about 1:0.7 by weight.
27. The soluble chitosan of claim 24, wherein subjecting the aqueous-chitin
10 mixture to shearing and heating produces a water-soluble chitosan.
28. The soluble chitosan of claim 27, wherein the water-soluble chitosan has about thirty (30) to about fifty-five (55) percent available acetyl groups.
- 15 29. The soluble chitosan of claim 24, wherein subjecting the aqueous-chitin mixture to shearing and heating includes producing an acid-soluble chitosan having the substantially amorphous structure.
30. The soluble chitosan of claim 29, wherein the acid-soluble chitosan has
20 about twenty (20) percent or less available acetyl groups and the substantially amorphous structure.
31. The soluble chitosan of claim 29, wherein subjecting the aqueous-chitin mixture to shearing and heating includes producing a mixture of the acid-
25 soluble chitosan and a water-soluble chitosan each having the substantially amorphous structure.
32. The soluble chitosan of claim 24, comprising subjecting the aqueous-chitin mixture to shearing forces to decrease a particle size of the chitin source
30 and produce sheared chitin prior to subjecting the aqueous-chitin mixture to shearing and heating; and

providing the base to the aqueous-chitin mixture having the sheared chitin.

33. The soluble chitosan of claim 32, wherein subjecting the aqueous-chitin mixture to shearing and heating produces a water-soluble chitosan having the substantially amorphous structure.

34. The soluble chitosan of claim 33, wherein the water-soluble chitosan has about thirty (30) to about fifty-five (55) percent available acetyl groups.

35. The soluble chitosan of claim 32, wherein subjecting the aqueous-chitin mixture to shearing and heating produces an acid-soluble chitosan having the substantially amorphous structure.

36. The soluble chitosan of claim 35, wherein the acid-soluble chitosan has about twenty (20) percent or less available acetyl groups.

37. The soluble chitosan of claim 35, wherein subjecting the aqueous-chitin mixture to shearing and heating produces a mixture of the acid-soluble chitosan and a water-soluble chitosan each having the substantially amorphous structure.

38. The soluble chitosan of claim 32, wherein subjecting the aqueous-chitin mixture to shearing forces produces a particle size of the chitin source of five (5) millimeters or smaller.

39. The soluble chitosan of claim 38, wherein subjecting the aqueous-chitin mixture to shearing forces includes maintaining a temperature of the aqueous-chitin mixture at or below one hundred 100 degrees Celsius.

40. The soluble chitosan of claim 24, including adding the base to the chitin-water mixture in an amount to produce a ratio of base to the chitin source of less than about 1:0.33 by weight.

41. The soluble chitosan of claim 24, wherein heating includes heating at a temperature of at least 100 degrees Celsius.
- 5 42. The soluble chitosan of claim 24, wherein the time effective to deacetylate the chitin and produce soluble chitosan is less than about four minutes.
43. A soluble chitosan having an amorphous structure.
- 10 44. The soluble chitosan of claim 43, wherein the soluble chitosan is water-soluble.
45. The soluble chitosan of claim 44, wherein the water-soluble chitosan has
15 about thirty (30) percent to about fifty-five (55) percent available acetyl groups randomly distributed throughout the water-soluble chitosan.
46. The soluble chitosan of claim 43, wherein the soluble chitosan is acid-soluble.
- 20 47. The soluble chitosan of claim 46, wherein the acid-soluble chitosan has about twenty (20) percent or less available acetyl groups randomly distributed throughout the acid-soluble chitosan.
- 25 48. A method of fat removal, comprising:
providing an effective amount of a soluble chitosan having a substantially amorphous structure to bind fat; and
binding fat molecules with the soluble chitosan.
- 30 49. The method of claim 48, wherein providing includes providing an effective amount of a water-soluble chitosan to bind fat, where the water-soluble chitosan includes about thirty (30) to about fifty-five (55) percent

available acetyl groups randomly distributed throughout the water-soluble chitosan.

50. The method of claim 48, wherein providing includes providing an
5 effective amount of an acid-soluble chitosan to bind fat, where the acid-soluble chitosan includes about twenty (20) percent or less available acetyl groups randomly distributed throughout the acid-soluble chitosan.

51. The method of claim 48, wherein providing includes providing an
10 effective amount of a mixture of a water-soluble chitosan and an acid-soluble chitosan to bind fat, where the water-soluble chitosan has about thirty (30) to about fifty-five (55) percent available acetyl groups randomly distributed throughout the water-soluble chitosan, and the acid-soluble chitosan has about
15 twenty (20) percent or less available acetyl groups randomly distributed throughout the acid-soluble chitosan.

52. A composition formulated for use in a mammal comprising a soluble chitosan having an amorphous structure.

20 53. The composition of claim 52, wherein the soluble chitosan includes a water-soluble chitosan having about thirty (30) to about fifty-five (55) percent available acetyl groups randomly distributed throughout the water-soluble chitosan.

25 54. The composition of claim 53, wherein the soluble chitosan includes an acid-soluble chitosan having about twenty (20) percent or less available acetyl groups randomly distributed throughout the acid-soluble chitosan.

55. The composition of claim 52, further comprising a pharmaceutically
30 acceptable carrier.

56. The composition of claim 52, wherein the composition is formulated for use as a fat binding composition in the mammal.
57. The composition of claim 56, formulated for oral administration to the
5 mammal.
58. The composition of claim 52, wherein the composition is formulated for use as a food composition in the mammal.
- 10 59. The composition of claim 58, further comprising a food grade acceptable carrier.
60. The composition of claim 58, formulated for oral administration to the mammal.
15
61. The composition of claim 52, wherein the composition is formulated for use as a pharmaceutical composition in the mammal.
62. The composition of claim 61, further comprising a pharmaceutically
20 acceptable carrier.
63. The composition of claim 61, formulated for oral administration to the mammal.
- 25 64. The composition of claim 52, wherein the composition is formulated for use as a cosmetic composition in the mammal.
65. The composition of 64, further comprising a cosmetically acceptable carrier.
30
66. The composition of claim 52, wherein the composition is formulated for use as a nutraceutical composition in the mammal.

67. The composition of claim 66, further comprising a nutraceutically acceptable carrier.

5 68. The composition of claim 66, formulated for oral administration to the mammal.

69. A composition comprising chitosan having a substantially amorphous structure.

10

70. The chitosan of claim 69, wherein the chitosan is a water-soluble chitosan.

15 71. The chitosan of claim 70, wherein the water-soluble chitosan has about thirty (30) percent to about fifty-five (55) percent available acetyl groups randomly distributed throughout the water-soluble chitosan.

72. The chitosan of claim 69, wherein the chitosan is an acid-soluble chitosan.

20

73. The chitosan of claim 72, wherein the acid-soluble chitosan has about twenty (20) percent or less available acetyl groups randomly distributed throughout the acid-soluble chitosan.

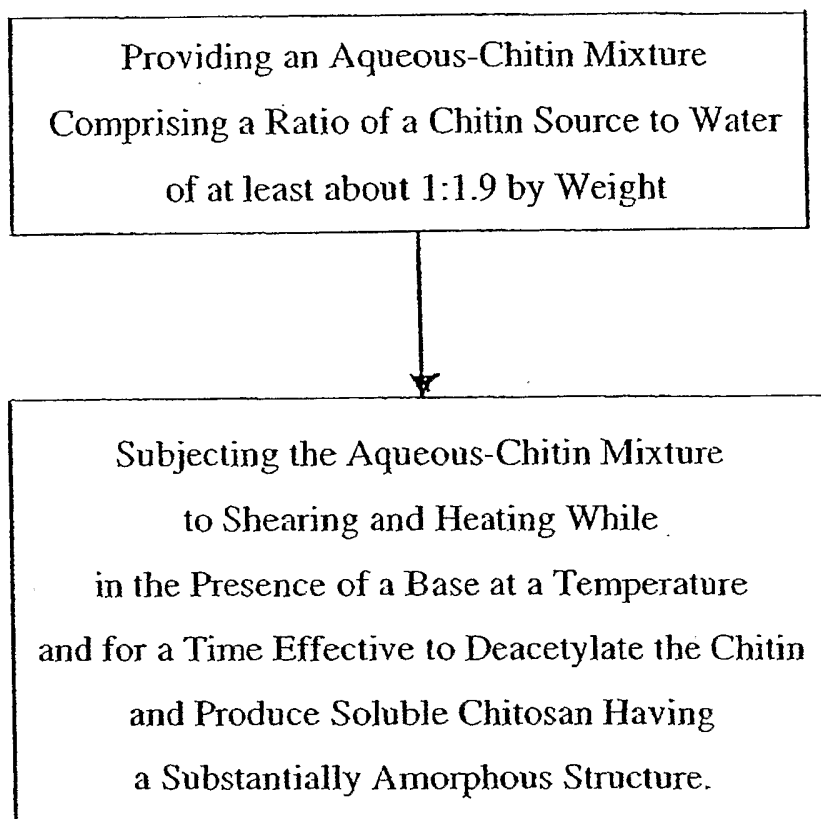


FIG. 1

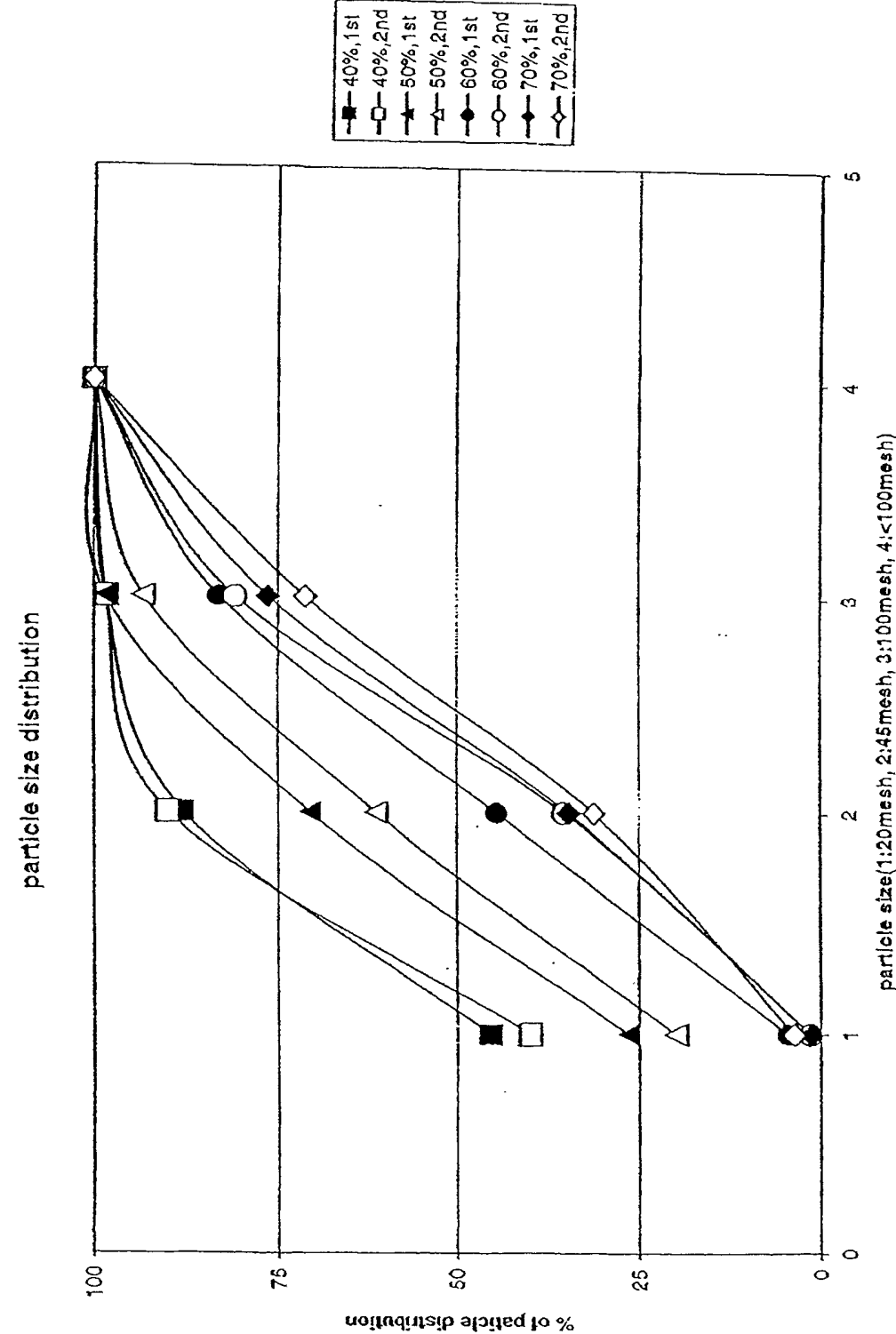


Fig. 2

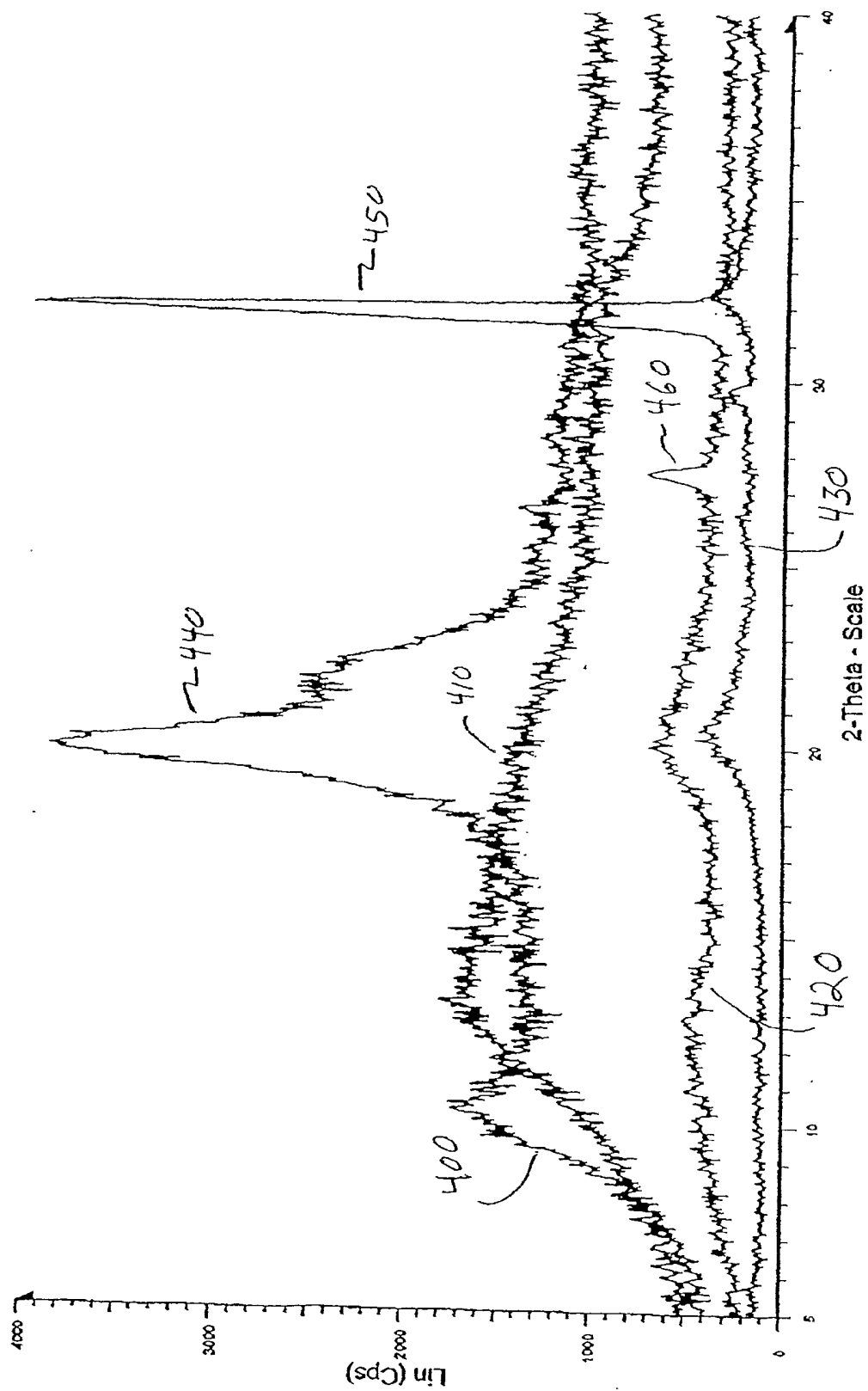


Fig. 3

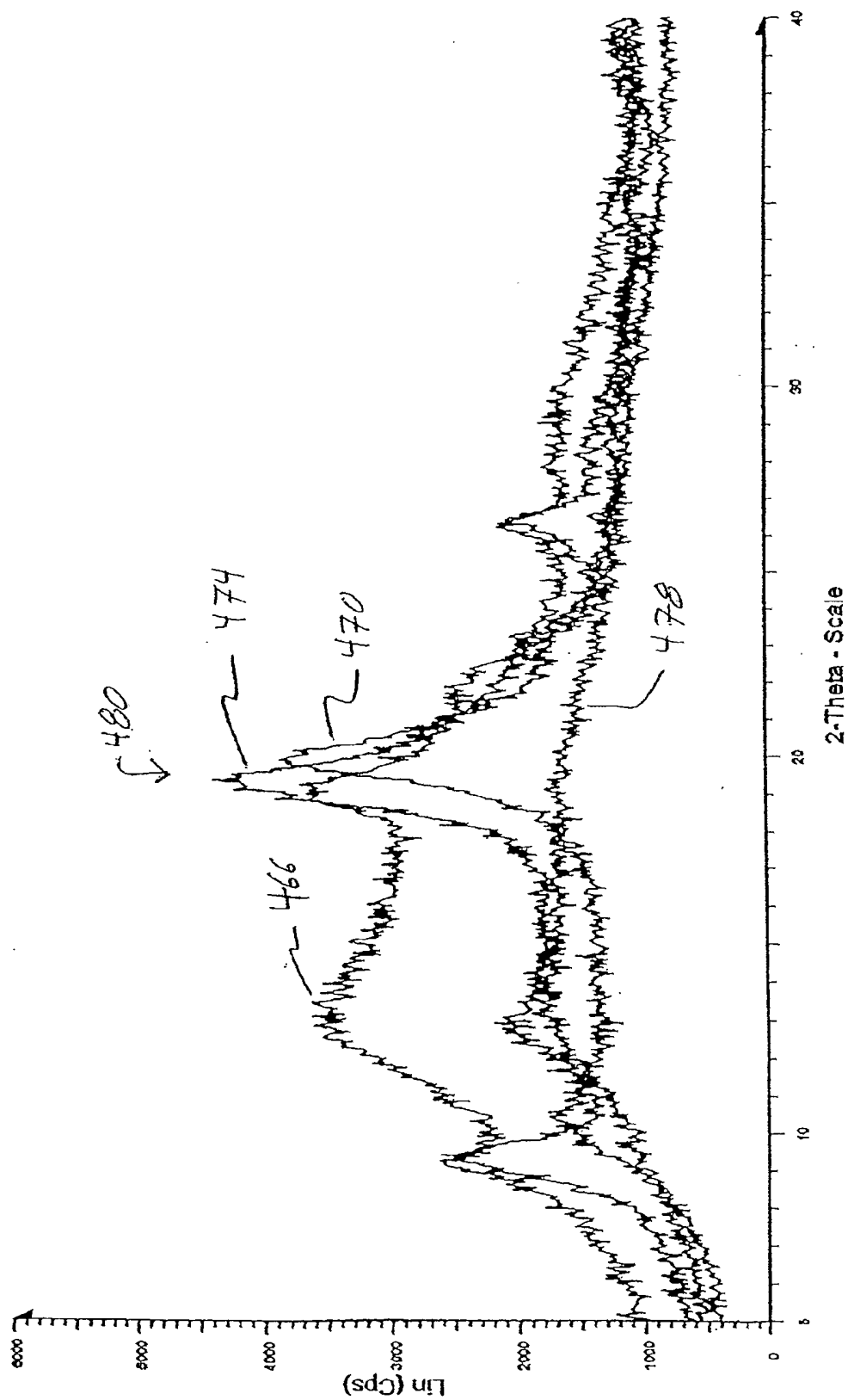


Fig. 4

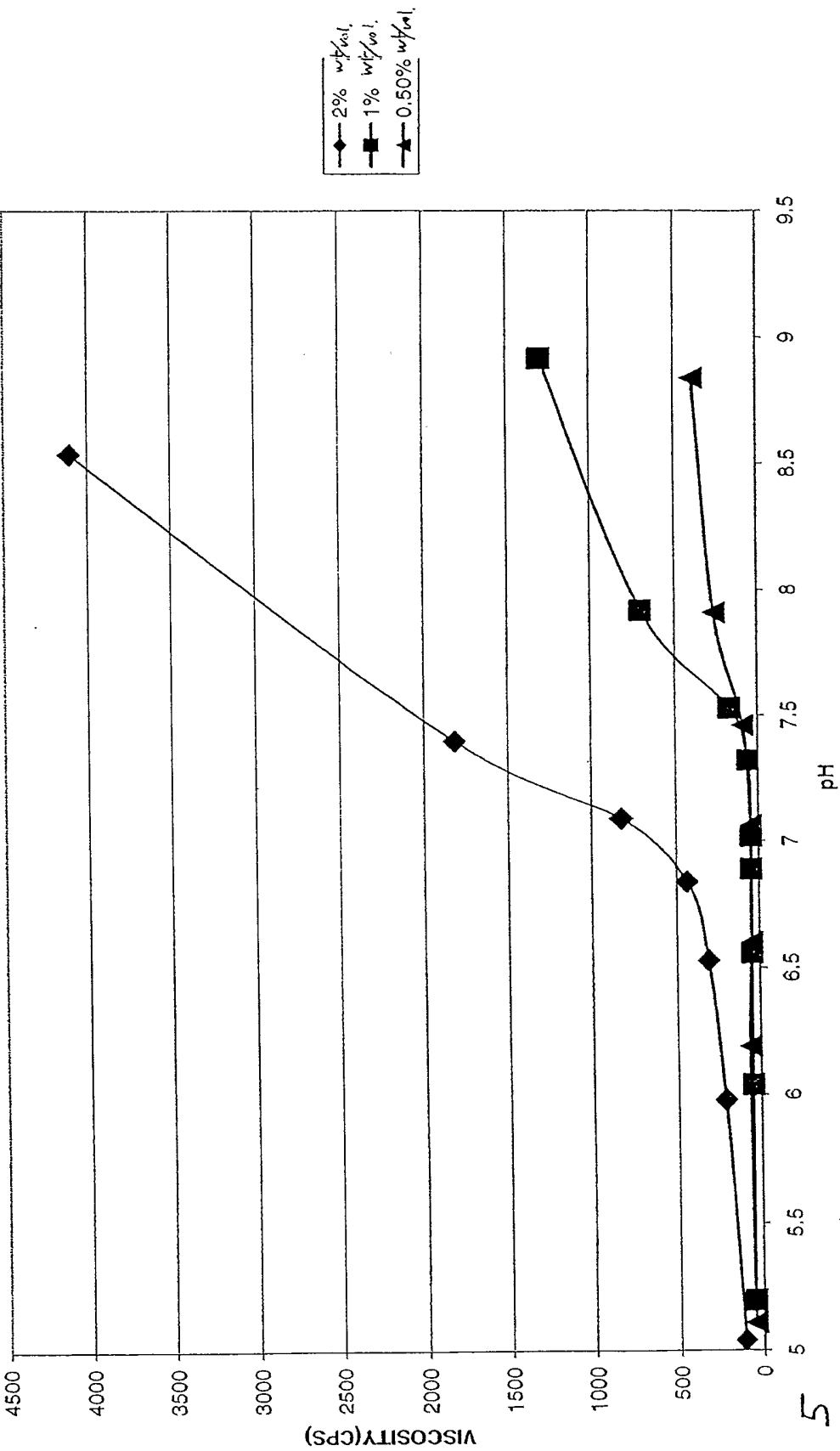


Fig. 5

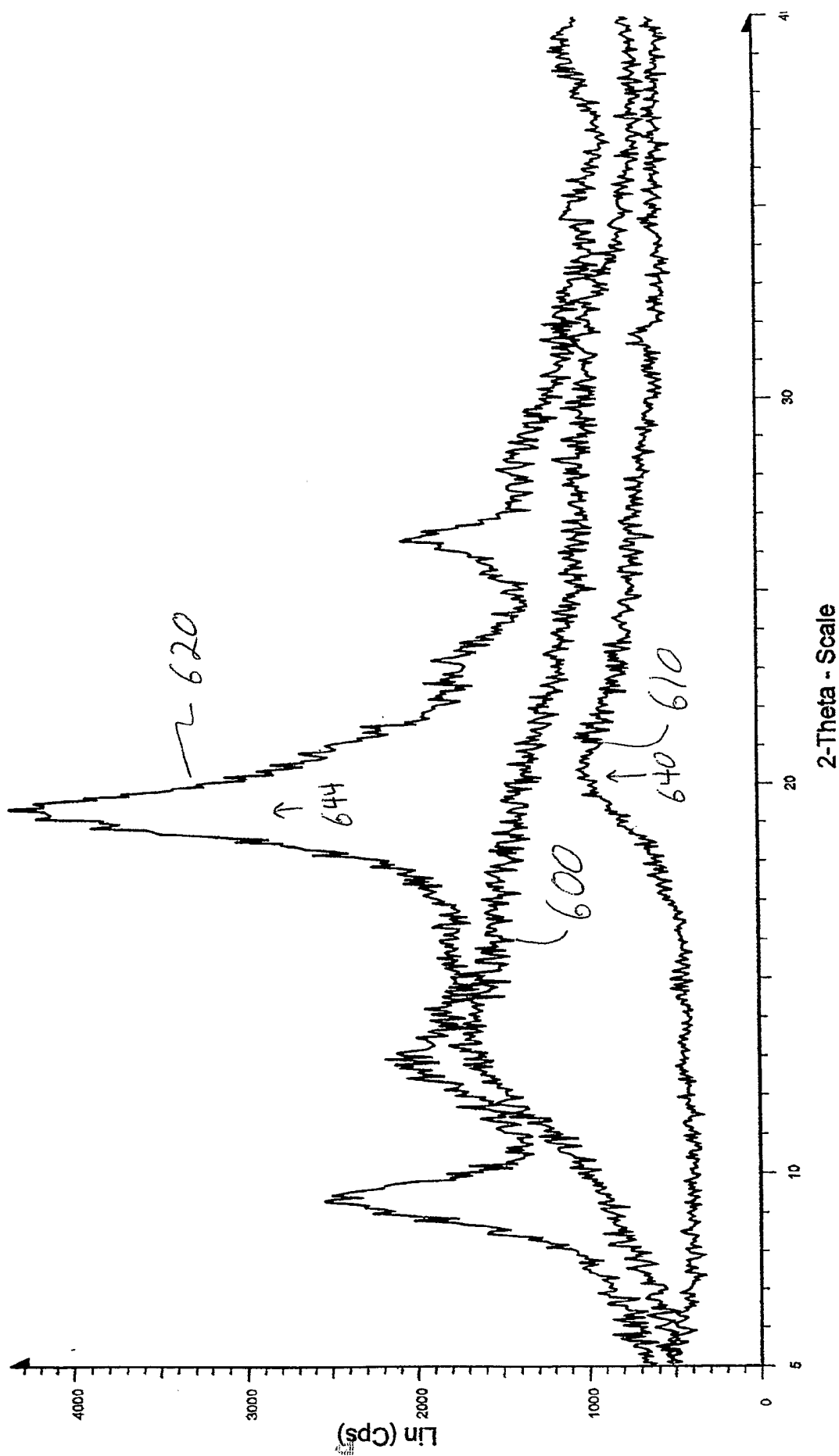


FIG. 6A

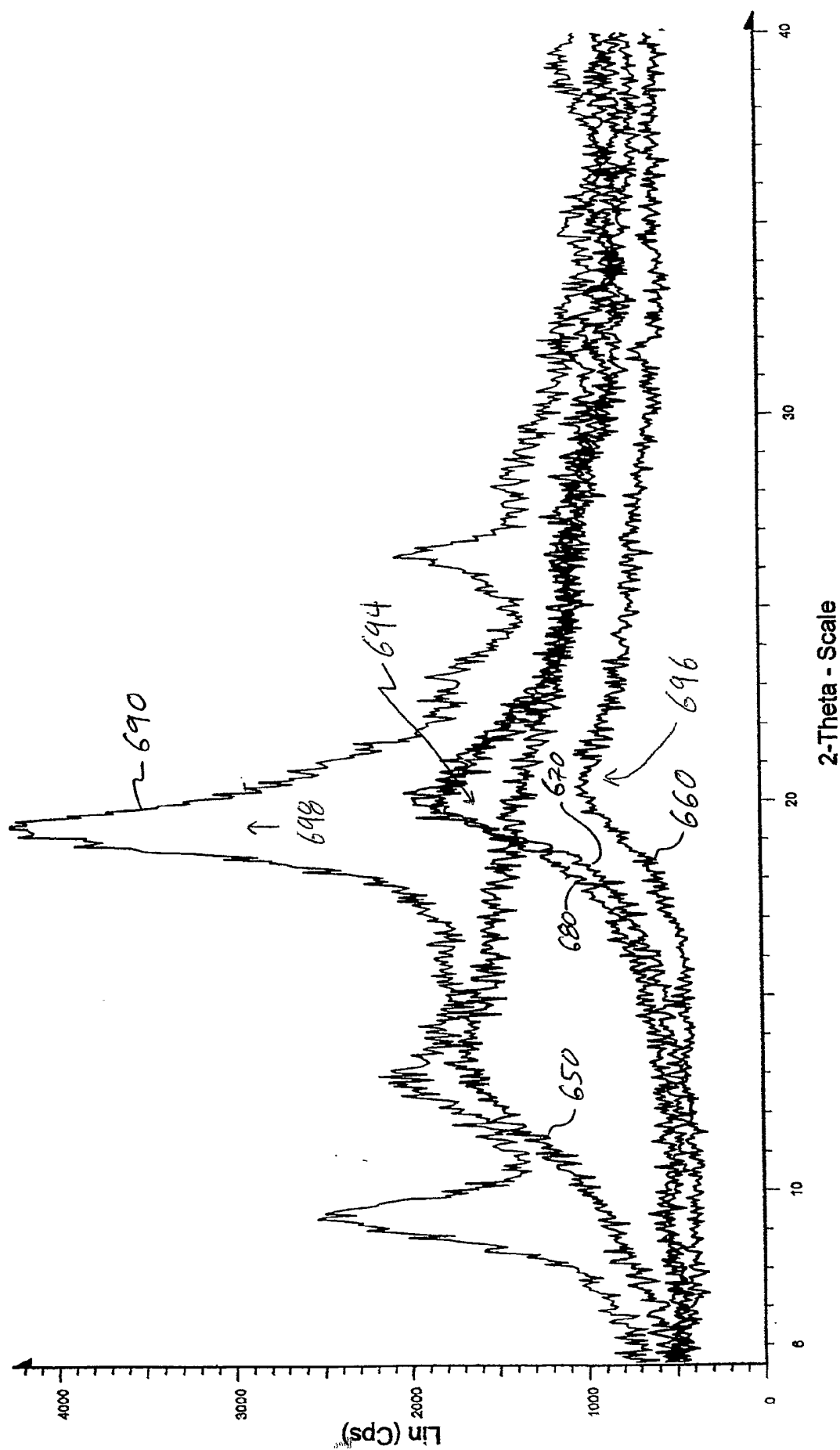


FIG. 6B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/18442

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A01N 43/04; C08B 37/08

US CL : 514/23, 55; 536/20; 424/434

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/23, 55; 536/20; 424/434

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WEST, CAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6,130,321 A (JOHNSON et al.) 10 October 2000, see entire document.	1-73
Y	US 5,232,842 A (PARK et al.) 3 August 1993, see entire document.	1-73
Y	US 6,277,792 B1 (HOUSE) 21 August 2001, see entire document.	1-73



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T"

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y"

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&"

document member of the same patent family

Date of the actual completion of the international search

28 September 2003 (28.09.2003)

Date of mailing of the international search report

05 NOV 2003

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