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GLUCOMANNAN SPONGEOUS MATRICES

(57) Abstract

The invention relates to articles of manufacture comprising spongy matrices which may have a controlled pore size and/or distribution, formed of: Component (a) glucosamine; and Component (b) at least one other aqueous gel-forming polysaccharide; and optionally Component (c) at least one water soluble hydrocolloid other than the foregoing. The invention also relates to processes for fabricating the spongy matrices and their use as plant culture media, as surgical sponges, and as packaging material.
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GLUCOMANNAN SPONGEOUS MATRICES

This invention relates to articles of manufacture comprising spongeous matrices formed from coprocessed mixtures of a glucomannan, especially konjac derived glucomannan, and at least one other hydrocolloid; processes for fabrication of the spongeous matrices; and uses for the spongeous matrices.

Gels prepared from aqueous sols of konjac glucomannan are known in the art. Japanese published patent application 59-146560 discloses a spongy, porous konjac glucomannan as a food additive. Japanese published patent application 60-83564 discloses a water-resistant food wrap containing konjac which "becomes a water-resistant gel upon freezing." The thawed gel is then dried to produce the food wrap. Similarly, Japanese published patent application 60-141250 discloses the production of konjac which is water insoluble and which does not absorb water, by drying or freezing an alkaline sol or paste of konjac. Japanese published patent application 59-227267 relates to a method of freezing konjac to produce a product stable at a pH of 10.0 to 12.2. See also Japanese published patent application 59-227267 which discloses a method of freezing, drying or dehydrating konjac to produce a water insoluble or non-plastic product.

Gels containing agar or agarose are also known to the art. U.S. patent 4,755,377 discloses aerated agar gels formed by mixing gaseous components into the sol before gelation. Gels containing agarose are commercial products and are the subject of various patents.

Also known in the art are multi-component gels containing konjac and one or more other components such as starch (Japanese published patent application 62-259550), alginic acid or starch with xanthan or
galactomannan (Japanese published patent application 01-166378), locust bean gum, plant protein and millet jelly (Japanese published patent application 63-79572), a milk-type material (Japanese published patent application 62-107751), alginic acid and propylene glycol, starch and sodium glycolate, and starch and a sodium stearate ester (Japanese published patent applications 62-272952 and 62-195264), frozen bean curd (Japanese published patent application 62-118859), and various carrageenans (U.S. patent 4,427,704).

In addition, gels made from certain derivatives of konjac (with or without other components) are also known (Japanese patents 01-160467, 01-160468 and 02-150402).

In a first article embodiment, this invention comprises an article of manufacture which is a spongyous matrix, whose pores may be controlled as to size and/or distribution, when desired. The article of manufacture is formed from a coprocessed mixture of (a) konjac glucomannan and (b) at least one other aqueous gel-forming polysaccharide.

Component (a) can be any glucomannan, of which glucomannan derived from konjac is particularly preferred. The konjac glucomannan is not limited as to source and may be obtained from crude konjac flour, or from purified or clarified konjac flour, or from konjac glucomannan which has been chemically or physically derivatized or modified.

Component (b) comprises a polysaccharide other than a glucomannan which is capable of forming an aqueous gel. Component (b) preferably comprises: agar, agaroids, agarose, algin, alginates, carrageenan, curdian, gellan, a gel-forming chemical or physical derivative of any of the foregoing, or a mixture thereof. Component (b) more preferably comprises agar, agarose, kappa-carrageenan, a gel-forming chemical
derivative or physical modification of any of the foregoing, or a mixture thereof. Most preferably, component (b) is agar, agarose, kappa-carrageenan, or a mixture thereof.

Components (a) and (b) are present in a ratio [a:b] based on their respective dry weights, of 1:0.125-8.0, preferably 1:0.25-4.0, more preferably 1:0.5-2.

In a second article embodiment the article of manufacture of this invention may be formed including an additional component (c) which comprises one or more water soluble polysaccharides other than (a) or (b), and which need not form an aqueous gel. Component (c) preferably comprises: guar gum, gum arabic, karaya gum, locust bean gum, starch, tragacanth, or a mixture thereof; more preferably: guar gum, locust bean gum, starch, or a mixture thereof; and most preferably starch.

Component (c) may be present in up to 200.0 % by dry weight, preferably 0.5 to 200.0 % by dry weight compared to the combined dry weight of (a) and (b). When component (c) is starch, it preferably is present in 5.0 to 100.0 %, more preferably 20.0 to 75.0 % all by dry weight compared to the combined dry weight of (a) and (b).

In a third article embodiment, the spongyous matrix may have a coating. The coating may be comprised of one or more of the materials suitable for forming the spongyous matrix itself, in which instance the coating is in a non-spongyous form. Alternatively, the coating may comprise a material other than that used for forming the spongyous matrix. The coating may be a dried water-soluble substance that otherwise would be added to the matrix during use, for example a nutrient medium, buffer salt, catalyst, reagent, or the like; to which water would be added to activate or reactivate. The coating may be water insoluble or soluble and independently may
be water permeable or impermeable, all depending upon
the use for which the inventive article is intended and
the environment in which the inventive article is
placed. Virtually all known water insoluble and/or
impermeable compositions such as silicone, resins, oils,
varnishes, and the like can be employed, depending on
the compatibility of the coating composition with the
intended use of the spongeous matrix. The coatings
themselves do not constitute a part of this invention
except when combined with the inventive spongeous
matrix.

In a fourth article embodiment, the inventive
spongeous matrix can be at least partially saturated
with a carrier medium. The preferred carrier is water,
although other liquids that do not adversely affect the
spongeous matrix for its intended purpose also may be
used. The substance carried may be medium soluble or
dispersible or may be medium borne particulate matter.
Depending upon the intended use of the inventive
spongeous matrix, the carrier medium may remain therein,
or may be partially or completely removed. Examples of
carried substances include, but are not limited to: cell
growth or sustenance nutrient mediums, reagents,
pharmaceuticals, flavorings, colors, scents, cosmetics,
air fresheners, deodorants, adjuvants to any of the
foregoing, affinity or ion-exchange particulate, living
or dead cells or cellular matter, activated charcoal, or
mixtures thereof. This invention includes all
combinations of the inventive spongeous matrices with
these carried substances, although the substances are
themselves known.

In a fifth article embodiment, the inventive article
comprises the spongeous matrix of the above embodiments
in substantially dry form. For the purposes of this
embodiment "substantially dry" is defined as having no
readily expressible water, or as generally 20 % or less
by weight of water based on the total weight of the
spongesous matrix. The percentage weight of water will
vary depending upon the ambient atmospheric humidity.

Another group of embodiments of this invention are
processes for fabricating the above articles of
manufacture.

The first embodiment for fabricating articles of
manufacture according to this invention comprises the
sequential steps of:

A forming an aqueous sol of above ingredients (a),
(b), [and optionally (c)] present in the above
part by dry weight ratios;

B forming a gel by treatment of the sol with a
base of a strength and in a quantity sufficient
to make the sol of basic pH; [in a known but
less preferred gel-forming variation, when the
sol is retorted the pH can be acid, as low as
6.0, for example it can be 6.66 using a
phosphate buffer];

C freezing the formed gel; and

D thawing the frozen gel, resulting in the
inventive spongesous matrix.

In step A the respective concentrations in water of (a)
and (b), are independently of each other 0.25 to 2.0 wt
%, preferably 0.5 to 2.0 wt %, more preferably 0.5 to
1.0 wt %, all based upon the total weight of the sol.
The formation of the sol in step A preferably may be
accompanied by heating, although this is not required.

It is important that during freezing the cooling
gradient of the gel is relatively uniform and in
particular that local areas of greater cooling intensity
are avoided. A local "cold spot" during freezing may
prevent the production of a satisfactory spongesous
matrix by producing a local zone of extremely small
pores whose appearance is similar to a gel.
Another factor affecting the nature of the spongy matrices is their rate of freezing during formation. It has been observed that for a given component mixture and ratio, a faster freezing rate usually results in smaller pores and conversely that a slower freezing rate usually results in larger pores. This observation fits well with the above cold spot observation, since such cold spots would be expected to produce faster local freezing rates.

The second fabrication embodiment according to this invention comprises including in step A of the first embodiment the water soluble polysaccharide identified above as component (c), present in the above dry weight percentages relative to (a) and (b) combined.

The third fabrication embodiment according to this invention comprises including in any of the other embodiments an optional step E of the process, in which at least a portion, preferably substantially all, of the water content of the spongy matrix is removed.

The fourth fabrication embodiment according to this invention comprises sterilizing the spongy matrix of any of the foregoing processes, by any known method such as gamma radiation, microwave exposure, or preferably heating.

The fifth fabrication embodiment according to this invention comprises using potassium carbonate as the base used to form the gel in step B.

The sixth fabrication embodiment according to this invention comprises adding sufficient base in step B to result in a sol pH of 9.0 to 12.0.

The seventh fabrication embodiment according to this invention comprises coating the inventive spongy matrix to form the product described in the third article embodiment, by applying a coating comprising a substance other than that of the spongy matrix itself. The coating application can be in any known
manner, including dipping, spraying, and painting.

The eighth fabrication embodiment according to this invention comprises coating the inventive spongyous matrix to form the product described in the third article embodiment, by applying a coating comprising a substance disclosed above as comprising the spongyous matrix itself, either in sol or in thin gel form, but without forming a spongyous matrix of the coating by the inventive steps of freezing and thawing. The coating application can be in any known manner, including dipping, spraying, and painting.

Another group of embodiments of this invention are uses of the inventive articles of manufacture.

The first use embodiment for the inventive articles of manufacture comprises employing the above-described spongyous matrices for the growth or storage of a living plant material, optionally at least partially saturated with a suitable growth or storage medium. The living plant material may be a seed, embryo, graft, cutting, young plant, callus, or any viable grouping of cells.

The second use embodiment relates to plant culture and comprises the sequential steps of:

A - adding an aqueous plant nutrient medium to a spongyous matrix according to this invention; [the matrix and other ingredients optionally may be sterilized];
B - inserting a living plant material into the spongyous matrix; and
C - growing or maintaining the plant material under known suitable respective growth or maintenance physical conditions, for example temperature, humidity, and radiation including actinic to encourage either dormancy or growth.
D - optionally, when growing plant material, after a root system sufficient to maintain the
plant is established, the entire spongeous matrix and contained plant may be placed into a soil-containing growth medium, hydroponic system, or the like.

The third use embodiment comprises the continuous or intermittent replenishment or addition of plant nutrient medium when employing the second use embodiment.

The fourth method of use embodiment comprises employing the spongeous matrix of this invention as a support for tissue or cell culture in a manner similar to its use for plant material.

The fifth use embodiment comprises employing the spongeous matrices as surgical sponges, [optionally utilizing an above-described carried substance therein].

This use takes advantage of the inventive matrices slow dissolution in aqueous fluids including body fluids and/or its biodegradability. Naturally, the spongeous matrices used for this purpose are first sterilized by known means.

The sixth use embodiment comprises forming the spongeous matrices into suitable spacer shapes and employing them in substantially dry form as a packing or packaging material, [taking advantage of their inherent biodegradability for eventual disposal].

The seventh use embodiment comprises employing the spongeous matrices of this invention as filters for gases and/or fluids.

Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein are to be understood as modified in all instances by the term "about".

It should be understood that the components comprising the inventive article of manufacture are complex organic polymers of natural origin and therefore
cannot be defined by exact chemical formulae. Most of the hydrocolloids and/or polysaccharides useful as components in the inventive articles of manufacture are genera rather than species, and where a genus is referred to all known species thereof are probably useful and are included unless otherwise indicated. Illustrative of the foregoing, the Webster’s New World Dictionary of the American Language, second edition (World Pub. Co., New York, 1972) defines gum arabic as being obtained from several African acacias and tragacanth as coming from any of various, especially Asiatic, plants (genus A. stragalus) of the legume family.

Unless indicated otherwise, Components (a) and (b), and optional Component (c), refer to not only purified materials, but also crude or native materials in which the pure materials are present in an operative amount for the purposes of this invention.

As used herein, the terms "chemical derivative" or "chemically modified" used in referring to a component of the inventive spongeous matrix refer to that component having a substituent moiety, examples of which moieties include, but are not limited to, acetyl, C₁₋₄ alkyl, C₁₋₄ alkoxy1, C₂₋₄ hydroxyl, carboxy C₁₋₄ alkyl, or an alkali metal or alkaline earth metal salt thereof where appropriate. The removal of a pre-existing moiety such as by deacetylation is also a possible chemical modification.

As used herein, the terms "physical derivative" or "physically modified" used in referring to a component of the inventive spongeous matrix refer to that component having properties which have been modified by a physical manipulation which may or may not result in a change of the complex organic polymers. The product of depolymerization (degradation) in varying degrees is also considered a physical derivative.
Component (a) - Glucomannan

The type and physical form of glucomannan useful in this invention theoretically is not limited and includes glucomannans derived from trees. Because of commercial availability, and because of its known favorable properties, konjac-origin glucomannan is preferred, and references to "konjac" or "glucomannan" in this application should be understood as to konjac-derived glucomannan unless otherwise indicated.

Konjac glucomannan is a hydrocolloidal polysaccharide obtained from the tubers of various species of Amorphophallus. It is a high molecular weight, non-ionic glucomannan primarily consisting of mannose and glucose at a respective molar ratio of approximately 1.6:1.0. This slightly branched polysaccharide is connected by β-1,4 linkages and has an average molecular weight of 200,000 to 2,000,000 daltons. Acetyl moieties along the glucomannan backbone contribute to water solubility properties and are located, on average, every 9 to 19 sugar units.

Konjac flour is obtained by slicing, drying and then wet- or dry-milling the Amorphophallus tuber. This material is then pulverized, sifted and air-classified. This crude konjac flour contains numerous impurities including starches, cellulose and nitrogen-containing materials including proteins. Konjac flour is dispersible in hot or cold water and forms a highly viscous sol with a pH between 4.0 and 7.0. Solubility is increased by heat and mechanical agitation.

Although this crude konjac flour is suitable for producing the spongy matrices of the invention, it may optionally be further refined by such methods as alcohol washing or by dissolution followed by filtration.

Konjac flour is available as a commercial product

While konjac flour is preferred for use herein in either crude or refined form, konjac tubers and raw or semirefined konjac can also be employed to form the inventive spongeous matrices, since the glucomannan is contained within tiny sacs or granules in the tubers which rupture upon hydration.

For most spongeous matrices, a higher molecular weight konjac glucomannan is preferable, such as that afforded by a native or crude konjac flour. A more purified or a clarified konjac glucomannan is preferable for certain particular uses of the spongeous matrix (for example in medical, pharmaceutical or biotechnical utilization). Clarified (purified) konjac is an experimental product of FMC Corporation, Food Ingredient Division, Philadelphia, Pennsylvania, U.S.A. It forms a clear sol, as compared to the cloudy sol formed from more crude konjac glucomannan. Such konjac glucomannan usually has a lower molecular weight as a result of the purification or clarification process. In such instance, a higher concentration aqueous gel can be obtained, and is preferably used to provide better structural integrity of the spongeous matrix.

"Cold-melt" konjac, which is distinguished by its unexpected property of liquifying at lower (cold) temperatures and solidifying at higher temperatures also may be used as the konjac glucomannan component (a). Cold-melt konjac and clarified konjac and their manufacture are disclosed in pending United States patent application 07/742,136 and/or 07/742,260 and their corresponding Patent Cooperation Treaty
publications, the contents of which are incorporated herein by reference.

Component (b) - Aqueous Gel-Forming Polysaccharide

Among the component (b) substances "agar", sometimes referred to as agar-agar, is a phycocolloid derived from red algae, such as Gelidium and Gracilaria, and is primarily a polysaccharide mixture of a variety of galactan molecules containing varying amounts of ester sulfate and/or pyruvate and/or methoxyl groups, the least ionic being termed "agarose".

Methods for the isolation of agarose from agar are well known to the art. The particular source of the agar or agarose is not important to the practice of the present invention, although some minor variations in the properties of the spongeous matrices may exist depending on the source of the agar and/or agarose, and whether it is used unmodified, or as a chemical or physical derivative.

Carrageenan for use in the spongeous matrices of the invention can be a mixture of the known carrageenan fractions, but is preferably predominantly, (and most preferably entirely) the kappa fraction.

Component (c) - Optional Water-Soluble Polysaccharides

Not all polysaccharides are suitable as the optional third component (c). As noted in the following examples, it was determined that chitosan is unsuitable because it requires acid conditions to be water soluble and the spongeous matrix forming processes require alkaline conditions. Exposing a chitosan aqueous sol to the alkaline conditions used in forming the inventive spongeous matrices results in the chitosan coming out of the sol. Gum arabic, karaya gum, and tragacanth are each useful as component (c) when present at 5.0 % dry weight, but work poorly or not at all at 50.0 % dry
weight. These polysaccharides therefore are less desirable as component (c), but still form compositions at lower concentrations of up to approximately 25.0 % dry weight, or when combined with starch, and are useful in forming inventive articles of manufacture that have utility for certain applications.

Starches that can be employed herein can be obtained from any source, e.g. from corn, potatoes, tapioca, rice, and wheat, and can be high or low in amylose.

The term "spongeous matrix" used to characterize the articles of manufacture of this invention refers to matrices having a predominantly sponge-like character upon formation. For most (but not all) utilities, it is preferred that they possess excellent structural stability and mechanical strength, and in addition have a porous, mostly interconnecting pocket-like structure, from which liquid present can be readily expressed. By the term "mostly interconnecting" is meant that at least 60% and generally at least 75% of the pockets interconnect. In addition, many of the preferred spongeous matrices when compressed exhibit rapid return to their precompressed size and shape, with rapid uptake of aqueous solutions, for example where the pores of the spongeous matrix are substantially filled with the aqueous solution when compressed, immersed in the aqueous solution, and then pressure released. Moreover, most of the spongeous matrices of the invention exhibit at least some stability to short immersion in boiling water.

While spongeous matrices can sometimes be formed from the individual components of the present spongeous matrices, for example from konjac glucomannan alone or agar or agarose alone, such spongeous matrices do not possess all of the favorable characteristics of the spongeous matrices of the invention. For example, these single component spongeous matrices tend to be weaker
than those of the present invention, possessing little structural integrity and contain cavities more like channels or tunnels rather than the mostly interconnecting pockets characteristic of the inventive articles of manufacture. Single component spongy matrices specifically are excluded from this invention.

The spongy matrices of the invention have many uses. One important use is in agriculture. Solid aqueous nutrient gel media have been used previously as supports in the growth and storage of seeds, plant cells, tissues, explants, embryos, grafts, young plants, and callus cultures. However, lack of air space in such solid supports inhibits growth and provides a barrier to root development and growth for the plant cultures. In addition, it is often desirable to change the nutrient media at different stages of growth by adding or deleting specific nutrients or plant hormones. Such desired change in nutrient media is not feasible in solid media supports, but is with the inventive spongy matrices. Furthermore, prior to transplanting the young plants from solid media it has been necessary to separate them from the solid supports, a labor intensive procedure that generally damages the fragile root systems.

The inventive spongy matrices can be hydrated in aqueous nutrient media to provide a beneficial combination of aqueous nutrient media and air space, which also permits rapid continuous or intermittent nutrient media replacement and/or exchange. Moreover, plant root systems readily penetrate the spongy matrix supports, which need not be removed prior to transplanting since the spongy matrices are completely biodegradable. In addition to plugs, spongy matrices in strip form can be used as biodegradable seed tapes.

Another important use of the present spongy
matrices in sterile form is as surgical sponges and in the external treatment or packing of surface wounds or conditions, for example as ear packings or for wound cleaning in which medicaments can also be present in or on the matrices. Where surgical spongyous matrices of this invention purposely or accidentally are left in a wound, they may present little problem where they can be absorbed by body fluids, depending upon their component materials.

A particularly important use for the spongyous matrices of the invention is as packing or packaging filler and protective materials which after use can be reused, discarded or buried in landfills without concern since the spongyous matrices are biodegradable. For this purpose, the spongyous matrices can be formed in any shape including those conventionally used for packaging filler.

The inventive spongyous matrices can also be used in many other fields such as for reagent delivery substrates, adsorbents, controlled release devices, chromatography packing, perfusion/dialysis equipment, diagnostic reagent carriers, immobilized enzyme reactors, collagen substitutes, cosmetic and air freshener substrates, scent and flavoring carriers such as lobster or fish baits, filters, electrophoresis wicks, microbial growth supports and sampling devices, filtration media, controlled release of reactants, substrates for the release of insect repellants, and the like.

In the process for fabricating the inventive articles of manufacture, bases for forming the gels are well known and those that can be used include (but are not limited to) ammonia, alkali metal hydroxides, (especially sodium and potassium) and alkali metal carbonates, (especially sodium and potassium), potassium carbonate being most preferred. Sufficient base is
added to the sol to create an alkaline pH, preferably one having a pH of 9.0 to 12.0, and the resulting sol is then heated until it forms a uniform gel.

The thawed spongeous matrix, in an optional step E, may be compressed to remove most of the liquid content of the spongeous matrix. The spongeous matrix can be dried completely in known manner such as by drying at ambient or at an elevated temperature, by freeze-drying, and by drying under a vacuum.

Preferably, prior to heating, the spongeous matrix is rinsed by squeeze/swell cycles in water, and then in a C$_{1-6}$ alkanol or alkanol-water mixture, for example 65% 2-propanol.

The dry spongeous matrices of the invention can be sterilized as desired by using heat (e.g., by autoclaving while being protected from direct contact with steam).

It was found that when the gel that forms in step B is partially dried before the gel is frozen in step C, the resulting sponge has a tough surface layer or skin, which may be advantageous for some applications. Alternatively, the skin can be removed prior to use.

Alternative methods of forming a skin include: immersion of the formed spongeous matrix in a dessicating medium such as isopropyl alcohol or acetone, or coating the matrix with other (preferably water-insoluble) known agents.

**EXAMPLES**

The following examples are given to further illustrate the invention.

**Example 1**

**Konjac/Agarose Composite Spongeous Matrices**

A 1% w/v clarified konjac sol was divided into a
number of 50 ml aliquots. Various amounts of agarose powders were added to the aliquots and dissolving by heating and stirring. The agarose samples were; Seakem® LE low electroendosmosis agarose (0.5% w/v, 1%, and 2%), and Seakem Gold agarose (1% and 2%). [Seakem® agarose products are manufactured by FMC BioProducts, Rockland, Maine 04841 U.S.A.]. Each sample was mixed with 2 ml 5M NH₄OH, heated for 20 minutes in a boiling water bath and then frozen. The samples were slowly thawed at room temperature. The samples containing Seakem LE and Seakem Gold agaroses formed spongeous matrices with large cavities, which easily shed their liquid when squeezed. As agarose concentration increased, cavity size decreased. When squeezed flat and released, the matrices rebounded to full size indicating a vast increase in structural integrity over sponges formed from the individual components. When placed in water, the spongeous matrices completely filled by capillary action. All but the 0.5% agarose-containing spongeous matrix were soaked in 83% 2-propanol, drained and then dried for an hour at 80°C. When placed back in water, they quickly rehydrated and filled with water.

Example 2

Konjac/Agar Composite Spongeous Matrices

Crude konjac flour was used to prepare two 100 ml samples of a 1% aqueous sol. One gram of Gracilaria-derived agar was added to one sample and dissolved by heating to boiling in a microwave oven. The sample was then stirred in a hot water bath until fully dissolved. Two aliquots were removed; one of 25 g and one of 50 g. The smaller aliquot was diluted with water 1:1 thus giving a concentration of 0.5% w/v konjac and 0.5% agar. The second 100 ml konjac sol was mixed with 2 g of agar and treated in the same manner. The four 50 g samples (konjac/agar concentrations of: 0.5/0.5%, 1/1%, 0.5/1%
and 1/2%) were all mixed with 0.375 ml of a 1M K₂CO₃ solution and heated for 25 minutes in a boiling water bath. The resulting gels were covered and frozen overnight. The resulting spongeous matrix samples were thawed and examined. The spongeous matrices appeared very similar to the konjac/agarose matrices of Example 1 with minor exceptions. Whereas the agarose-containing matrices were covered with a strong skin and had to be forcibly squeezed to remove liquid, the konjac/agar matrices had a thin skin which allowed most of the internal liquid to drain away by gravity. The spongeous matrices were processed and dried as previously described. The dried samples all rehydrated and filled with water. The 0.5% konjac/1% agar matrix was the fastest to swell while the 0.5%/0.5% matrix was the slowest.

Example 3
Konjac/Agarose Composite Spongeous Matrices by Freeze Drying

A sol of 1% konjac from crude konjac flour containing 1% Seakem® LE agarose was prepared. A portion of this (150 ml) was cast into a crystallizing dish and gelled by adding 1.125 ml of 1M K₂CO₃ in a boiling water bath. The gel was placed in a freezer at -15°C for 1.5 hours and then transferred to a freezer at -80°C for 1 hour. The sample was placed in a lyophilizer on a glass plate and dried under vacuum (~ 0.1 Torr) with a shelf temperature of 100°F (about 38°C). After 3 days the sample was removed and weighed (3.59 g). The resulting spongeous matrix readily hydrated and filled when placed in water. It had numerous small uniform cavities.
Example 4
Process Variations in Konjac/Agarose Matrix Formation
The composite sponges of Examples 1-3 were formed by gelling the konjac component (a) through the use of a base and heat. The other component (b) solidified as the hot gel cooled. A 100 ml solution of 1% konjac/1% LE agarose was prepared and split into two equal aliquots. One sample was gelled as described in Example 2 by heating the alkaline solution and cooling. The second sample was mixed with the same amount of K₂CO₃ solution but was iced immediately afterward to rapidly set the agarose matrix. Once gelled, the sample was heated in a hot water bath (63°C - 70°C), a temperature below the melting point of the agarose for 60 minutes. Both gels were frozen and thawed as described in Example 2. The spongy matrix formed by the first procedure had large irregular cavities whereas the second gel produced a spongy matrix with smaller, more consistent-sized cavities.

Example 5
Effects of pH during Gel Formation on Matrix Properties
A sol of 1% konjac from crude flour and 1% low electroendoosmosis agarose was prepared as in example 1 and divided into seven 50 ml aliquots. Different amounts of 5M NH₄OH ranging from 0.1 to 4 ml was stirred into five of these aliquots. The pH of each was quickly measured and the aliquots were gelled for 20 minutes in a boiling water bath. The last 2 aliquots were mixed with 0.1 and 0.2 ml volumes of 5M NaOH and gelled in the same manner. Their pH was also measured with a pH meter (model 155 pH/ion meter; Corning Science Products; Halstead, Essex; England). The volumes and type of alkali used, as well as the corresponding pH's, are shown in the following table:
All gels were frozen and thawed as previously described, producing spongy matrices. Several general trends were observed in the matrices. When the pH increased the number of spongy cavities increased, the size of the cavities decreased, and the homogeneity of the spongy matrix appeared to increase; the reverse would be expected to be obtained upon decreased pH.

Example 6

Konjac / Agar Spongy Matrix Concentration Variations
Comparison of Properties

A series of nine konjac (from crude konjac flour) + agar spongy matrices were prepared according to the procedure of Example 2. Concentrations of the components were either 0.5%, 0.75% or 1%. The matrices were compared based on: 1) strength of the matrix, 2) whether the spongy matrix retained its original size or shrank (before and after drying), and 3) whether the spongy matrix would rebound after being squeezed and released.

The most desirable spongy matrices were full sized, durable and fully rebounded after a squeeze/release cycle resulted from the following konjac / agar concentrations: 0.75%/1%, 0.5%/1% and 1%/0.75%. Intermediate samples were 1%/1% and 0.75%/0.75%. The remaining matrices were generally soft and shrunken, remaining flat after squeezing, but swelling when placed in water.
Example 7

A. **Spongeous Matrix Stability in Boiling Water**

Three 200 ml spongeous matrices containing 1% konjac (from crude konjac flour) and 1% of either agarose or agar were prepared as described in Examples 1 and 2 respectively. The spongeous matrices were dried and accurately weighed. The matrices were placed into 500 ml of hot water for 20 minutes and then boiled for an additional 60 minutes. The agarose and agar containing matrices appeared little changed. The spongeous matrices were squeezed to remove excess liquid, rinsed and treated with 83% 2-propanol for 30 minutes and dried in a hot air oven. The samples were reweighed to determine weight loss. The results are given below.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Initial Weight</th>
<th>Final Weight</th>
<th>% Loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>konjac/agarose</td>
<td>3.711 g</td>
<td>2.758 g</td>
<td>25.7%</td>
</tr>
<tr>
<td>konjac/agar</td>
<td>3.530 g</td>
<td>2.597 g</td>
<td>26.4%</td>
</tr>
</tbody>
</table>

The dried spongeous matrices were placed in water and observed. Although the spongeous matrices did fill with water, they lacked their initial structural integrity and were partially collapsed.

B. **Coated Spongeous Matrix Stability In Boiling Water**

Two 1% konjac (from crude konjac flour) / 1% agar spongeous matrices were formed and dried as described above. The dried matrices were accurately weighed. As a coating material, a 50 ml volume of a 1% crude konjac solution was diluted to 200 ml or 0.25%. To this 1.5 ml of $1\text{M} \text{K}_2\text{CO}_3$ was added and the sample gelled in a boiling water bath for 35 minutes. The gel was placed in an ice bath where it cold melted, forming a cold-melt konjac. This cold-melt sol was filtered through a cloth filter to remove debris such as the sacs which are found in konjac flour. One of the dry spongeous
matrices was placed in this filtered cold-melt sol coating material and allowed to hydrate. The matrix was massaged to remove air bubbles and to allow thorough coating. The spongeous matrix was removed, squeezed to remove excess liquid and hardened briefly in 99% 2-propanol. The matrix was transferred to a 92°C oven for 30 minutes to partially dry, removed, and recoated as before. Excess cold-melt liquid was removed by squeezing and the spongeous matrix redried at 75°C overnight (no alcohol used). The matrix was reweighed. Each matrix was placed in 300 ml of boiling water for 60 minutes. Excess liquid was squeezed from each spongeous matrix and both were then dried overnight at 75°C. The dried matrices were reweighed to calculate the % weight loss due to leaching.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial Weight</th>
<th>Final Weight</th>
<th>Weight Loss</th>
<th>% Weight Loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncoated</td>
<td>1.678 g</td>
<td>1.039 g</td>
<td>0.639 g</td>
<td>38%</td>
</tr>
<tr>
<td>Coated</td>
<td>1.849 g</td>
<td>1.481 g</td>
<td>0.369 g</td>
<td>19%</td>
</tr>
</tbody>
</table>

Example 8

Three Component Spongeous Matrices

Two 50 ml samples of 1% konjac sol (from crude konjac flour) were taken from a larger volume. To the first sample, 0.25 g of agar and 0.25 g of kappa carrageenan were mixed in and dissolved. The final composition was 1% konjac, 0.5% agar and 0.5% carrageenan. The first sample was gelled by heating in a boiling water bath for 20 minutes after the addition of 0.5 g KCl and 0.375 ml 1M K₂CO₃.

To the second sample, 0.25 g agar and 0.25 g soluble starch were mixed in and dissolved. This was gelled by adding 0.375 ml of 1M K₂CO₃ and heating as with the first sample. The final gel composition was
1% konjac, 0.5% agar and 0.5% starch.

The gels were frozen and thawed as described in Example 1, to form spongy matrices. The matrix containing agar and carrageenan was very firm and thus hard to squeeze. When placed in water, it swelled slowly. The starch/agar containing matrix was softer and easier to squeeze. It was also quicker to swell in water than the first matrix. The starch containing spongy matrix was covered with a thin but leathery skin.

Example 9

Konjac/Kappa Carrageenan Composite Spongy Matrices

A. An aqueous sol of 1% (w/v) konjac (from crude konjac flour) containing 3% kappa carrageenan was prepared and divided into three 200 ml aliquots. To the first aliquot, 2 g (1%) KCl was added under heating to initiate gelation of the carrageenan. The hot aliquot was allowed to cool and then frozen. The second aliquot was mixed with 1.5 ml 1M K₂CO₃ (for konjac gelation) and 0.89% KCl (for carrageenan gelation). The aliquot was heated in a boiling water bath for a period of 30 minutes. The gel was allowed to cool and then frozen. The third aliquot was mixed with 0.5 ml of 1.0 M NaOH, heat-set for 30 minutes and frozen overnight.

After thawing, the first sample (KCl only) was transparent, with a rubbery, thick-walled corrugated skin. The sample was firm, containing numerous large irregular cavities, and would rebound to full size after squeezing and releasing. It did not absorb much water through capillary action and would only fill if squeezed and released under water. The sample was found to be hot-water soluble.

The second sample (KCl and K₂CO₃) was white, rubbery, contained few cavities and had a thin skin.
The sponge would rebound after a squeeze/release cycle. The sample did break up somewhat when placed in boiling water but did not dissolve. There was evidence that at least a portion of the carrageenan did leach out into the water.

The third sample (NaOH) was very rubbery and tough with very few cavities and looked and felt more like a slightly fractured gel.

B. A series of duplicate 1% kappa carrageenan aqueous gels containing konjac (from crude konjac flour), in increments of 0.2% (0.2 -> 1.0%), were prepared as described above. One set of gels was set with KCl only while the second was gelled with KCl, K$_2$CO$_3$ and heat. The gels were frozen and thawed as previously described.

For the first series (with KCl only), all gels were transparent, rubbery with thick walls, tough and would rebound after a squeeze/release cycle. (Only the sample with 0.8% konjac did not rebound after squeezing.) The cavities were irregular and generally large. For those gels set with both alkali and KCl, the gels were white, soft, thin walled and full of small cavities. They would remain flat after a squeeze/release cycle (except the sample which contained 0.6% konjac) but would swell to full size in water. The sponge containing 0.2% konjac was weak and partially broke up when squeezed. As the konjac concentration increased, the matrices became somewhat more durable.

The spongeous matrix containing 1% konjac and 1% kappa carrageenan was dried and weighed. The matrix was then placed into 500 ml of hot water for 20 minutes and then boiled for an additional 60 minutes. The sponge turned white in color and became slimy. The sponge was redried and reweighed to determine weight loss. The result was:
initial weight - 3.564 g
final weight - 2.754 g
percentage loss - 22.7%.
The dried matrix was then placed in water and observed. The matrix was slimy with a thick rubber feel and possessed no sponge-like characteristics.

Example 10
Konjac/Gellan Gum Composite Spongeous Matrix
On hundred ml of a 2% (w/v) hot solution of gellan gum was admixed with 100 ml of a 2% (w/v) hot solution of konjac, to prepare 200 ml of 1% konjac / 1% gellan. The mixture was cooled to 60°C and 1.5 ml of 1M K₂CO₃ and MgSO₄ •7H₂O were admixed therein. A 150 mg portion of the sample was introduced into a 6.5 x 6.5 x 10 cm plastic container. The sample was heated in an 85°C water bath for 60 minutes. Then it was removed and cooled at room temperature overnight, after which it was frozen for 24 hours at -15°C. The sample was thawed at room temperature and examined. It was fully spongeous. Although the sample was only about 75% of its initial size, when squeezed and thawed it rebounded fully at a moderate rate.

Example 11
Konjac/Sodium Alginate Composite Matrices
Two hundred ml of 1% konjac / 1 % sodium alginate (w/v) mixture was prepared by admixing 100 ml of a 2 % (w/v) hot solution of sodium alginate with 100 ml of a 2% (w/v) hot solution of konjac. The sol was cooled to 60°C and 1.5 ml of 1M K₂CO₃ and 0.4 g CaCl₂ were admixed. The alginate component began precipitating immediately and its gel structure was disrupted by subsequent mixing. Despite this, a 150 g portion of the sol was placed in a 6.5 x 6.5 x 10 cm plastic container and covered. The sample was heat-set for 60
minutes in an 85°C water bath. The gel was removed and cooled overnight at room temperature and then frozen for 24 hours at -15°C. The sample was thawed at room temperature and examined. The sample was completely porous while the matrix was soft and filled with many small, fine pores. When squeezed and released, the sample did not rebound significantly. Additionally, the matrix crumbled quite easily, especially along its edges.

A duplicate 200 ml sample of 1% konjac / 1% sodium alginate was prepared in the above manner. When cooled to 60°C, only the K₂CO₃ was added. The sol was gelled by heating in the above manner. The gel was then removed from the container and placed in 750 ml of 2% CaCl₂ for 48 hours to set the alginate component. The gel, which shrank during this, was frozen and thawed in the above manner. The matrix was fully porous, yet was filled by much larger cavities. It was also much firmer and quickly rebounded after being compressed and released.

Example 12
Use of Inventive Spongeous Matrices for plant culture
A. A 150 ml volume of an aqueous sol containing 1% crude, alcohol-washed konjac (from crude konjac flour) and 1% agar was prepared as described in Example 2. The sample was mixed with 1.125 ml of 1M K₂CO₃ and cast into a 6.5 x 6.5 x 10 cm plastic plant embryo culture container (type GA 7, Magenta Corp.; Chicago, IL.). The sol was then gelled by heating in an 85°C hot water bath for 35 minutes. The sample was frozen at -15°C overnight and thawed slowly at room temperature. The resulting matrix was squeezed flat, placed in clean tap water and allowed to reswell. This was repeated six times, changing the water each time. The matrix's capacity of absorption by capillary action was tested
by slowly adding measured volumes of water and pouring off the excess. Capacity was determined to be 37 ml although the maximum would be much higher if air bubbles were squeezed out and the sponge completely filled. The sample was rinsed briefly in 2-propanol and dried in a forced air oven at 55°C.

The spongeous matrix was hydrated with 25 ml of Murashige and Skoog salt base buffer (Hazelton Research Products; Denver, PA). Two small slits were cut into the surface of the sponge and a Kentucky Wonder® pole bean seed was inserted into each. The seeds began germinating after 2 days. After 10 days, a complex root system had developed and leaves had appeared. In some places where the thin skin covering the sponge was ripped or torn, roots had penetrated through to the exterior. Throughout this period, the sponge was kept moist by additional buffer. Two weeks after planting the seeds, the seedlings were transplanted outside by placing the entire sponge into the ground and slightly covering it with soil. After nine days, the plants were carefully excavated to examine the root system and sponge. Roots which had already penetrated the skin had begun to develop smaller tap roots whereas those still within the sponge had not yet exited the matrix which was beginning to show signs of deterioration. After 7 weeks the plant was again excavated. There was very little left of the spongeous matrix. What little remained was a soft gel-like material which readily fell away from the extensive root system which had developed.

B. Two 1% konjac (from crude konjac flour)/1% agar spongeous matrices were prepared by freeze/thawing gel which were prepared in 50 ml disposable centrifuge tubes. The samples were processed and dried as described above. The dried matrices (~ 5 cm long) were hydrated in water and holes were cut through them along their long axis. Cuttings from a Coleus sp. house
plant (~13 cm in length) were inserted 4 cm into the matrices. The matrices/cuttings were placed back into centrifuge tubes containing water. Within 2 weeks numerous roots had sprouted from the stem and had grown throughout the sponge matrix.

Example 13

Spongeous Matrices prepared varying Component (c)

A solution of 1% glucomannan as crude konjac [Component (a)] containing 0.75% agar [Component (b)] was prepared by dissolving 15 g konjac and 12 g agar in 1.5 l distilled water over 60 minutes in a hot water bath. The sample was split into 100 g aliquots.

Various Component (c) gums were incorporated as dry powders either by adding 0.088 g (5% w/w based on konjac and agar) or 0.875 g (50% w/w). Each aliquot was stirred and heated to effect dissolution. Samples were cooled slightly and 0.75 ml of 1M K2CO3 was admixed. Each sample was heat-set in a boiling water bath for 30 minutes. Gels were removed, cooled to room temperature and then frozen overnight. The frozen materials were thawed at room temperature and examined. The results are summarized below.

<table>
<thead>
<tr>
<th>Component (c)</th>
<th>Conc.</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guar gum</td>
<td>5%</td>
<td>spongy; slow, incomplete swelling when placed in water, no rebound after squeeze/release</td>
</tr>
<tr>
<td>Guar gum</td>
<td>50%</td>
<td>as above but with slimy skin on top surface</td>
</tr>
<tr>
<td>CLBG*</td>
<td>5%</td>
<td>seemed normal; full-sized after squeeze/release</td>
</tr>
<tr>
<td>CLBG*</td>
<td>50%</td>
<td>&quot;</td>
</tr>
<tr>
<td>Arabic, gum</td>
<td>5%</td>
<td>spongy; swelled quickly in water; no rebound after squeeze/release</td>
</tr>
</tbody>
</table>
Arabic, gum 50% mush; did not work
Karaya gum 5% similar to that with 5% arabic
Karaya gum 50% similar to arabic at 50%, did not work

* = clarified locust bean gum

An attempt to incorporate chitosan as Component (c) was not successful. Chitosan is only soluble under acidic conditions, and was dissolved in aliquots of the konjac/agar by adding acetic acid. However, when alkali was added to adjust the pH for heat-setting, the chitosan precipitated. An attempt was then made to gel the sample by cooling the sol and allowing the agar component to gel. At this point, the gel was soaked in alkali to raise the pH. While this was in process, much of the chitosan leached out of the gel and precipitated in the alkaline buffer. Further efforts to incorporate chitosan into a konjac/agar sponge were then abandoned.

Comparison Example A
Attempts At Matrix Formation Using Only Konjac

Three 90 ml aliquots of 1% (w/v) clarified konjac sol were prepared and then gelled by adding 3.6 ml of 5M NH₄OH and heating for 20 minutes in a boiling water bath. One gel was frozen directly. The other two samples were cold melted in an ice bath and one such cold-melt sol was frozen directly. The second cold-melt sol was adjusted to pH 3.5 with 5M HCl and then frozen. All samples were frozen overnight (18 hours) at x -15°C. The three samples were thawed slowly at room temperature. All three produced copious amounts of syneresate. The syneresate from two of the samples (frozen gel and frozen alkaline cold-melt sol) produced a slight coag when dropped in 99% 2-propanol. The
syneresate from the acidified sample produced no such coag. All three samples formed fibrous, spongy matrices which had little structural integrity and which were quite similar in texture and appearance. One exception was that the matrix formed from acidified cold-melt sol reswelled in liquid more rapidly than the others after squeezing.

Comparison Example B

Effect of Concentration on Freeze/Thaw Behavior in Matrices Comprising Only Konjac

Three crude konjac sols were prepared at 1%, 2% and 3% concentrations. The 1% sample (150 ml volume) was mixed with 1.5 ml 1M K$_2$CO$_3$ while the other 2 samples (200 ml each) were mixed with 2 ml of the alkaline solution. The samples were gelled 30 minutes in a boiling water bath, covered and frozen overnight. The samples were thawed and examined. The 1% sample was soft and squeezable. When compressed, liquid slowly oozed out leaving a soft fibrous mat which was quite tough and resilient, indicating insufficient structural integrity. The mat remained flat after squeezing but did swell somewhat when placed in water. This matrix was full of very tiny pores. The 2% and 3% samples were essentially unaffected by the freeze/thaw procedure and appeared to be entirely intact. The samples were very tough and rubbery and if anything, appeared to be stronger than the initial gels. When squeezed, there was very little (2%) or no (3%) liquid expressed. There were no cavities or pores visible.

Comparison Example C

Drying and Rehydration of Matrices Comprising Only Konjac

Duplicate 50 ml samples of 2% and 4% clarified konjac were gelled with NH$_4$OH and heat. The gels were
cooled and frozen overnight and then slowly thawed. The samples, which were firm and covered with a tough skin, had to be squeezed and massaged to remove the liquid trapped within the numerous cavities of the sponge. When squeezed flat, the sample was very tough and rubbery and when placed in water would swell back to its original size. The samples were rinsed by repeated squeezes/swell cycles in water and then in 65% 2-propanol. The samples were then dried in a 55°C oven. The dried sponges were lightweight and durable. When placed in water, the samples rehydrated slowly to their original size of 2 cm x 5 cm. No structural integrity was evidenced by these matrices.

Comparison Example D

Attempt At Matrix Formation Using Only Agarose

A solution of 1% Seakem® LE agarose was prepared and used to fill two 150 ml crystallizing dishes. The samples were allowed to gel and were then frozen overnight. The frozen gels, which had constricted somewhat, were thawed slowly at room temperature. The samples were covered with a smooth skin, which burst open when squeezed. The resulting matrix was fibrous and weak and had insufficient structural integrity, but did swell when placed in water. The sample was rinsed, treated with propanol and dried as described in example 2. The dried sample did not rehydrate when placed in ambient water, but dissolved in hot water.

Comparison Example E

Attempts at Matrix Formation Using Only Agarose

Gel Concentration Variations

100 ml samples of 1%, 2% and 3% Seakem® LE agarose were prepared. The samples were allowed to gel, cooled to room temperature and were then frozen overnight. The samples were thawed and examined. All samples were
full sized although the 1% sample’s sides were slightly constricted. The 1% sample had a corrugated surface, a few large irregular cavities, had no structural integrity, was soft and easily broke up. The sample did swell when placed in water. The 2% and 3% samples resembled fractured gels with no visible cavities. The samples were not compressible (3%) or released very little water. Both were soft, yet somewhat brittle and thus broke up very easily.

Comparison Example F
Attempt At Matrix Formation Using Only Agar
Three agar gels were prepared at 1%, 2% and 3% concentrations. The starting material was a Gracilaria-derived agar (Algas Marinas; Santiago, Chile). The three gels were cooled and then covered, frozen overnight and then thawed at room temperature. The 1% sample was much like the 1% agarose sample in Comparison Example E. It was however softer and weaker. After squeezing, the sample did not rebound and remained compressed but did swell when placed in water. The matrix, which contained some large and randomly distributed cavities, was weak and easily broke apart if force was used. The 2% sample was fully compressible and when released, partially rebounded. This sample also swelled in water. The matrix was still rather weak and contained some cavities which were smaller, more numerous and evenly distributed. The 3% sample was much like the 2% agar matrix although it fully rebounded after being squeezed. Although it was slightly stronger, the matrix was still fragile.

Comparison Example G
Attempt At Matrix Formation Using Only Kappa-Carrageenan
Two samples of kappa-carrageenan were prepared by dispersing 1 and 2 gram quantities into 100 ml volumes of deionized water. Both samples were heated to boiling to dissolve the material. Once fully dissolved, 1 g of potassium chloride was added to both the 1% and 2% w/v kappa-carrageenan sols. The samples were allowed to cool, were frozen overnight and were then slowly thawed at room temperature. Both samples were very soft and mushy when squeezed. The somewhat spongy matrix was transparent and possessed very little mechanical strength. Pores/cavities were generally small but were somewhat irregular in size and placement. The samples broke up very easily and were hot-water soluble.

Comparison Example H
Attempts at Spongy Matrix Formation Using Other Materials As Component (b)

A number of other materials were tried as potential Component (b) when mixed with konjac glucomannan from crude konjac flour in attempts to form spongy matrices of adequate structural integrity and/or meeting other acceptable parameters of this invention. Some did not work at all while others produced poor results. In some cases, the matrices formed were interesting but lacked properties seen in the examples according to this invention. In each of the following examples (except where indicated), three gels were prepared with concentrations of (konjac/other material) of 1%/0.5%, 1%/1% and 0.5%/1%. The samples were prepared by dissolving the other material in a prepared solution of konjac, adding alkali and heating for 20 minutes in a boiling water bath. The gels were then frozen and thawed as in Example 1.
H(1) Clarified Locust Bean Gum (CLBG)
The thawed matrix samples were very soft, slimy and had very small, uniform pores. They were also quite weak and thus broke up readily during squeezing. Samples remained flat after squeezing and did not swell in water, even after drying. The liquid which was expressed formed a coagulum when dropped into 83% 2-propanol.

H(2) Clarified Guar
These samples were very similar to those formed with CLBG although the empty matrix was tougher, more resilient.

H(3) Polyvinyl Alcohol (PVA)
These samples were prepared with PVA (88% hydrolyzed) with an average molecular weight of 125,000 (Aldrich Chemical Co., Inc.; Milwaukee, Wisconsin). The thawed material was very weak, soft and slimy but did contain many small uniform pores. When gently squeezed, liquid would seep out. The empty matrix was flat and quite tough and remained flat after squeezing and would not swell in water before or after drying.

H(4) Methyl Cellulose
These samples were prepared from material rated at 400 centipoise viscosity (2% @ 25°C) from Sigma Chemical Co. (St. Louis, MO). During the heating phase, the samples foamed significantly generating many tiny air bubbles. These samples were much like those prepared with CLBG; soft, slimy and weak. Only the sample containing 1% konjac/0.5% methyl cellulose was squeezed flat. After drying, it hydrated but did not swell in room temperature water. However, when the water was heated for 45 minutes, the matrix did partially swell.
H(5) Gelatin

Knox® gelatin was used to prepare the 3 composite samples which were gelled, frozen and thawed as previously described. The thawed materials had little structural integrity and were not squeezable.

H(6) Polyethylene-Polyoxypropylene Block Copolymer

In this series of gels, Pluronic® F-127 polyethylene-polyoxypropylene block co-polymer (BASF Corp.; Parsippany, N.J.) was employed as an additive. The thawed samples had a jelly-like texture and squeezing them was difficult, especially the 1%/1% sample which could not be compressed. The liquid expressed from the other 2 samples formed a coagulum in 2-propanol. The flat matrices would not swell in water, yet after being dried, they did swell appreciably but only in hot water.

H(7) Xanthan

For this experiment, concentrations of konjac/xanthan (Kelco Keltrol® T) were 1%/1% and 0.5%/0.5%. A duplicate of the 1%/1% was prepared and allowed to gel without added alkali and heat. The first 2 samples were gelled as previously described. All gels were frozen and thawed. None of the thawed samples displayed any sponge-like characteristics and were either mush or gel-like.

H(8) Carboxymethyl Agarose (CMA)

A sample of CMA was used to prepare 2 gels. One was 1% konjac containing 1% CMA and the second sample was 1% konjac/0.5% CMA/0.5% low eeo (electroendoosmosis) value agarose. After freeze/thawing the gels, the first sample (1%/1%) was observed to be mushy, was not squeezable, and there
were no visible cavities. The sample began breaking up after a very small amount of fluid was expressed. The second sample had more structural integrity and did contain some visible cavities. It could be squeezed flat but would remain that way until placed in water where it would swell.

H(9) Polyaerylamide (PAA)
A solution containing 0.5% konjac/5% PAA (36:1 acrylamide:bis-acrylamide) was prepared by dissolving 0.25 g konjac, 2.432 g acrylamide (Sigma Chemical Co.; St. Louis, MO.) and 0.068 g of bis-acrylamide (American International Chemical; Natick, MA) in 50 ml water at room temperature. Once dissolved, 0.375 ml of 1 M K$_2$CO$_3$, 12.5 μl N,N,N',N'-tetramethylethylendiamine (TEMED) and 250 μl of 10% ammonium persulfate (both from FMC Corporation, Philadelphia, PA) were added and the sample gelled for 20 minutes in a hot water bath (~80°C). This sample was frozen and thawed as previously described. The thawed sample was gel-like and very rubbery. There were no cavities visible and no liquid could be squeezed from the sample.

Comparison Example I

Inhibition of Matrix formation in Composite Gels

I(1) Addition of 10% NaCl
A 1% konjac/1% Seakem LE agarose sol (200 ml) was prepared as described in Example 1. To this, 20 g (10% w/v) NaCl was added. The sample was gelled by adding 1.5 ml 1 M K$_2$CO$_3$ and heating for 30 minutes in a boiling water bath. A control was also prepared without the added salt. Both gels were covered, frozen overnight and thawed at room temperature. The control had formed a typical sponge as described in Example 1. The sample which contained 10% NaCl was a firm strong gel with no evidence of cavities or fracturing. The sample was
frozen again at -80°C overnight and thawed. There was some very slight fracturing but no sponge-like properties.

5  I(2) Addition of 5% Glycerol

A 200 ml solution of 1% konjac/1% agar was prepared to which 10 g (5% w/v) of glycerol (EM Science; Cherry Hill, N.J.) was added. The sample was gelled by adding 1.5 ml of 1M K₂CO₃ and heating in a boiling water bath for 30 minutes. The gel was covered, frozen overnight and thawed. The sample was largely intact, showing only minimal fracturing. The sample was not compressible and there was no release of liquid.
CLAIMS:

1. An article of manufacture characterized in that it is a spongy matrix whose pores are controlled as to one or more of size and distribution, formed from a coprocessed mixture comprising:
(a) glucomannan, and
(b) at least one other aqueous gel-forming polysaccharide.

2. The article of claim 1 characterized in that:
(a) is konjac-derived glucomannan; and
(b) is at least one gel forming: agar, agaroid, agarose, alginate, carrageenan, curdlan, gellan, pectin, gel-forming chemical derivative of the foregoing, or a mixture thereof.

3. The article of claim 1 characterized in that
(b) is: agar, agarose, carrageenan, or a mixture thereof.

4. The article of manufacture of claim 2 characterized in that said coprocessed mixture comprises:
(a) said konjac glucomannan present in 1.0 part by dry weight, and
(b) said at least one other gel-forming polysaccharide present in about .125 to 8.0 parts by dry weight; and
(c) optionally one or more water soluble polysaccharides other than (a) or (b), which may be present in up to about 200.0 % by dry weight compared to the combined dry weight of (a) and (b).

5. The article of manufacture of claim 4 characterized in that (c) is present and is in at least 0.5 % by dry weight compared to the combined dry weight
of (a) and (b) and is: guar gum, gum arabic, karaya gum, locust bean gum, starch, tragacanth, or a mixture thereof.

6. The article of manufacture of claim 5 characterized in that (c) is: guar gum, locust bean gum, starch, or a mixture thereof.

7. The article of manufacture of claim 5 characterized in that (c) is starch.

8. The article of manufacture of claim 5 characterized in that (c) is starch and is present in about 20.0 to 75.0 % by dry weight compared to the combined dry weight of (a) and (b).

9. The article of manufacture of claim 1 characterized in that (a) is a substantially undegraded konjac.

10. The article of manufacture of any one of claims 4 through 9 characterized in that (b) is present in about 0.25 to 4.0 parts by dry weight.

11. The article of manufacture of any one of claims 4 through 9 characterized in that (b) is present in about 0.5 to 2.0 parts by dry weight.

12. The article of manufacture of claim 4 characterized in that said spongy matrix is at least partially saturated with a carrier medium and a carried substance which is medium-soluble, medium-dispersible, or medium-borne particulate matter.

13. The article of manufacture of claim 12 characterized in that said carrier medium is water.
14. The article of manufacture of claim 13 in which said carrier medium has been at least partially removed.

15. The article of manufacture of claim 13 characterized in that the carried substance is a: nutrient medium, reagent, pharmaceutical, flavoring, color, scent, cosmetic, air freshener, deodorant, adjuvant to any of the foregoing, affinity or ion-exchange particulate, cell or cellular particulate, activated charcoal, or mixture thereof.

16. The article of manufacture of claim 4 comprising a biodegradable sponge.

17. The article of manufacture of claim 4 in substantially dry form.

18. The article of manufacture of claim 1 or 4 or 12 coated with a material selected from:
(a) a material other than that comprising said spongyous matrix, or
(b) the same material that comprises said spongyous matrix but in a non-spongyous form.

19. The article of manufacture of claim 18 characterized in that said coating is water-insoluble.

20. The article of manufacture of claim 18 characterized in that said coating is water-permeable.

21. The article of manufacture of claim 19 characterized in that said coating is water-permeable.

22. A process for fabricating an article of
manufacture which is a spongy matrix formed from a coprocessed mixture of the components:
(a) glucomannan,
(b) at least one other aqueous gel-forming polysaccharide, and optionally
(c) one or more water soluble polysaccharides other than (a) or (b);
said process characterized by the sequential steps of:
A - forming an aqueous sol comprising components
(a) and (b) present in a ratio a:b of about 1:0.125-8.0 by dry weight, and optionally component (c) which may be present in up to about 200.0 % by dry weight compared to the combined dry weight of (a) and (b);
B - gelling said aqueous sol by addition of sufficient base to result in a sol pH above 7.0, and optionally a gelling agent for component (a) or component (b);
C - freezing said gel; and
D - thawing said gel to form said spongy matrix.

23. The process of claim 22 characterized in that component (c) is present in said spongy matrix.

24. The process of claim 22 or 23 characterized in that the respective concentrations in water of components (a) and (b) independently are 0.25 to 2.0 wt %, based upon the total weight of said sol.

25. The process of claim 22 or 23 characterized in that the respective concentrations in water of components (a) and (b) independently are 0.5 to 2.0 wt %, based upon the total weight of said sol.

26. The process of claim 22 or 23 characterized in that the respective concentrations in water of
components (a) and (b) independently are 0.5 to 1.0 wt %, based upon the total weight of said sol.

27. The process of claim 22 or 23 characterized in that component (b) consists essentially of: agar, agaroid, agarose, alginate, carrageenan, curdlan, gellan, pectin, gel-forming chemical derivatives of the foregoing, or a mixture thereof.

28. The process of claim 22 or 23 characterized in that component (b) is: agar, agarose, carrageenan, or a mixture thereof.

29. The process of claim 23 characterized in that component (c) is present.

30. The process of claim 22 or 23 characterized in that the pH of said sol is adjusted to about 9.0 to 12.0 by the addition of said base.

31. The process of claim 30 characterized in that said base is ammonium hydroxide, an alkali metal hydroxide or carbonate, or an alkaline earth metal hydroxide or carbonate.

32. The process of claim 30 characterized in that said base is ammonium hydroxide, potassium carbonate, potassium hydroxide, sodium carbonate, or sodium carbonate.

33. The process of claim 30 characterized in that said base is potassium carbonate.

34. The process of claim 22 or 23 characterized in that the sponges matrix is partially dewatered after step D.
35. The process of claim 22 or 23 characterized in that the spongy matrix is substantially dewatered after step D.

36. The process of claim 35 characterized in that the substantially dewatered spongy matrix is sterilized.

37. A method for the growth or maintenance of a plant material characterized by the steps of:
   A. adding an aqueous nutrient medium to a spongy matrix according to claim 1 or 2;
   B. inserting the plant material into the nutrient-containing spongy matrix; and
   C. growing or maintaining in viable condition the plant material in the spongy matrix.

38. The method of claim 37 characterized in that said plant material is grown and comprises a callus, seed, embryo, explant, graft, or young plant.

39. The method of claim 38 characterized in that the plant-containing spongy matrix is placed into a soil-containing growth medium following Step C.

40. The method of claim 38 characterized by the intermittent or continuous addition of nutrients to the aqueous nutrient medium in the spongy matrix.

41. A surgical sponge characterized in that it comprises a sterilized biodegradable spongy matrix according to claim 1.

42. A surgical sponge according to claim 41 further characterized in that it is coated with or
contains at least one physiologically active substance.

43. Biodegradable material for the packaging of articles for storage or shipment characterized in that it comprises one or more spongyous matrices according to claim 1.
A. CLASSIFICATION OF SUBJECT MATTER

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<th>Please See Extra Sheet.</th>
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<td>According to International Patent Classification (IPC) or to both national classification and IPC</td>
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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

| U.S.        | 424/488, 426; 604/364, 368; 47/58, 80; 426/573, 46; 252/315.3 |

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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

APS/JPO FREEZ# AND (GLUCOMANNAN# or Konja?)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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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 part of particular relevance
  *E* earlier document published on or after the international filing date
  *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
  *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

X 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: 15 DECEMBER 1993

Date of mailing of the international search report: 05 JAN 1994

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks

Box PCT
Washington, D.C. 20231

Authorized officer: DANIEL S. METZMAIER

Facsimile No. NOT APPLICABLE

Telephone No. (703) 308-0451

Form PCT/ISA/210 (second sheet)(July 1992)
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<td>US, A, 4,943,444 (Nozaki et al.) 24 July 1990, See entire document, claims in particular.</td>
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<td>A</td>
<td>Derwent Abstract, AN 92-170036/21, 18 March 1992, JP0 4084868-A</td>
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<td>A</td>
<td>US, A, 4,769,945 (Motoyama et al.) 13 September 1988 (note col. 2, line 63 to col. 4, line 37)</td>
<td>37-40</td>
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<td>A</td>
<td>US, A, 3,653,383 (Wise) 04 April 1972, See entire document</td>
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<td>A</td>
<td>US, A, 4,676,976 (Toba et al.) 30 June 1987, See entire document</td>
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**INTERNATIONAL SEARCH REPORT**

**Box I** Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II** Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.
A. CLASSIFICATION OF SUBJECT MATTER:

IPC (5):

A61F 13/15; A61K 9/14; A01C 1/00; A01G 31/00

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

I. Claims 1-11, 16-17, 22-36, and 43 are drawn to a sponge matrix and a method of making said sponge matrix, classified in Class 424, subclass 488.

II. Claims 12-15, 18-21, and 41-42 are drawn to a sponge matrix impregnated with a substance, a sponge matrix coated with a coating, a surgical sponge which is coated or impregnated, classified in Class 604, subclass 634.

III. Claims 37-40 are drawn to a method of growth maintenance of the plant material, classified in Class 47, subclass 58.

Unity exist between claims in group I involving a sponge matrix and methods of making said matrix. The claims of group II lack unity with the claims of group I which does not include the special technical features further included in the compositions of Group II. The claims of Group III lack unity as corresponding to one of multiple uses of the spongeous matrices.