This paper includes:

- Part 1: General questions (20% of marks)
- Part 2: Case A, Novelty (40% of marks)
  - Annex 1 Patent Application
  - Annex 2 Priority Document
  - Annex 3 Search Results (Documents 1-5)
- Part 3: Case B, Infringement (40% of marks)
  - Documents 1-9
General instructions for completing the exam

(1) Candidates will not need to undertake any additional searching in the course of completing any one of Parts 1-3 of Paper B.

(2) Candidates will need to give their answers in English.

(3) Candidates shall accept the facts given in the examination paper and limit themselves to those facts. Whether and to what extent the provided facts are used shall be the responsibility of each candidate. Candidates should not use any special knowledge they may have of the technical field of the invention.
Part 1: General Questions (20% of marks)

1. Are the following priority claims valid? (3 marks)
   a. A Brazilian patent application filed 22 April 2010, claiming priority from a US application filed 22 April 2009?
   b. A German patent application filed 8 April 2010, claiming priority from a German utility model application filed 8 September 2009?
   c. A PCT application filed 17 May 2010, claiming priority from a PCT application filed 17 January 2010?

2. Describe six differences between a utility model and a patent? (3 marks)

3. An EP-patent with a filing date of February 2000 is claiming priority from a Canadian patent application filed in April 1999. Which date should be considered when reviewing the results of an invalidation search and why? Please assume that grace period provisions available in Europe do not apply. (3 marks)

4. In a family of patents the following family members are found. What is the expiry date of each of these members of this family, assuming that all necessary (annuity) fees have been or will be paid? (7 marks)
   e. A PCT-application (WO-E) filed 30 December 1998, claiming priority from US-B;
   f. A Japanese patent (JP-F), which is the national phase of the PCT application noted in e) above, filed with the JPO on 10 July 2000 and granted on 7 October 2004.

5. As a result of the increasing importance of China in the field of Chemistry, you are often asked to assess the content and legal status of Chinese patent documents during FTO analysis. Assuming that you do not speak Chinese, how might you go about this? If the filing date of a Chinese application of potential relevance was 29 March 2008 and it has not yet been granted would you still cite the document for FTO if performing the analysis today? (3 marks)

6. The expiry of a patent will normally be twenty years after the filing date. Name two situations in which the patent expiry date can be extended. (2 marks)

7. A prior art document contains compounds according to the following Markush formula (I):

   \[
   \begin{align*}
     &R_1 \text{CH}_2 \text{C} \text{N} \text{CH}_2 \text{R}_3 \\
     &\text{O} \quad \text{R}_2
   \end{align*}
   \]

   in which
   
   \(R_1\) is selected from the group of H, OH, lower alkyl;
   
   \(R_2\) is lower alkyl;
   
   \(R_3\) is lower alkyl;
R₃ is selected from the group of H, OH, lower alkyl, phenyl.

Only the following compounds are exemplified:

<table>
<thead>
<tr>
<th>Example</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Example 1</td>
<td><img src="" alt="Example 1" /></td>
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<tr>
<td>Example 2</td>
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<td>Example 3</td>
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<tr>
<td>Example 4</td>
<td><img src="" alt="Example 4" /></td>
</tr>
</tbody>
</table>

wherein said compositions are claimed for alleviating chronic pain in cancer patients.

Is the prior art relevant, from a novelty perspective, to the following claims: (5 marks)

1. A compound according to Markush formula (X)

   ![Markush formula](attachment:markush_formula.png)

   wherein
   - R₁ is selected from the group of H, OH, NH₂ or lower alkyl;
   - R₂ is lower alkyl.

2. A compound according to claim 1, wherein said compound is NH₂·CH₂·C(=O)·N·(CH₂·CH₃)·CH₃

3. A compound according to claim 1, wherein said compound is CH₃·CH₂·CH₂·C(=O)·N·(CH₂·CH₃)·CH₂·Ph

4. A compound according to claim 1, 2 or 3 for use in therapy.

5. A compound according to claim 1, 2 or 3 for use in treatment of Alzheimer's disease.
8. List at least four different types of publications that could be cited as relevant in a novelty or patentability search? (2 marks)

9. Please describe how the ‘grace period’ functions under the differing jurisdictions of the European Patent Convention, US patent law (prior to the recent enactment of the America Invents Act: Leahy-Smith Amendments) and under Japanese patent law. (6 marks)

10. Which information in or on a patent document is of interest for a Freedom To Operate analysis and why? Is there any other kind of information, apart from that in or on the patent document itself, that is of interest? (4 marks)

11. Which of the following documents may pose a potential patent infringement risk in Germany, assuming that the claimed subject matter in each of the listed documents is the same as the proposed product or process/method? (4 marks)
   
   a. A granted in force German patent
   b. A granted European patent with corresponding in force national patents in GB, FR, CH and DE
   c. A granted and in force German utility model
   d. A pending German patent application
   e. PCT application for which the earliest priority date claimed is 1 July 2009
   f. German registered and renewed trade mark
   g. a, b and c
   h. a, b, c, d, e
   i. any one of a-f

12. Suppose that a European patent was granted; how could you determine whether the patent is still in force? (4 marks)

13. What are the different “patent family” notions that you know. Give at least two definitions. (4 marks)
Part 2: Case A: Novelty (40% of marks)

You have recently been employed by a global food company, HealthChoice. The company has spent $500,000 dollars developing a high fibre and great tasting bread that is marketed as SonnePan. The bread contains tiny encapsulated particles that release both live *Lactobacillus acidophilus* culture as well as the daily recommended dose of dietary fibre directly in the intestines of the person or animal that consumes it. The food scientists at HealthChoice have managed to create the nutritional particles for the bread by encapsulating a dietary fibre and pro-biotic bacteria within a sugar based shell using a spray-drying process.

On 19 December 2000, HealthChoice filed worldwide the enclosed patent application directed at SonnePan which claims priority from the enclosed priority document that was filed on 22 December 1999. The patent application is currently pending in all countries. Health Choice is about to close a $30 million dollar deal that would involve selling all its rights relating to SonnePan to Puritan Foods. Before Puritan Foods will sign the deal they have asked HealthChoice’s CEO for supporting evidence of his assertion that the claims will be granted as filed because in the CEO’s opinion "there is no relevant prior art". The CEO has approached you to conduct a novelty search to verify his assertion. You have conducted the search and the final results of your search are attached as Annex 3.

Please report your findings to the CEO by categorising the results, according to whether you consider each document relevant or irrelevant, on the basis of novelty only. Please explain the reasons for how each document has been categorised.

List of Annexes

Annex 1- Sonnepan patent application
Annex 2- Sonnepan priority document
Annex 3- Search Result Document 1 (D1)- JP2002522403
Search Result Document 2 (D2)- Company News
Search Result Document 3 (D3)- US Patent Publication ’414
Search Result Document 4 (D4)- Industrial Processes journal article in part
Search Result Document 5 (D5)- WO Patent Publication ’745
Encapsulated Multifunctional Biologically Active Food Component

The invention relates to a multifunctional encapsulated biologically active food component which consists of a core that comprises at least one dietary fiber, which core is surrounded by at least one biologically active substance and in which the core and the biologically active substance are surrounded by at least one shell-forming substance. The stability of the multifunctional food component is based on interactions of the components with one another.

BACKGROUND OF THE INVENTION

[0002] Biologically active substances in nutrition are physiologically important components. They can have the most varied functions in the organism and as a result make a positive contribution to health. Biologically active substances can act, for example, as classical nutrients, can stimulate immune activity or have protective activity or intervene in physiological processes in the body. Biologically active substances can include, inter alia, probiotic microorganisms, prebiotic substances, nutrients or secondary plant constituents. Enrichment of the diet with components of this type in a stable and in particular bioavailable form is therefore desirable from the nutritional aspect.

[0003] Dietary fibers are a heterogeneous product group. Many dietary fiber preparations are based on plant fibers and consist predominantly of water-insoluble polysaccharides in addition to pectin, lignin and plant gums (for example wheat fibers, oat dietary fibers, rice dietary fibers, apple fibers, citrus dietary fibers etc.). In addition there are also soluble dietary fibers which are mostly made up of complex carbohydrates (for example fructo- or galactooligosaccharides, β-glucans etc.). Dietary fibers taken in via the diet are distinguished by the fact that they are indigestible constituents for humans. Because of their inert character, dietary fibers reside in the intestine and can there optimally exert their physiological effects, for example increasing intestinal peristalsis, effects on cholesterol absorption, prebiotic activities etc. An increased intake of dietary fibers is desirable for nutritional reasons. A daily intake of 25-30 g of dietary fiber is recommended by nutritionists.

[0004] For the reasons described it is expedient to provide dietary fibers and biologically active substances in a stable and nutritionally utilizable form via the diet or other delivery route to the organism. An optimal release of the biologically active substances, for the reasons described above, is not desirable until after passage through the stomach in the lower digestive tract. Furthermore, it is desirable that adverse sensory perceptions of nutritionally valuable substances in foods do not occur. This is, in the case of insoluble dietary fibers, frequently a marked sensory perception of particles in the food matrix ("scratchy off-taste").

[0005] The technique of microencapsulation has long been used commercially predominantly in the pharmaceutical industry. For some time, however, there have also been studies on the use of encapsulation in food technology (Jackson, Lee, 1991, Kanawija et al. 1992, Hegenbart, 1993, Arshady, 1993, Dewettinck, 1997, Pegg, Shahidi, 1999). The spray-drying process is one of the most frequently used processes for encapsulating various substances in the food industry. It can be considered as one of the essential advantages here that spray-drying is suitable for processing heat-sensitive materials. In addition, the process is inexpensive and offers the advantage that an existing technology can be utilized. In order to make possible targeted utilization for the various application sectors in the food industry, studies of the customary materials in different combinations would have to be carried out, since single materials cannot comply with the complex requirements which a food makes of the capsule material. Studies to date were concerned predominantly with decreasing the volatility of substances and their oxidation by embedding them into a suitable material. The effects resulting from the physicochemical properties of the capsule materials and of the physical conditions of this process require further study (Re, M. I., Drying Technology, 1998, 16(6), 1195-1236).

[0006] Most patents or patent applications relate to fields of application in the pharmaceutical industry. The major roles are taken here by the capsule materials used (generally in combination with specific activities or
effects), the controlled release of substances via the application of the encapsulating technique and the stabilization of substances. In the food sector there are far fewer patents, which, however, are essentially determined by these three directions. In the case of microencapsulation of cells or cell free extracts (CFE), physiological stability in combination with their use as a pharmaceutical product play the major roles.

[0007] Microencapsulation with the purposes of a) use of dietary fibers, in particular fibers as a support material for biologically active substances, in particular microorganisms with simultaneous increase of the nutritional value, b) support material/microorganism interactions, c) prevention of support material/capsule material interactions, d) stabilization of the biologically active substances in the product and in the food with correspondingly extended shelf life, has not been described to date.

[0008] Immobilization of lactococcus, which are less sensitive than lactobacillus species, is possible in a matrix made of alginate/polyamino acids. In this experiment a study was to be made as to what extent the stabilization, handling and storage of microorganisms may be improved by the immobilization. Lactic acid production, as an indicator of metabolic activity, was reduced, however, after handling and storage of the microorganisms in comparison with microorganisms which had not been embedded. No information was provided, however, on interactions of the capsule material with its direct environmental surroundings (for example foods) or with the microorganisms or the use of support materials (Larisch, B. C., Poncelet, D., Champagne, C. P., Neufeld, R. D., J. Microenc., 1994, Vol. 11, No. 2, 189-195).

[0009] The encapsulation of lactic acid bacteria of the genera Streptococcus, Lactobacillus, Pediococcus, has also been achieved in various materials using extrusion. In this case a study was made of the viability of the encapsulated organisms in acidic media simulating the gastrointestinal tract and of the shelf life and stability of the encapsulated microorganisms at various temperatures. It was found in this case that the survival rates depend on the physicochemical properties of the capsule materials. The shelf-life studies at different temperatures found increased survival rates of the encapsulated organisms at temperatures above 22° C. in comparison with the unencapsulated bacteria. No statements were made about metabolic performance and/or metabolic activity. (Kim, H. S., Kamara, B. J., Good, I. C., Enders Jr., G. L., J. Indust. Microbiol., 1988, 3, 253-257).

[0010] Encapsulation techniques for lactic acid bacteria are also described in various patents. WO-A 9716077 refers to probiotic formulations which can be used as food ingredients. In this case the microorganisms are mixed with a second substance as carrier or shell substance, which in the latter case leads to an improved stability to passage through the stomach. Production processes for formulations to increase the stability of such probiotic microorganisms in the gastrointestinal tract are also described in CN-A 1113515, CN-A 1124773, WO-A 9920745 or WO-A 9952511. In these cases, especially, encapsulation techniques are used in order to protect the microorganisms against gastric acid. A disadvantage of these processes is that the resultant formulations are not stable in most food applications.

[0011] WO-A 9608261, WO-A 9734615 and WO-A 9734592 describe the microencapsulation of probiotic microorganisms with modified and unmodified starches. In these cases the starch acts as transport medium for the probiotic microorganisms into the stomach. WO-A 9826787 describes a method in which improved passage through the gastrointestinal tract for probiotic microorganisms is achieved using β-glucan as support.

[0012] The spray-drying process can also be used to increase the stability and storage life of bacteria. Thus in the agricultural field, in the case of seed material, by spray-drying a strain of rhizobacteria, improved preservation and protection of the seed material against infection with disease and premature germination are achieved. Encapsulation was achieved using spray-drying with the use of various materials all of which except for one combination (modified starches) are not permitted for the food sector. (Amiet-Charpentier, C., Gadille, P., Digat, B., Benoit, J. P., J. Microenc., 1998, Vol. 15, No. 5, 639-659).

[0013] In the food sector, lactic acid bacteria were also stabilized via various drying processes. Thus WO-A 9957242 describes a process which gives preparations suitable for foods made from lactic acid bacteria with additional carbon-dioxide-generating additives via various drying processes. Increased stability of
encapsulated microorganisms was demonstrated when algal polysaccharides were used as capsule material. In this case the aerobic microorganisms were embedded into the capsule material by crosslinking. The resultant microorganisms can be used in the environmental sector and displayed similarly good performance with respect to PCP degradation as non-embedded microorganisms. (Hammill, T. B., Crawford, R. L., Can. J. Microbiol., 1997, 43, 1091-1095).

[0014] A disadvantage of the above described processes and substances is that they are not stable in most food applications. When in particular microorganisms are added to foods, the resulting survival rates of the microorganisms are still too low to develop nutritional effects. Furthermore, with many biologically active formulations, adverse sensory perceptions occur. In addition, it is disadvantageous that the biologically active formulations, although they can pass to their destination (intestine), because of the pH, they cannot fully develop their activity there.

[0015] The object of the present invention was therefore to provide a system which simultaneously

[0016] prevents an interaction with the surrounding food (for example in some cases prevents unwanted swelling properties of the dietary fibers)

[0017] provides nutritionally valuable substances without adverse sensory perceptions,

[0018] ensures increased storage stability of nutritionally valuable substances and

[0019] optimizes the quantitative supply and localization of release of the biologically active substances at the desired site (optimized bioavailability).

DESCRIPTION OF THE INVENTION

[0020] This object is achieved by a multifunctional encapsulated biologically active food component which consists of a core which comprises at least one dietary fiber, which core is surrounded by at least one biologically active substance, the core and the biologically active substance being surrounded by one or more shell-forming substances, which preferably form stable complexes with the core materials and/or the biologically active substances. Since in most cases the biologically active substances are labile compounds whose activity decreases during storage as the pure substance, during processing and during storage in the processed state, the universal stabilization method described below and the resultant substances are a considerable improvement compared with the prior art. In addition, the bioavailability of the abovementioned substances is frequently a problem. Using the method described, biologically active substances may be formulated such that the bioavailability and thus the utility to health is considerably increased.

[0021] For the purposes of the present invention, food components are natural and synthetic constituents of the human and/or animal diet. In addition, the food components are taken to mean constituents which are added specifically to the preparations and which supplement the human and/or animal diet (dietary supplements). This term also includes substances which are used in pharmaceuticals as nutritional components.

[0022] "Multifunctional" for the purposes of the invention means that the encapsulated food component fulfills two or more nutritional functions. These also include technical functions, for example delayed release of nutritionally active substances at the site of action or an improved sensory perception of the components in the food.

[0023] Encapsulated means that the substance in question is surrounded on all sides by a shell. Shell substances which can be used are in particular compounds which are able to form stable complexes with the core materials and/or the biologically active substances. Examples of these are mono-, di- and
polysaccharides (hydrolyzed starches, microbial polysaccharides, plant polysaccharides, acidic plant gums, pectins, celluloses), emulsifiers, peptides, proteins and prebiotic substances/substrates.

[0024] Stable complexes with the core materials and/or the biologically active substances are formed, in particular, when said materials interact with one another. Interactions here are taken to mean molecular and particulate interactions.

[0025] A dietary fiber is a substance defined by food law as a nutrient which is not metabolized at all or only to a small extent by the organism in question. Because of its inert character, dietary fibers reside in the intestine and can there exert optimally their physiological effects, such as increasing intestinal peristalsis, affecting cholesterol absorption, prebiotic effects etc. Examples of inventively usable dietary fibers are plant fibers (wheat fibers, oat fibers, rice dietary fibers, apple fibers, citrus fibers etc.), water-insoluble celluloses and hemicelluloses, but also water-soluble polysaccharides (for example β-glucans, fructo-and galactooligosaccharides), pectins, lignins or plant gums.

[0026] Hereinafter, "biologically active substances" are taken to mean materials which have the most varied nutritional functions in the organism and as a result make a positive contribution to the state of health. Biologically active substances can act, for example, as classical nutrients, can have immune-stimulating activity or protective activity or else can intervene in physiological processes in the organism. Substances which can act as biologically active substances are, inter alia, probiotic microorganisms, prebiotic substances, enzymes, nutrients (vitamins, minerals, trace elements, proteins, amino acids etc.), natural or synthetic secondary plant constituents (for example carotenoids) or substances having antioxidant activity (for example flavonoids).

[0027] The encapsulated food components are spherical or polygonal structures having a mean diameter, in the unprocessed state, of from 1 μm to 200 μm, preferably from 20 to 100 μm, in particular <50 μm. In the processed state, the particle diameter remains unchanged, but it can also increase up to 5 fold.

[0028] The core content in the food component is, depending on the sought-after effect in the product in which the food components are to be used, from 10 to 90% by weight, preferably greater than 50% by weight. The content of the biologically active substance in the food component depends on its dose-dependent physiological activity and can be from less than 1% by weight to more than 50% by weight, preferably from 10 to 20% by weight. The content of the shell materials of the food component is determined by the target-product-oriented functionality and is up to 50% by weight, but preferably below 10% by weight.

[0029] To produce the inventive food components, expediently a procedure is followed such that the biologically active substance or the mixture of two or more biologically active substances is introduced into a medium which comprises one or more shell-forming substances. The resultant mixture is then enriched with the dietary fiber or the dietary fibers and homogeneously mixed and then freed from the solvent or dispersion medium.

[0030] If the biologically active substance is a microorganism suspension, it is produced by a fermentation, in which it must be ensured that sufficiently high cell densities are achieved, preferably >1·10^9 per ml CFU (colony forming units). If necessary, the microorganism suspension can also be concentrated by centrifugation, filtration or other concentration processes corresponding to the prior art. This step is necessary, in particular, if, at the customarily achievable cell densities, an adequately high concentration in the encapsulated food components cannot be achieved. Concentration can be carried out by two powers of ten to approximately 10^{11} CFU per ml, but preferably by one power of ten.

[0031] In addition, it is expedient that components of the fermentation medium make a contribution to shell formation. Such substances can be, inter alia, proteins, peptides, carbohydrates or minerals.

[0032] When the biologically active substances are introduced into the solvent or dispersion medium, it must be ensured that a homogeneous distribution is achieved and, after the partial or complete removal of the
solvent or dispersion medium, the sought-after quantitative ratio between dietary fiber, shell materials and biologically active substances is formed. If expedient, the shell-forming substance can also be charged first and the biologically active substances added to it.

[0033] When the mixture is produced, the sequence of addition of the individual components is of no importance, but care must be taken to ensure that unwanted aggregations do not occur. This applies in particular in the case of addition of minerals which dissociate in solution. Not until the following drying process, as a consequence of specifically intended interactions between the components of the shell materials, the biologically active substances and the dietary fibers, does formation of complexes occur, which complexes are of importance for stabilizing the encapsulated food components. The solvent or dispersion medium is removed by known drying processes, for example spray-drying, fluidized-bed drying, freeze drying etc., but preferably by spray-drying. In the cases where all constituents of the food component to be encapsulated are present in a dispersion, a single-component nozzle is used for spraying, which ensures the formation of sufficiently small particles during the spraying operation. Preferably, nozzles having a nozzle diameter of from 0.1 to 2.0 mm are used. However, it can also prove to be expedient that the shell material is not to come into contact with the mixture until immediately after encapsulation, so that they are combined in the drier via a two-component nozzle.

[0034] The advantage of the invention is that bioactive substances, by using dietary fibers, preferably fiber materials, can be processed to form food components in such a manner that they achieve high stability after the drying process, in the food in which they are incorporated, during storage of the food and in the gastrointestinal tract. The release of the food components with their physiologically multifunctional properties does not take place until at the optimum position in the gastrointestinal tract. Furthermore, technologically functional properties may be achieved in the food by incorporating the food components which lead to sensory enhancement, for example due to increased creaminess of the end product.

[0035] The invention can be used in very many food groups, such as milk products (fermented milk products, fresh cheese, cheese preparations), meat processing products (uncooked sausage, scalded-emulsion sausage, cooked-meat sausage, meat pies, meat salads), fruit and vegetable products (jams, jellies, fruit juices, vegetable purees, vegetable juices), bakery products (bread, rolls, patisserie products), beverages, but also in food supplements in animal nutrition (domestic and small animals; farm animals) and in cosmetics and in pharmaceuticals etc.

[0036] The invention is illustrated below on the basis of Examples.

**EXAMPLE 1**

[0037] 1% by weight of wheat fibers is suspended in water, MRS broth is added and the mixture is autoclaved. This mixture is inoculated with a microorganism culture (Lactobacillus acidophilus) and fermented for 24 hours at 37° C. until a cell count of 10^9 CFU ml^{-1} is achieved. Culture and wheat fibers are centrifuged off and rinsed once with a 0.1% strength by weight maltose solution. The supernatant is removed and the residue is taken up with 0.1% strength by weight maltose solution. In a second batch, the capsule material B, in this example 4% by weight of gum arabic, is suspended in water. By adding the microorganism/wheat fiber mixture to the capsule material, a sprayable dispersion is produced. This dispersion, during the subsequent spray-drying is vigorously stirred at 500 rpm to ensure uniform distribution of the material to be dispersed. The drying process was carried out using the following parameter settings: 1 Drying air temperature: 170-185° C. Exhaust air temperature: 55-60° C. Spraying pressure: 1 bar Intake pressure: 0.01 bar

[0038] The resultant fine white powder was suspended in aqueous solution to determine the survival rate of the encapsulated microorganisms. The survival rate was >60% by weight.

[0039] In this example, the wheat fiber serves as a multifunctional food ingredient, firstly owing to its dietary fiber character, and to its simultaneous function as a support material for the microorganisms. Gum arabic acts as capsule material and maltose as processing aid and also as an additional C source for the bacteria.
EXAMPLE 2

[0040] The growth and fermentation conditions for the microorganisms are similar to those of Example 1. The suspension of the capsule materials is supplemented in this example by a network-forming protein with 2% by weight of gelatin, so that a copolymer of fixed capsule structures is formed. After production of the dispersion, consisting of microorganism suspension and capsule material suspension, this is dried as in Example 1.

EXAMPLE 3

[0041] The capsule material of this example is composed of a plurality of substances as followed: 3% by weight of gum arabic, 1% by weight of gelatin, 0.5% by weight of xanthan, 0.3% by weight of citric acid. The substances were dispersed in water with constant stirring and mixed with the suspension of microorganisms prepared according to Example 1. This mixture is dried under the same conditions as described in Example 1. The result is a finely crystalline powder having a microorganism density of >60% by weight, based on the starting amount.

[0042] Gum arabic and gelatin form a copolymer whose formation is supported by adding xanthan and citric acid. Xanthan can act simultaneously as energy source for the microorganisms, due to the amounts of acetate of pyruvate present therein.

EXAMPLE 4

[0043] In this example, in a targeted manner individual constituents of the culture medium (MRS broth) were integrated into the formation of a stable capsule/microorganism complex. The substances used here are ®Tween 80 at a concentration of 0.1% by weight as growth promoter for the bacteria, in addition 1% by weight of maltose, replacing glucose as C source, and, as complexing aid, 0.09% by weight of calcium acetate are used instead of sodium acetate. The bacterial suspension prepared according to Example 1 is taken up with a dispersion of the abovementioned substances in water. An aqueous 1-2% strength by weight alginate solution is then injected into the resultant mixture. The product obtained in this manner is dried in the spray tower as described in Example 1.

EXAMPLE 5

[0044] Similarly to the procedure in Example 4, certain constituents of the culture medium are replaced by substances which firstly can serve for capsule formation and secondly can serve as substrate for the microorganism. In this case the following were used: albumin as capsule material and as N source, lecithin as emulsifier and binding aid between protein and sugar, and as P source and maltose as aid during capsule formation and as C source. The substances are dissolved in water in the following concentrations: albumin 4% by weight, lecithin 0.5% by weight and maltose 0.5% by weight. The bacterial suspension produced as in Example 1 is added to this mixture. The dispersion is sprayed under the standard conditions specified in Example 1. A voluminous finely crystalline powder is obtained as a result of this process.

EXAMPLE 6

[0045] The microorganisms were cultured and fermented as in Example 1. To form the protective shell, in a first step, 3% by weight of pectins containing 0.5% by weight of inulin are suspended in water. The prepared bacterial suspension is added to this mixture. In a third step approximately 0.1% by weight of calcium chloride is added with constant stirring. The resultant mixture is spray-dried under standard conditions. In this example, pectin acts as capsule material, inulin is used as processing aid with prebiotic properties. By adding calcium chloride, the pectin forms what is termed a calcium-bridged gel.

EXAMPLE 7
A high-viscosity dispersion is given using 2.5% by weight of maltodextrin, 0.5% by weight of lecithin and 1% by weight of guar gum as capsule materials. This dispersion is mixed with the bacterial suspension produced according to Example 1 and sprayed under standard conditions. The substances maltodextrin and lecithin form a gel-like network, the gelation capacity of the polysaccharide used being increased by the guar gum used.

**EXAMPLE 8**

As described under Example 1, in this case also a microorganism suspension is prepared containing wheat fiber as support material. This suspension is taken up as follows using an aqueous 0.5% strength by weight xanthan solution for stabilization. The resultant mixture is mixed with a previously prepared dispersion of 2.5% by weight of albumin and 1% by weight of carboxymethylcellulose in water with constant stirring. The albumin used forms a complex with the carboxymethylcellulose, which complex acts as capsule material.

**CLAIMS (ENGLISH)**

What is claimed is:

1. A multifunctional encapsulated biologically active food component consisting of a core which comprises at least one dietary fiber, which core is surrounded by at least one biologically active substance, in which the core and the biologically active substance(s) are encapsulated by one or more shell-forming substance(s).

2. The food component as claimed in claim 1, wherein the dietary fiber is selected from plant fibers (wheat fibers, apple fibers, oat fibers etc.), water-insoluble polysaccharides (celluloses) and water-soluble polysaccharides, pectins, lignin and plant gums.

3. The food component as claimed in claim 1, wherein the shell substance(s) is (are) able to form a stable complex with the core material or the biologically active substance(s) or the core material and the biologically active substance(s).

4. The food component as claimed in claim 3, wherein the shell substance is selected from one or more of the following substances: mono-, di- and polysaccharides (hydrolyzed starches, microbial polysaccharides, plant polysaccharides, acidic plant gums, pectins, celluloses), emulsifiers, peptides, proteins and prebiotic substances.

5. The food component as claimed in claim 1, wherein the dietary fiber is selected from one or more of the following substances: plant fibers (wheat fibers, oat fibers, rice dietary fibers, apple fibers, citrus fibers etc.), water-insoluble celluloses and hemicelluloses, water-soluble polysaccharides (for example β-glucans, fructo- or galactooligosaccharides), pectins, lignins or plant gums.

6. The food component as claimed in claim 1, wherein the biologically active substance is selected from one or more of the following substances: probiotic microorganisms, prebiotic substances, enzymes, nutrients (vitamins, minerals, trace elements, proteins, amino acids), natural or synthetic secondary plant constituents (for example carotenoids) and substances having antioxidant activity (for example flavonoids).

7. The food component as claimed in claim 1, which has a spherical or polygonal shape having a mean diameter, in the unprocessed state, of from about 1 μm to about 200 μm.

8. The food component as claimed in claim 1, wherein the core content of the food component is from about 10 to about 90% by weight.

9. The food component as claimed in claim 1, wherein the content of the biologically active substance in the food component is from <1% by weight to >50% by weight.
10. The food component as claimed in claim 1, wherein the content of shell materials in the food component is \( \leq 50\% \) by weight.

11. A process for producing a food component as claimed in claim 1, which comprises introducing a biologically active substance or a mixture of two or more biologically active substances into a medium which comprises one or more shell-forming substances, then enriching the resultant mixture with one or more dietary fiber(s) and homogeneously mixing the mixture and then freeing it from solvents or dispersion media.
Annex 2 (Sonnepan Priority Document)

Multifunctional Food Particle

The invention relates to a multifunctional encapsulated biologically active food component which consists of a core that comprises at least one dietary fiber, which core is surrounded by at least one biologically active substance and in which the core and the biologically active substance are surrounded by at least one shell-forming substance. The stability of the multifunctional food component is based on interactions of the components with one another.

BACKGROUND OF THE INVENTION

Biologically active substances in nutrition are physiologically important components. They can have the most varied functions in the organism and as a result make a positive contribution to health. Biologically active substances can act, for example, as classical nutrients, can stimulate immune activity or have protective activity or intervene in physiological processes in the body. Biologically active substances can include, inter alia, nutrients or secondary plant constituents. Enrichment of the diet with components of this type in a stable and in particular bioavailable form is therefore desirable from the nutritional aspect.

Dietary fibers are a heterogeneous product group. Many dietary fiber preparations are based on plant fibers and consist predominantly of water-insoluble polysaccharides in addition to pectin, lignin and plant gums (for example wheat fibers, oat dietary fibers, rice dietary fibers, apple fibers, citrus dietary fibers etc.). In addition there are also soluble dietary fibers which are mostly made up of complex carbohydrates (for example fructo- or galactooligosaccharides, β-glucans etc.). Dietary fibers taken in via the diet are distinguished by the fact that they are indigestible constituents for humans. Because of their inert character, dietary fibers reside in the intestine and can there typically exert their physiological effects, for example increasing intestinal peristalsis, effects on cholesterol absorption, prebiotic activities etc. An increased intake of dietary fibers is desirable for nutritional reasons. A daily intake of 25-30 g of dietary fiber is recommended by nutritionists.

For the reasons described it is expedient to provide dietary fibers and biologically active substances in a stable and nutritionally utilizable form via the diet or other delivery route to the organism. An optimal release of the biologically active substances, for the reasons described above, is not desirable until after passage through the stomach in the lower digestive tract. Furthermore, it is desirable that adverse sensory perceptions of nutritionally valuable substances in foods do not occur. This is, in the case of insoluble dietary fibers, frequently a marked sensory perception of particles in the food matrix (“scratchy off-taste”).

The technique of microencapsulation has long been used commercially predominantly in the pharmaceutical industry. For some time, however, there have also been studies on the use of encapsulation in food technology (Jackson, Lee, 1991, Kanawija et al. 1992, Hegenbart, 1993, Arshady, 1993, Dewettinck, 1997, Pegg, Shahidi, 1999). The spray-drying process is one of the most frequently used processes for encapsulating various substances in the food industry. It can be considered as one of the essential advantages here that spray-drying is suitable for processing heat-sensitive materials. In addition, the process is inexpensive and offers the advantage that an existing technology can be utilized. In order to make possible targeted utilization for the various application sectors in the food industry, studies of the customary materials in different combinations would have to be carried out, since single materials cannot comply with the complex requirements which a food makes of the capsule material. Studies to date were concerned predominantly with decreasing the volatility of substances and their oxidation by embedding them into a suitable material. The effects resulting from the physicochemical properties of the capsule materials and of the physical conditions of this process require further study (Re, M. I., Drying Technology, 1998, 16(6), 1195-1236).

Most patents or patent applications relate to fields of application in the pharmaceutical industry. The major roles are taken here by the capsule materials used (generally in combination with specific activities or effects), the controlled release of substances via the application of the encapsulating technique and the stabilization of substances. In the food sector there are far fewer patents, which, however, are essentially determined by
these three directions. In the case of microencapsulation of cells or cell free extracts (CFE), physiological stability in combination with their use as a pharmaceutical product play the major roles.

Microencapsulation with the purposes of a) use of dietary fibers, in particular fibers as a support material for biologically active substances b) support material interactions, c) prevention of support material/capsule material interactions, d) stabilization of the biologically active substances in the product and in the food with correspondingly extended shelf life, has not been described to date.

The object of the present invention was therefore to provide a system which simultaneously prevents an interaction with the surrounding food (for example in some cases prevents unwanted swelling properties of the dietary fibers) provides nutritionally valuable substances without adverse sensory perceptions, ensures increased storage stability of nutritionally valuable substances and optimizes the quantitative supply and localization of release of the biologically active substances at the desired site (optimized bioavailability).

DESCRIPTION OF THE INVENTION

This object is achieved by a multifunctional encapsulated biologically active food component which consists of a core which comprises at least one dietary fiber, which core is surrounded by at least one biologically active substance, the core and the biologically active substance being surrounded by one or more shell-forming substances, which preferably form stable complexes with the core materials and/or the biologically active substances. Since in most cases the biologically active substances are labile compounds whose activity decreases during storage as the pure substance, during processing and during storage in the processed state, the universal stabilization method described below and the resultant substances are a considerable improvement compared with the prior art. In addition, the bioavailability of the abovementioned substances is frequently a problem. Using the method described, biologically active substances may be formulated such that the bioavailability and thus the utility to health is considerably increased.

For the purposes of the present invention, food components are natural and synthetic constituents of the human and/or animal diet. In addition, the food components are taken to mean constituents which are added specifically to the preparations and which supplement the human and/or animal diet (dietary supplements). This term also includes substances which are used in pharmaceuticals as nutritional components.

"Multifunctional" for the purposes of the invention means that the encapsulated food component fulfills two or more nutritional functions. These also include technical functions, for example delayed release of nutritionally active substances at the site of action or an improved sensory perception of the components in the food.

Encapsulated means that the substance in question is surrounded on all sides by a shell. Shell substances which can be used are in particular compounds which are able to form stable complexes with the core materials and/or the biologically active substances. Examples of these are mono-, di- and polysaccharides (hydrolyzed starches, microbial polysaccharides, plant polysaccharides, acidic plant gums, pectins, celluloses), emulsifiers, peptides, proteins and prebiotic substances/substrates.

Stable complexes with the core materials and/or the biologically active substances are formed, in particular, when said materials interact with one another. Interactions here are taken to mean molecular and particulate interactions.

A dietary fiber is a substance defined by food law as a nutrient which is not metabolized at all or only to a small extent by the organism in question. Because of its inert character, dietary fibers reside in the intestine and can there exert optimally their physiological effects, such as increasing intestinal peristalsis, affecting cholesterol absorption etc. Examples of inventively usable dietary fibers are plant fibers (wheat fibers, oat fibers, rice dietary fibers, apple fibers, citrus fibers etc.), water-insoluble celluloses and hemicelluloses, but also water-soluble polysaccharides (for example .beta.-glucans, fructo- and galactooligosaccharides), pectins, lignins or plant gums.

Hereinafter, “biologically active substances” are taken to mean materials which have the most varied nutritional functions in the organism and as a result make a positive contribution to the state of health.
Biologically active substances can act, for example, as classical nutrients, can have immune-stimulating activity or protective activity or else can intervene in physiological processes in the organism. Substances which can act as biologically active substances are, inter alia, enzymes, nutrients (vitamins, minerals, trace elements, amino acids etc.), natural or synthetic secondary plant constituents (for example carotenoids) or substances having antioxidant activity (for example flavonoids).

The encapsulated food components are spherical or polygonal structures having a mean diameter, in the unprocessed state, of from 1 \( \mu \text{m} \) to 200 \( \mu \text{m} \), preferably from 20 to 100 \( \mu \text{m} \), in particular <50 \( \mu \text{m} \). In the processed state, the particle diameter remains unchanged, but it can also increase up to 5 fold.

The core content in the food component is, depending on the sought-after effect in the product in which the food components are to be used, from 10 to 90% by weight, preferably greater than 50% by weight. The content of the biologically active substance in the food component depends on its dose-dependent physiological activity and can be from less than 1% by weight to more than 50% by weight, preferably from 10 to 20% by weight. The content of the shell materials of the food component is determined by the target-product-oriented functionality and is up to 50% by weight, but preferably below 10% by weight.

To produce the inventive food components, expediently a procedure is followed such that the biologically active substance or the mixture of two or more biologically active substances is introduced into a medium which comprises one or more shell-forming substances. The resultant mixture is then enriched with the dietary fiber or the dietary fibers and homogeneously mixed and then freed from the solvent or dispersion medium.

When the biologically active substances are introduced into the solvent or dispersion medium, it must be ensured that a homogeneous distribution is achieved and, after the partial or complete removal of the solvent or dispersion medium, the sought-after quantitative ratio between dietary fiber, shell materials and biologically active substances is formed. If expedient, the shell-forming substance can also be charged first and the biologically active substances added to it.

When the mixture is produced, the sequence of addition of the individual components is of no importance, but care must be taken to ensure that unwanted aggregations do not occur. This applies in particular in the case of addition of minerals which dissociate in solution. Not until the following drying process, as a consequence of specifically intended interactions between the components of the shell materials, the biologically active substances and the dietary fibers, does formation of complexes occur, which complexes are of importance for stabilizing the encapsulated food components. The solvent or dispersion medium is removed by known drying processes, for example spray-drying, fluidized-bed drying, freeze drying etc., but preferably by spray-drying. In the cases where all constituents of the food component to be encapsulated are present in a dispersion, the sought-after quantitative ratio between dietary fiber, shell materials and biologically active substances is formed. If expedient, the shell-forming substance can also be charged first and the biologically active substances added to it.

When the mixture is produced, the sequence of addition of the individual components is of no importance, but care must be taken to ensure that unwanted aggregations do not occur. This applies in particular in the case of addition of minerals which dissociate in solution. Not until the following drying process, as a consequence of specifically intended interactions between the components of the shell materials, the biologically active substances and the dietary fibers, does formation of complexes occur, which complexes are of importance for stabilizing the encapsulated food components. The solvent or dispersion medium is removed by known drying processes, for example spray-drying, fluidized-bed drying, freeze drying etc., but preferably by spray-drying. In the cases where all constituents of the food component to be encapsulated are present in a dispersion, a single-component nozzle is used for spraying, which ensures the formation of sufficiently small particles during the spraying operation. Preferably, nozzles having a nozzle diameter of from 0.1 to 2.0 mm are used. However, it can also prove to be expedient that the shell material is not to come into contact with the mixture until immediately after encapsulation, so that they are combined in the drier via a two-component nozzle.

The advantage of the invention is that bioactive substances, by using dietary fibers, preferably fiber materials, can be processed to form food components in such a manner that they achieve high stability after the drying process, in the food in which they are incorporated, during storage of the food and in the gastrointestinal tract. The release of the food components with their physiologically multifunctional properties does not take place until at the optimum position in the gastrointestinal tract. Furthermore, technologically functional properties may be achieved in the food by incorporating the food components which lead to sensory enhancement, for example due to increased creaminess of the end product.

The invention can be used in very many food groups, such as milk products (fermented milk products, fresh cheese, cheese preparations), meat processing products (uncooked sausage, scalded-emulsion sausage, cooked-meat sausage, meat pies, meat salads), fruit and vegetable products (jams, jellies, fruit juices, vegetable purees, vegetable juices), bakery products (bread, rolls, patisserie products), beverages, but also in food supplements in animal nutrition (domestic and small animals; farm animals) and in cosmetics and in pharmaceuticals etc.
Claims

1. A multifunctional encapsulated biologically active food component consisting of a core which comprises at least one dietary fiber, which core is surrounded by at least one biologically active substance, in which the core and the biologically active substance(s) are encapsulated by one or more shell-forming substance(s).

2. The food component as claimed in claim 1, wherein the dietary fiber is selected from plant fibers (wheat fibers, apple fibers, oat fibers), water-insoluble polysaccharides (celluloses) and water-soluble polysaccharides, pectins, lignin and plant gums.

3. The food component as claimed in claim 1 or 2, wherein the shell substance(s) is (are) able to form a stable complex with the core material and/or the biologically active substance(s).

4. The food component as claimed in one or more of claims 1 to 3, wherein the biologically active substance is selected from one or more of the following substances: enzymes, nutrients (vitamins, minerals, trace elements, amino acids), natural or synthetic secondary plant constituents (for example carotenoids) and substances having antioxidant activity (for example flavonoids).

5. The food component as claimed in one or more of claims 1 to 4, which has a spherical or polygonal shape having a mean diameter, in the unprocessed state, of from 1 μm to 200 μm.

6. A process for producing a food component as claimed in claim 1, which comprises introducing a biologically active substance or a mixture of two or more biologically active substances into a medium which comprises one or more shell-forming substances, then enriching the resultant mixture with one or more dietary fiber(s) and homogeneously mixing the mixture and then freeing it from solvents or dispersion media.
**Annex 3 (Search Results)**

**Document 1 (for Part 2 of the exam)**

Publication number: JP2002522403  
Publication date: 2002-04-16  
Also published as:  
BRPI9909153A (on 2000-12-05)  
EP10673986A1 (on 2001-01-03)  
US6515427BA (on 2003-02-04)  
WO9952311A1 (on 1999-10-21)

**English abstract:** The invention relates to starch capsules which protect various substances, such as living microbes or enzymes, against the effect of the environment or the intestines, and to a method for manufacturing such capsules. A fraction of a suitable size category is chosen from the starch granules, the porosity of the granules is improved by hydrolyzing, and the granules are filled with desired substances, such as living microbes and/or enzymes. When desired, the starch granules can be coated with a suitable biopolymer, such as starch or amylase.

**Machine translation of JP JP2002522403 claims 1-10:**

1. The starchy capsule which features that it possessed porous structure, as a result of hydrolysis the microbe or the microbe and/or filled up with the polypeptide, or the protein includes the starchy granule.

2. The starchy granule, by using the enzyme adding water in the claim 1 which features that it is disassembled the starchy capsule of statement.

3. The starchy granule, α-[amiraze] and beta - the amylase and/or in the claim 1 which features that with the gluco amylase adding water it is disassembled or 2 the starchy capsule of statement.

4. The starchy granule, being biopolymer, coating from the claim 1 which features that it is done either of 3 in one section the starchy capsule of statement.

5. The starchy granule, the cellulose, the pectin and the protein, the starch and/or with the amylase coating from the claim 1 which features that it is done either of 4 in one section the starchy capsule of statement.
6. The starchy granule, the cellulose, the pectin and the protein, the starch and/or with the blend of the amylose and the membrane coating material which can be allowed medicine coating from the claim 1 which features that it is done either of 5 in one section the starchy capsule of statement.

7. The starchy granule, the barley, the potato and the wheat, the oats, the pea bean and the corn, the tapioca and the sago, the United States or the similar tuber vegetable, or the corn crop, desirably the potato, the barley, the wheat or the corn, to be most desirable from the claim 1 which features that it does from the potato either of 6 in one section the starchy capsule of statement.

8. Size of the starchy granule 10~100 micro m, desirably from the claim 1 which features that it is 50~100 micro m either of 7 in one section the starchy capsule of statement.

9. The starchy granule, the bacterium, the yeast or from the claim 1 which features that it has filled up with the microbe like the Hyphomycetes either of 8 in one section the starchy capsule of statement. less than DP N=0003 greater than less than TXF FR=0001 HE=246 WI=152 LX=0300 LY=0300 greater than

10. The starchy granule, lactic acid bacillus being attached, streptococcus being attached, [pedeiokotsukasu] being attached, [rakutokotsukasu] being attached, [roikonosutotsuku] being attached and [korinebakuteriumu] being attached, from the claim 1 which features that it has filled up with the lactobacillus the [enterokotsukasu] being attached way or the lactobacillus which belongs to [bihuidobakuteriumu] being attached either of 9 in one section the starchy capsule of statement.
From our correspondent

The US-company Procter & Gamble has announced the introduction of a special kind of energy drink for people and animals suffering from autoimmune diseases like rheumatoid arthritis (RA).

Phosphatidylinositol and phosphatidylcholine can alleviate the symptoms of RA and pharmaceuticals with these compounds are available on the market. Yet, these compounds are also available in the average diet of people that eat healthy foods, such as milk, fruits, yoghurt and probiotics. Scientists at P&G found that the human body is not able to take full advantage of the presence of the compounds in these food products since they are excreted without being extracted from the food. They have now found a revolutionary way to make use of other food components, such as fibers to enable the uptake of these compounds into the body.

Mary Roseblum (head of Animal Nutrition Research at P&G) explains: “In essence it was a simple idea. We used pectin (formed as little globules) to let phosphatidylinositol and phosphatidylcholine precipitate on those globules. In this way we were able to overconcentrate these compounds. The next step was to disperse these globules in drinking liquid, such as milk”.

Jeff Richmaster of the Formulations Department added: “This was only half of the solution, since it appeared that the globules did not cross the stomach, but were degraded there, which prevented the advantageous effect of the compound. Thus we had to coat these globules with cellulose to enable them to get through the stomach unharmed”.

Once P&G had found how to stabilize the active ingredients and to shield them from degradation in the stomach, they could start to develop the current product; a milk-based solution in which the little globules are dispersed, and which by the presence of these globules has a smooth mouth-feel, like yoghurts or creams.

It is envisaged that this health drink will alleviate the symptoms of RA and patients suffering from other autoimmune diseases. And that simply by drinking a yoghurt-like product. This truly is ‘nutraceutics’ in the way it should be.
ABSTRACT
A nutritional product having a solid matrix containing protein, fat and carbohydrate has disposed therein particles of dietary fiber encapsulated in zein. The preferred dietary fiber is guar. Such a nutritional product may be used for reducing the serum cholesterol in a mammal.

DETAILS

The present invention relates to a nutritional product with a solid matrix containing encapsulated dietary fiber, which reduces serum cholesterol in mammals.

Atherosclerosis is a disease of the arteries which begins as a lipid filled lesion in the intima of the arterial wall and progresses gradually with the eventual formation of a fibroatheromatous plaque over a period of years. The disease often affects the coronary arteries which perfuse the heart. Once significant encroachment on the vessel lumen occurs, coronary flow may be insufficient to meet myocardial oxygen demands, causing thoracic pain (stable angina pectoris). Eventually, the plaque may fissure or rupture, with or without overlying thrombosis. Plaque disruption may cause an abrupt reduction in coronary perfusion, leading to unstable angina, myocardial infarction (necrosis of the heart muscle resulting from interruption of the blood supply to the area) or ischemic sudden death, presumably due to ventricular arrhythmia. Heart disease can be the result of several etiologies, but it is most often due to atherosclerotic obstruction of large coronary arteries. More than half the deaths related to heart disease can be attributed to atherosclerosis.

The risk factor hypothesis of atherosclerosis has become well accepted in the medical world: the majority of people who die or are disabled as a result of atherosclerosis exhibit one or more identifiable characteristics called risk factors. If a person has a risk factor, he or she is more likely to develop clinical manifestations of atherosclerosis and is likely to do so earlier than is a person with no risk factors. The following parameters have been shown to be associated with coronary heart disease (CHD) in the Framingham study and other large epidemiological studies and are now well accepted: age (the relation of age to CHD is also dependent on sex) and family history of premature CHD, hypercholesterolemia and specifically high blood levels of low
density lipoprotein (LDL) cholesterol, low levels of high density lipoprotein (HDL) cholesterol, cigarette smoking, hypertension, and diabetes each contributes to increasing the risk of disease over baseline rates by a factor of 2 to 6-fold. When these characteristics are combined, the combined risks of coronary heart disease are additive (Dauber, "The Epidemiology of Atherosclerotic Disease", THE HARVARD UNIVERSITY PRESS, 1980; Kannel, "New Perspectives on Cardiovascular Risk Factors", AMERICAN HEART JOURNAL, 114:213-219, 1987; Matthews et al., "Menopause and Risk Factors for Coronary Heart Disease", THE NEW ENGLAND JOURNAL OF MEDICINE, 321(10):641-646, 1989; "Report of the National Cholesterol Education Program Expert Panel on detection, evaluation, and treatment of high blood cholesterol in adults", ARCHIVES OF INTERNAL MEDICINE, 148:36-69, 1988)

Just as important as the identification of cholesterol as a risk factor is the fact that when blood cholesterol and specifically LDL cholesterol levels are decreased in hypercholesterolemic subjects, there is a decrease in risk of heart disease. The results of the Lipid Research Clinic trials indicate that for every percentage point decrease in cholesterol levels, the risk of coronary heart disease decreases by 2%. ("Lipid Research Clinics Program. The Lipid Research Clinics Primary Prevention Trial Results I. Reduction in incidence of coronary heart disease", JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION, 251(3)351-364, 1984; "Lipid Research Clinics Program. The Lipid Research Clinics Coronary Primary Prevention Results II. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering", JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION, 251(3)365-374, 1984)

The blood cholesterol raising effects of dietary saturated fat and cholesterol are well accepted. Therefore, the American Heart Association and the National Cholesterol Education Program recommend as their "Step 1" diet a cholesterol-lowering program consisting of a reduction of total fat to less than 30% of calories as fat, a reduction of saturated fatty acids to less than 10% of calories and a reduction of cholesterol to less than 300 mg per day. It is also accepted that polyunsaturated fat lowers blood cholesterol, but because of the relatively small amount of data on the long term use of diets with very high polyunsaturated fat content, the American Heart Association and the National Academy of Sciences do not recommend that polyunsaturates exceed 10% of calories. (Expert Panel, "Summary of the Second Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II)", JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION, 269(23):3015-3023, 1993; Nutrition Committee of the American Heart Association, "Dietary Guidelines for Healthy American Adults", CIRCULATION, 77(3):721A-724A, 1988; Food and Nutrition Board, National Research Council, RECOMMENDED DIETARY ALLOWANCES, 10TH EDITION, National Academy Press, Washington, D.C., 1989). The National Cholesterol Education Program guidelines also state that monounsaturates should make up 10-15% of the diet, protein 10-20%, carbohydrate 50-60%, and dietary fiber 15-25 g/day. The type of dietary fiber is not specified.

For people who are eating an average American diet, switching to a Step 1 diet means decreasing their intake of fat. In the Multiple Risk Factor Intervention Trial, dietary changes according to a Step 1 diet were initiated leading to decreases in serum cholesterol in the range of 5-7%. (Banks et al., "Dietary Management of the Patient with Atherosclerosis: Are the New National Cholesterol Education Panel Recommendations Enough?", JOURNAL OF THE NATIONAL MEDICAL ASSOCIATION, 81(5):493-495, 1989). That means that if the ultimate goal is to reduce total cholesterol levels to less than 200 mg/dL, individuals who are consuming an average American diet and have serum cholesterol levels over 215-220 mg/dL will need to rely on more than a Step 1 diet.

A more stringent dietary recommendation of the National Cholesterol Education Program is the "Step 2 diet" which further restricts saturated fat to 7% of calories and cholesterol to less than 200 mg per day. One drawback of the Step 2 diet and other very low fat diets in that in addition to lowering LDL they lower HDL. (Jones et al., "Effect of dietary fat selection on plasma cholesterol synthesis in older, moderately hypercholesterolemic humans", ARTERIOSCLEROSIS AND THROMBOSIS, 14(4):542-548, 1994; Grundy et al., "Comparison of monounsaturated fatty acids and carbohydrates for reducing raised levels of plasma cholesterol in man", AMERICAN JOURNAL OF CLINICAL NUTRITION, 47:965-969, 1988)
The Step 1 diet does not provide specific recommendations for any other components of the diet which might enhance its cholesterol-lowering or antiatherogenic potential. Additional cholesterol-lowering components include vegetable oils that contain non-saponifiable components, cholesterol-lowering dietary fibers, and vegetable proteins. Rice bran oil contains a sizeable unsaponifiable fraction, i.e., non-acyl glycerol portion that contains 2 general classes of compounds: (a) sterols, and triterpene alcohols and (b) tocotrienols, which are similar to tocopherols, but with 3 double bonds in the side chain. The sterols and triterpene alcohols in rice bran oil are often esterified to ferulic acid and are known as "oryzanols". Studies in rats, primates and humans indicate that rice bran oil lowers serum cholesterol and may lower serum triglycerides. (Nicolosi, et al., "Rice bran oil lowers serum total and low density lipoprotein cholesterol and Apo B levels in nonhuman primates", ATHEROSCLEROSIS, 88:133-142, 1991; Lichenstein et al., "Rice bran oil consumption and plasma lipid levels in moderately hypercholesterolemic humans", ATHEROSCLEROSIS AND THROMBOSIS, 14(4):549-546, 1994) Two different mechanisms may be involved. First, plant sterols and oryzanols may interfere with cholesterol or saturated fat absorption and/or the absorption of bile acids. Second, in studies in chicks, pigs, and quail, tocotrienols have been reported to reduce cholesterol synthesis in the liver. It is also possible that plant sterols may prevent atherosclerosis by other mechanisms. (Mattson et al., "Optimizing the Effect of Plant Sterols on Cholesterol Absorption in Man", THE AMERICAN JOURNAL OF CLINICAL NUTRITION, 35:697-700, 1982; Qureshi et al., "The Structure of an Inhibitor of Cholesterol Biosynthesis Isolated from Barley", THE JOURNAL OF BIOLOGICAL CHEMISTRY, 261(23):10544-10550, 1986)

The cholesterol-lowering effects of dietary fiber have been summarized in several recent reviews. (Jenkins et al., "Fiber in the treatment of hyperlipidemia", Handbook of Dietary Fiber in Nutrition, G. Spiller, Ed., CRC Press, 1986 pp. 327-344; Sugano et al., "Dietary Fiber and Lipid Absorption", Dietary Fiber: Chemistry, Physiology, and Health Effects, Kritchevsky et al., Ed. Plenum Press, 1988 pp. 137-155; Anderson et al., "Dietary Fiber and Coronary Heart Disease", CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION, 29(2):95-147, 1990. Viscous soluble fibers are effective cholesterol-lowering agents when compared to non-viscous insoluble fibers or digestible carbohydrates, but the effect is variable. Table 1 summarizes data from over 50 studies of human subjects. As indicated in Table 1, one can expect a decrease in serum cholesterol in the range of 10% to 15% with doses of an appropriate source of fiber ranging from 6 g/day to 50 g/day. The variability of the response could be due to differences in the dose, timing of administration, types of subjects enrolled, and purities and chemical compositions of the fiber sources. In general, guar gum has advantages over the other fibers listed in Table 1 as it is more consistently high level of dietary fiber than pectin, more readily available at higher quality than psyllium, and much more consistently effective than oat and soy products.

TABLE 1

<table>
<thead>
<tr>
<th>CHOLESTEROL LOWERING EFFECT OF VARIOUS FIBER SOURCES</th>
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<tbody>
<tr>
<td>Total Cholesterol</td>
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<tr>
<td>LDL Cholesterol</td>
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<td>Number</td>
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<td>Mean decrease or</td>
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</tbody>
</table>
Fiber Source

range range Studies

Guar Gum-11.2%-17% 22
Pectin-12.4%--14
Psyllium-13.1%-20% (one study) 10
Soy-6.5%--2
Poly-
saccharide

Oat Products

-3 to-36% 0 to-58% 13

The most likely mechanisms for the cholesterol lowering effect of fiber according to Anderson et al., "Dietary fiber and coronary heart disease", CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION, 29 (2):95-147, (1990) are: (1) modification of bile acid reabsorption in terminal ileum (interruption of the entero-hepatic cycle of bile acids); (2) interference with lipid absorption; and (3) down-regulation of the liver's capability to synthesize cholesterol.

Many soluble fibers are extensively or completely degraded by bacteria in the cecum, yet bile acids are not well reabsorbed once they are released from dietary fiber. This may be partly due to the products of fermentation. The production of short chain fatty acids (SCFA) causes a drop in colonic pH which may decrease the solubility and the passive reabsorption of bile acids. (Remesy et al., "Cecal fermentations in rats fed oligosaccharides (insulin) are modulated by dietary calcium levels", AMERICAN JOURNAL OF PHYSIOLOGY, 264:G855-G862, 1993)

Cholesterol biosynthesis in the liver is known to be regulated by intracellular cholesterol levels, but dietary fiber does not cause an increase in cholesterol biosynthesis in the liver that is commensurate with the requirements for bile acid synthesis. Several studies support the hypothesis that propionate generated by bacterial fermentation of fiber could exert a rate-controlling effect on liver cholesterol synthesis. (Chen et al., "Propionate may mediate the hypocholesterolemic effects of certain soluble plant fibers in cholesterol-fed rats", PROCEEDINGS OF THE SOCIETY OF EXPERIMENTAL BIOLOGY AND MEDICINE, 175:215-218, 1984; Ebihara et al., "Hypcholesterolemic effect of cecally infused propionic acid in rats fed a cholesterol-free, casein diet", NUTRITION RESEARCH, 13:209-217, 1993) Other data dispute the validity of this concept. (Evans et al., "Relationship between structure and function of dietary fibre: a comparative study of the effects of three galactomannans on cholesterol metabolism in the rat", BRITISH JOURNAL OF NUTRITION, 68:217-229, 1992; Nishina et al., "Effects of propionate on lipid biosynthesis in isolated rat hepatocytes", JOURNAL OF NUTRITION, 120:668-673, 1990)
Plant proteins such as zein protein can lower cholesterol. The cholesterol-lowering mechanism of zein protein is unclear. Part of the effect can be explained by the amino acid composition of proteins. (Huff et al., "Plasma cholesterol levels in rabbits fed low fat, cholesterol-free, semipurified diets: Effects of dietary proteins, protein hydrolysates and amino acid mixtures", Atherosclerosis 28:187-195, 1977) Although the substitution of a supplement containing 5-10 g of plant protein for part of the animal protein in the diet would be unlikely by itself to cause a medically significant decrease in blood cholesterol levels, the use of zein protein as part of a combination of dietary cholesterol-lowering ingredients might contribute to a medically significant decrease in LDL cholesterol.

There is very little published scientific information on diets that combine known cholesterol lowering ingredients.

There is provided in accordance with the present invention a nutritional product having a solid matrix comprising protein, fat and carbohydrate, said matrix having disposed therein dietary fiber encapsulated in zein. In one embodiment, the matrix is a food bar. The preferred dietary fiber in the food bar is guar encapsulated with a coating of at least about 20% add-on zein. The protein is preferably soy protein and may further include calcium caseinate, and/or oat protein. The fat is preferably selected from the group consisting of vegetable oils containing less than 25% saturated fatty acids, by weight. Examples of such vegetable oils are rice bran oil, canola oil, and corn oil.

FOOD BAR EXAMPLE 1

Many attempts were made to manufacture an acceptable food bar matrix containing unencapsulated guar gum and free of partially or fully hydrogenated fat. For example the order of adding ingredients and mixing times was varied, but without satisfactory results. Food Bar Prototype Number 1 is typical of these attempts.

__________________________________________

RECIPE FOR FOOD BAR PROTOTYPE NUMBER 1

CONCENTRATION

BY PERCENT

INGREDIENT WEIGHT OF BAR

---------------------------------------------

High Fructose Corn Syrup

25.71

Oat Bran.sup.1 21.17

Guar.sup.2 12.35

Soy Protein 11.44

Rice Bran Oil 10.13

Polydextrose 6.62

Glycerin 6.18
Crisp Rice 4.45
Dicalcium Phosphate
0.97
Lecithin 0.59
Citric Acid 0.39

....sup.1 The "oat bran" used was actually a mixture comprising, by weight,
26.25% oat fiber, 62.128% oat flour and 11.622% soy protein.

....sup.2 The guar was obtained from TIC Gums and was designated by their
product code "8/22A", which is described below in the paragraph preceding
"ENCAPSULATION EXPERIMENT 1".

Manufacturing Procedure:

Food Bar Prototype Number 1 was prepared in a Hobart mixer. All ingredients were placed in the mixer and
mixed at room temperature (24. degree..+-. 10° C.). The first ingredients placed in the mixer were the soy
protein, dicalcium phosphate, and citric acid and they were mixed until blended. The rice bran oil, and lecithin
were then added to the other ingredients and mixed until blended. The guar was then added to the ingredient
blend and mixed therewith until blended. The polydextrose, oat bran, and crisp rice, were then added and
mixed until blended. The final ingredients added to the blend were the high fructose corn syrup, and glycerin
which were mixed with the other ingredients until blended. The batch was then dumped on to the bench top
and rolled out using a typical rolling pin to a uniform thickness. The batch was cut into bars with a spatula then
cooled in a refrigerator to between 0. degree. and 10° C. At no time were the food bars -or the blend of
ingredients subjected to elevated temperatures for cooking or baking. Of course, friction due to mixing could
elevate the temperature of the blend a few degrees. The bars were then packaged in a low density
polyethylene/foil wrap.

The texture of this prototype food bar and all of the other prototype food bars described herein was
determined using a Stevens L.F. R.A. Texture Analyzer. This instrument measures the amount of "grams of
force"[that it takes to move a probe 3 mm into a bar at a speed of 0.2 mm/sec. The sample size is one bar,
with five measurements taken per bar. The five measurements are averaged together and recorded as grams
of force. The texture of food bar prototype number 1 was determined several times over a period of weeks
and the results are presented in TABLE 5.

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TEXTURE TESTING FOR FOOD BAR PROTOTYPE

NUMBER 1

FOOD BAR
Inasmuch as a hardness of 400 or greater is unacceptably difficult to chew, this prototype and others containing unencapsulated guar and desirable levels of hydrogenated fat were not acceptable as a commercial product. Other problems which were observed were food bars becoming dried out, hardened, crumbling, or even turning into a powder.

ENCAPSULATION EXPERIMENT 2

The purpose of this experiment was to determine if the use of a different plasticizer with the zein would allow thinner coatings which would be as good of a moisture barrier as thicker coatings while giving the acceptable mouth feel of a thinner coating (smaller particles) when incorporated in a food product.

A solution of coating material was prepared comprising zein F4000 plus Captex® 355 equaling 20% of the zein, as a 12.5% by weight solution of ethanol/water at a 90/10 weight/weight ratio. In a 4"/6" fluid bed unit, the 8/22 guar gum (small guar particles) was initially granulated with a bottom spray nozzle and then coated using a bottom spray with a Wurster column insert. The coating solution was applied to 1000 g of the guar gum at a rate of 9 g/min. The atomizing air pressure for the spray nozzle was 15 psig. The fluidizing inlet air temperature varied between 44.4° and 50.9° C. with a corresponding air discharge temperature of about 21.1° to 27.2° C. After 10% zein by weight of the weight of the guar gum was applied, the guar gum was sieved to remove particles above 840 μm and below 125 μm. The guar gum was then coated using the Wurster column insert with the same processing conditions. Samples were removed at zein levels of 20% and 40% by weight of the guar gum. At each sampling point the coated guar gum was sieved to remove particles greater than 840 μm before being returned to the unit for more coating. The encapsulation process was stopped after 60% zein by weight of the guar was applied. This process resulted in less agglomeration than encapsulation experiment 1, but the powder flow within the chamber was slower which may have been due to the higher oil content in the coating solution.

ENCAPSULATION EXPERIMENT 3

This experiment was conducted to evaluate the coatability of larger size guar particles.

Guar gum was encapsulated in 20% add on zein using a 18" Wurster coater. The guar gum 8/22A used in this experiment has a slightly larger particle size, as described above. A solution of a coating material was prepared comprising zein F4000 plus Durkex® 500 equaling 20% of the zein, as a 15% by weight solution of ethanol/water at a 90/10 weight/weight ratio. The coating solution was applied to 35 kg of guar gum at an initial rate of 200 g/min and was gradually increased to 250 g/min over a 40 minute period. The atomizing air pressure for the spray nozzle was 80 psig. The fluidizing inlet air temperature varied between 44.4° and 46.7° C. with a corresponding air discharge temperature of between 26.1° to 38.9° C. After 20% zein by weight of
the weight of the guar gum was applied, the process was stopped. The product was dried for 5 minutes and then removed from the column. 99% of the product was less than 40 mesh.

It was determined that larger size guar particles yielded better encapsulated particles of more uniform size than those obtained by coating the smaller size guar particles.

ENCAPSULATION EXPERIMENT 4

The objective of this experiment was to attempt to produce a final product of smaller encapsulated particles by using a series of sieving steps.

Guar gum was encapsulated in different levels of zein using a 18" Wurster coater. Guar gum 8/22 (small guar particles) was used in this experiment. A solution of a coating material was prepared comprising zein F4000 plus Durkex® 500 equaling 20% of the zein, as a 23.5% by weight solution of ethanol/water at a 90/10 weight/weight ratio. The coating solution was applied to 35 kg of the guar gum at a rate of 250 g/min. The atomizing air pressure for the spray nozzle was 80 psig. The fluidizing inlet air temperature varied between 43.9° and 46.7. degree. C. with a corresponding air discharge temperature of between 24. 4° to 40° C. After 10% zein by weight of the weight of the guar gum was applied, the process was stopped. The product was sieved to remove product greater than 420 μm and less than 150 . μm. The sieved guar gum was returned to the Wurster column insert and coated with the same processing conditions. After a zein level of 20% add on was applied, the system was stopped. The product was dried for 5 minutes and then removed from the column. 97.5% of the product was less than 40 mesh.

While the yield of acceptable end product was high, this procedure would be cost prohibitive because of extra processing steps.

ENCAPSULATION EXPERIMENT 5

The objective of this experiment was to evaluate the use of rice bran oil as a hydrophobic material in the zein coating.

For this experiment the larger size guar gum (8/22A) was encapsulated in 25% add on zein with rice bran oil as the plasticizer. A solution of a coating material was prepared comprising zein F4000 plus rice bran oil equaling 20% of the zein by weight, as a 23.5% by weight solution of ethanol/water at a 90/10 weight/weight ratio. In a 4"/6" fluid bed unit, the guar gum was coated using a bottom spray with a Wurster column insert. The coating solution was applied to 500 g of the guar gum 8/22A at a rate of 9 g/min. The atomizing air pressure for the spray nozzle was 15 psig. The fluidizing inlet air temperature varied between 43.9° and 45° C. with a corresponding air discharge temperature of between 26.9° and 34.4° C. After 25% zein by weight of the weight of the guar gum was applied, the guar gum was sieved to remove particles above 420 μm and below 125 μm. 85.3% of the product was in the correct size range.

Rice bran oil did not appear to facilitate as good a coating process as the other plasticizers, but this could possibly be improved with changes in process and/or formulation.

ENCAPSULATION EXPERIMENT 6

The objective of this experiment was to evaluate the coating of larger guar particles with increased levels of zein.

For this experiment, the larger size guar gum particles (8/22A) were encapsulated in 30% add on zein with Durkex® 500 as the plasticizer. A solution of a coating material was prepared comprising zein F-4000 plus Durkex® 500 equaling 20% of the zein, as a 23.5% by weight solution of ethanol/water at a 90/10 weight/weight ratio. In a 4"/6" fluid bed unit, the guar gum was coated using a bottom spray with a Wurster
The coating solution was applied to 500 g of the guar gum 8/22A at a rate of 9 g/min. The atomizing air pressure for the spray nozzle was 15 psig. The fluidizing inlet air temperature varied between 42.8° and 46.1° C. with a corresponding air discharge temperature of between 29.4° to 35. 6° C. After 30% zein by weight of the weight of the guar gum was applied, the guar gum was sieved to remove particles above 420 μm and below 125 μm. 89.4% of the product was in the correct size range.

When the microencapsulated guar manufactured in this experiment was incorporated into food bars, the bars were crumbly and had an unacceptable "sandy" mouth feel.

ENCAPSULATION EXPERIMENT 7

The purpose of this experiment was to evaluate the use of carnauba wax as a coating material to reduce processing time with a dual coating process.

For this experiment, some of the product from experiment 3 was overcoated with carnauba wax. The carnauba (No. 120, Frank B. Ross Co. Inc., Jersey City, N.J., U.S.A.) was melted in a beaker and held at a temperature of 104. 4° C. In a 4"/6" fluid bed unit, 500 g of the product from example 4 was coated using a bottom spray without a Wurster column insert. The molten wax was pumped at a temperature between 98.9. degree and 104.4° C. The atomizing air pressure was 15 psig. The fluidizing inlet air temperature varied between 51.6° and 53.3. degree. C. with a corresponding outlet temperature of 39.4° to 41. 7° C. After 75 g of the wax was applied the coating process was stopped.

When the microencapsulated particles manufactured in this experiment were incorporated into food bars, the resultant food bars became unacceptably hard within two months after manufacture.

ENCAPSULATION EXPERIMENT 8

The objective of this experiment was to evaluate zein particles coated only with carnauba wax.

For this experiment, the larger size guar gum particles (8/22A) were encapsulated in 44.8% add on carnauba wax. The carnauba wax (No. 120) was melted in a beaker and held at a temperature of 104.4° C. In a 4"/6" fluid bed unit, the guar gum was coated using a bottom spray without a Wurster column insert. The molten wax was applied to 500 g of the guar gum 8/22A. The atomizing air pressure for the spray nozzle was 15 psig. The fluidizing inlet air temperature varied between 53.9° and 72.2° C. with a corresponding air discharge temperature of between 33.1° and 36.7° C. After the carnauba wax was applied, the product was removed.

The microencapsulated guar manufactured in this experiment was not used in food bars because of the results of experiment 7.

ENCAPSULATION EXPERIMENT 9

The objective of this experiment was to evaluate the use of beeswax as a coating material.

For this experiment, the larger size guar gum was encapsulated in 23% add on beeswax. The beeswax (Frank B. Ross Co. Inc., Jersey City, N. J. U. S.A. was melted in a beaker and held at a temperature of 107.2. degree. C. In a 4"/6" fluid bed unit, the guar gum was coated using a bottom spray without a Wurster column insert. The molten wax was applied to 500 g of guar gum 8/22A (large guar gum particles). The atomizing air pressure for the spray nozzle was 15 psig. The fluidizing inlet air temperature varied between 21.1° and 32.2° C. with a corresponding air discharge temperature of between 28.3° to 29.4. degree. C. After the 5 minutes, the guar gum was starting to agglomerate so the inlet air temperature was reduced to 21° C. The process was stopped after 23% add on because of flow problems within the chamber.
When the encapsulated guar manufactured in this experiment was incorporated in food bars, the bars were unacceptably hard and crumbly and caused packing around the teeth of persons eating the bar.

ENCAPSULATION EXPERIMENT 10

The objective of this experiment was to evaluate the use of paraffin wax as a coating material.

For this experiment, the larger size guar gum particles (8/22A) were encapsulated in 40% add on paraffin wax. The wax (Paraffin 150/160, Frank B. Ross Co., Inc., Jersey City, N.J. U.S.A.) was melted in a beaker and held at a temperature of 104.4° C. In a 4"/6" fluid bed unit, the guar gum was coated using a bottom spray without a Wurster column insert. The molten wax was applied to 500 g of the guar gum 8/22A. The atomizing air pressure for the spray nozzle was 15 psig. The fluidizing inlet air temperature varied between 26.1° and 27.2. degree. C. with a corresponding air discharge temperature of between 25.5° and 29.4. degree. C. After 40% add on was applied the process was stopped.

When the encapsulated guar manufactured in this experiment was incorporated in food bars the bars hardened very quickly and caused packing around the teeth of persons eating the bar.

ENCAPSULATION EXPERIMENT 11

The purpose of this experiment was to evaluate the prospect of coating xanthan gum, which is a soluble high viscosity fiber.

For this experiment, xanthan gum was encapsulated in 20% add on zein with Durkex® 500 as the plasticizer. A solution of a coating material was prepared comprising zein F4000 plus Durkex® 500 equaling 20% of the zein, as a 23.5% by weight solution of ethanol/water at a 90/10 weight/weight ratio. In a 4"/6" fluid bed unit, the xanthan gum was coated using a bottom spray with a Wurster column insert. The coating solution was applied to 500 g of the xanthan gum at a rate of 9 g/min. The atomizing air pressure for the spray nozzle was 15 psig. The fluidizing inlet air temperature varied between 45° and 52.2. degree. C. with a corresponding air discharge temperature of between 27.2° and 32.2° C. After 20% zein by weight of the weight of the xanthan gum was applied, the process was stopped.

To date the encapsulated xanthan gum has not been incorporated into a food product, but the coating process appears to have yielded a satisfactory product.

ENCAPSULATION EXPERIMENT 12

This experiment was conducted to evaluate the feasibility of larger scale (bigger batch size) coating of guar with zein using larger capacity coating equipment and a different plasticizer.

Guar gum was encapsulated in different levels of zein using a 18" Wurster coater. The small guar gum particles (8/22) were used in this experiment. A solution of a coating material was prepared comprising zein F4000 plus partially hydrogenated vegetable oil (Durkex. RTM. 500, Van den Bergh Foods Co., Lasle, Ill. U.S.A.) equaling 20% of the zein, as a 23.5% by weight solution of ethanol/water at a 90/10 weight/weight ratio. The coating solution was applied to 50 kg of the guar gum at an initial rate of 175 g/min and was gradually increased to 215 g/min over a 30 minute period. Periodically the liquid line was flushed with 90/10 ethanol/water, if the liquid line pressure increased. The atomizing air pressure for the spray nozzle was 80 psig. The fluidizing inlet air temperature varied between 45.6° and 46.7. degree. C. with a corresponding air discharge temperature of between 25. degree. and 33.3. degree. C. After 10% zein by weight of the weight of the guar gum was applied, the process was stopped to remove a sample. The guar gum (35 kg) was returned to the Wurster column insert and coated with the same processing conditions. After a zein level of 15% add on was applied, the system was stopped again, and a sample was removed. The encapsulation process was stopped after 20% zein by weight of the guar was applied. The product was dried for 5 minutes and then
removed from the column. The product was sieved to remove product over 40 mesh (420 μm). 84% of the product was less than 40 mesh.

It was determined that a scale-up of the coating process if feasible and that the hydrogenated vegetable oil is a good plasticizer that did not have any substantial effect on product taste.

ENCAPSULATION EXPERIMENT 13

The objective of this experiment was to further refine the coating process.

Guar gum was encapsulated in 25% add on zein using a 18" Wurster coater. A solution of a coating material was prepared comprising zein F4000 plus Durkex® 500 equaling 20% of the zein, as a 23.5% by weight solution of ethanol/water at a 90/10 weight/weight ratio. The coating solution was applied to 35 kg of large guar gum particles (8/22A) at a rate of 240 g/min. The atomizing air pressure for the spray nozzle was 80 psig. The fluidizing inlet air temperature varied between 45° and 47.2° C with a corresponding air discharge temperature of between 28.9° to 37.8° C. After 25% zein by weight of the weight of the guar gum was applied, the process was stopped. The product was dried for 5 minutes and then removed from the column. The product was sieved to remove product over 40 mesh and underneath 140 mesh. 89.8% of the product was in the right range.

The microencapsulated guar manufactured with this procedure was employed in food bar prototype number 4 which was used in the "HUMAN CLINICAL STUDY OF FOOD BAR" which is described below.

ENCAPSULATION EXPERIMENT 14

The objective of this experiment was to improve the coating process and produce better microencapsulated guar for use in a solid food product.

Large guar gum particles (8/22A) were encapsulated in 25% add on zein using a 18" Wurster coater. A solution of a coating material was prepared comprising zein F4000 plus Durkex® 500 equaling 20% of the zein, as a 15% by weight solution of ethanol/water at a 90/10 weight/weight ratio. The coating solution was applied to 35 kg of the guar gum particles at a rate of 240 g/min. The atomizing air pressure for the spray nozzle was 80 psig. The fluidizing inlet air temperature varied between 38.9° and 47.2° C, with a corresponding air discharge temperature of between 24.4° and 35.6° C. After 25% zein by weight of the weight of the guar gum was applied, the process was stopped. The product was dried for 5 minutes and then removed from the column. The product was sieved to remove product over 40 mesh. 97.2% of the product was less than 40 mesh.

CLAIMS (ENGLISH)

We claim:

1. A nutritional product comprising a solid matrix comprising fat, carbohydrate, and at least one protein selected from the group consisting of vegetable proteins and milk proteins, said solid matrix having dispersed therein particles comprising a dietary fiber which lowers Serum cholesterol in humans encapsulated in zein.

2. A nutritional product according to claim 1 wherein the dietary fiber is guar.

3. A nutritional product according to claim 1 wherein said particles comprise guar encapsulated in about 20% add-on zein.

4. A nutritional product according to any one of claims 1-3 wherein a source of protein is soy protein.
5. A nutritional product according to any one of claims 1-3 wherein a source of fat is selected from the group consisting of vegetable oils containing, by weight, less than 25% saturated fatty acids.

6. A nutritional product according to any one of claims 1-3 wherein the protein is soy protein and the fat is selected from the group consisting of vegetable oils containing, by weight, less than 25% saturated fatty acids.

7. A nutritional product according to any one of claims 1-3 wherein the protein is soy protein and oat protein.

8. A nutritional product according to any one of claims 1-3 wherein the protein is soy protein and calcium caseinate.

9. A nutritional product according to any one of claims 1-3 wherein the protein is soy protein, oat protein and calcium caseinate.

10. A nutritional product for lowering serum cholesterol in humans comprising a solid matrix comprising fat, carbohydrate, at least one protein selected from the group consisting of vegetable proteins and milk proteins, and vitamins and minerals, said solid matrix having dispersed therein a serum cholesterol reducing quantity of particles comprising guar encapsulated in zein.

11. A nutritional product according to claim 10 wherein the protein is soy protein.

12. A nutritional product according to claim 10 wherein the fat is selected from the group consisting of vegetable oils containing, by weight, less than 25% saturated fatty acids.

13. A nutritional product according to claim 11 wherein the fat is selected from the group consisting of vegetable oils containing, by weight, less than 25% saturated fatty acids.

14. A nutritional product for lowering serum cholesterol in humans comprising a solid matrix of soy protein, rice bran oil, and carbohydrate, said solid matrix having dispersed therein a serum cholesterol reducing quantity of particles comprising guar encapsulated in zein.

15. A nutritional product according to claim 14 wherein said particles comprise guar encapsulated by at least about 20% add-on zein.

16. A nutritional product according to claim 14 wherein said solid matrix further comprises oat protein.

17. A nutritional product according to claim 14 wherein said solid matrix further comprises calcium caseinate.

18. A nutritional product according to claim 14 wherein said solid matrix further comprises calcium caseinate and oat protein.

19. A nutritional product according to claim 14 wherein the solid matrix further comprises an acidulant selected from the group consisting of citric acid, malic acid, and fumaric acid.

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Document 4 (for Part 2 of the exam)


Sodium-Alginate Beads Coated with Polycationic Polymers: Comparison of Chitosan and DEAE-Dextran

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(Received 26 June 1995; accepted 6 August 1995)

The retention capacity of alginate beads coated by two polycationic polymers, chitosan or diethylaminoethyl-dextran (DEAE-dextran) was studied. When beads were stored in latex, the chitosan coating was stable for less than 2 years, whereas the DEAE-dextran was stable for 5 years. The greater the concentration of the polycationic polysaccharides in the formation solution of the beads, the greater the stability of the coated beads. Further the stability depended on the pH conditions of bead formation. With chitosan, the greatest stability was observed at pH 5.4. With DEAE-dextran the greatest stability was observed at pH 4.

**INTRODUCTION**

Alginate is a polysaccharide, extracted from brown algae, which contains guluronic and mannuronic acids. The alginate gelation process has its origin in a capacity of the polysaccharide molecules specifically to bind divalent cations and this results in conformational/aggregational changes. To improve stability, the microcapsules are sometimes coated with a polycationic polymer that forms a membrane at the bead surface. In fact, the formation of a polyelectrolyte complex has been demonstrated to occur when an anionic and a cationic polymer are simultaneously present in aqueous solution. Such a complex between two oppositely charged polymers has been studied by several groups, 2-5 and it was recognized that it could be formed between alginate (or any other anionic polysaccharide) and chitosan which is a polycationic polysaccharide derived from the natural polymer, chitin. However, chitosan presents the drawback of being insoluble at pH higher than 5.4 and this property limits its use when pigments that are unstable at low pH, have to be encapsulated. Therefore, we used another polycationic polymer, diethylaminoethyl-dextran (DEAE-dextran), and the resulting DEAE-dextran coated beads were compared to those prepared with chitosan. DEAE-dextran is soluble at any pH and its amine functions are capable of interacting with alginate. In this study, we report on the stability of coated and non-coated beads in aqueous based resins.

**METHODS**

**Bead formation**

Sodium alginate was dissolved in a solution of the material to be encapsulated (Trypan blue) to obtain an alginate final concentration of 1.8% (w/v). This viscous solution was introduced in a 10 ml syringe and extruded through a 0.15 mm diameter needle using compressed air. The droplets were pulled off in 150 ml of a 0.1% HCl chitosan (2-8 g/litre)-0.05 M CaCl₂ or DEAE-dextran (8-20 g/litre)-0.05 M CaCl₂ stirred solution whose pH was adjusted at a given value (2, 4, 5.4, 6 or 7) with 1 M NaOH. The beads were allowed to harden for 30 min. They were rinsed with distilled water, then transferred into a latex.

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Document 5 (for Part 2 of the exam)

[Publication Date: Dec, 24 2000]

[30] Priority:
DK Jun, 7 1999 DK1999XXA

[22] Application Date: Jun, 7 2000

[51] Int. Cl. 8: A23L000100 A23L00010524 A23L00010526 A23L00010528 A23L0001308

[52] ECLA: A23L000100P4 A23L00010524 ; A23L00010526 ; A23L00010528 ; A23L0001308

[57] ABSTRACT
A particulate fibre composition containing at least one first dietary fibre, coated by an insoluble dietary fibre or a dietary fibre with low solubility, serving to prevent dissolution of the fibre composition in the oral cavity and during passage through the oesophagus. The fibre composition has one or several inserted additional layers of at least one second dietary fibre between the at least one first dietary fibre and the coating of the insoluble dietary fibre/dietary fibre of low solubility. This fibre composition can be produced and made for individual and special purposes and applications in as much as the different properties in relation to solubility and fermentability of the fibres are utilized for the production of multilayer particles. The dietary fibre supplement can be applied as pharmaceuticals and in food products where high fibre content and small calorie content is given high priority. Furthermore, the dietary fibre supplement can be applied for replacement of part of the sugar in sugar coatings of generally known cereals.

DETAILS

Particulate fibre composition

The invention relates to a particulate fibre composition of such type containing at least one first dietary fibre surrounded by an insoluble dietary fibre or a dietary fibre of low solubility serving to prevent dissolution of the fibre composition in the oral cavity as well as during passage through the oesophagus.

In recent years, ready-made food has become an increasing part of the diet. As little as 50 years ago, the quantity of fibres in the diet was approximately 5 times higher than in today's Western World diets. This gradual change of food habits and the increased level of welfare, particularly in the Western World, has resulted in an increasing number of persons with diseases such as diabetes mellitus, gastrointestinal diseases, obesity, obstipation, hiatal hernia, cardiovascular diseases, intestinal polyps, arteriosclerosis and colon cancer and rectal cancer as well as ordinary digestive trouble.

So far, it is a well-known fact that a diet containing sufficient quantities of fibres facilitates the support of normal healthy body functions, thus decreasing the number of gastrointestinal diseases considerably.

Natural vegetable fibres are high-molecular polymers that form part of the vegetable cell wall such as e.g. cellulose, hemicellulose, pectin, etc. Vegetable fibres are indigestible or digest slowly in human beings. Consequently, no calories or a very small amount of calories are added to the food. Fibres are known to be a valuable contribution to the food, as they contain e.g. anti-oxidants and vitamins.

The vegetable fibres become voluminous at contact with liquid, resulting in a sense of satiety and reducing the desire for further intake of food. When the fibres absorb liquid, the vegetable fibres act as a lubricant for the
passage of food through the alimentary canal, thus protecting the mucosa. Hence, intake of food with high fibre content offers the possibility of adjusting digestion and reducing calorie intake.

Attempts to utilize this knowledge in various ways are made, e.g. by ingestion of unprocessed fibres directly via the food or as a dietary supplement, or ingestion of more or less processed fibres.

Frequently, however, the taste of unprocessed fibres is very unpleasant. The mouthfeel becomes sticky when the fibres swell and consequently they are very difficult to swallow. Hence, unprocessed fibres are extremely difficult to ingest and their useful effect difficult to utilize.

US Patent No. 4,619,831 discloses a composition of dietary fibres produced by coating an insoluble fibre with an easily soluble fibre. The insoluble fibre is chemically and enzymatically purified to provide a concentration of insoluble dietary fibre.

Subsequently, the concentrated fibre is encapsulated in an easily soluble fibre. Easily soluble vegetable fibres are inclined to absorb liquid. They therefore dissolve at the first contact with liquid, e.g. water, and the insoluble vegetable fibres will quickly begin to swell. When e.g. the humidity of air is absorbed, the nutritional supplement will gradually become soft and spongy and may offer favourable conditions of growth for microbial activity, hence a poor durability of the dietary fibre composition; on top of that, the vegetable fibres expand quickly.

Such disadvantages make heavy demands on storage facilities and reduce the applications of the product.

Another disadvantage is created when the composition of dietary fibres is consumed together with food, in as much as the fibres begin to swell at the first contact with saliva in the oral cavity. Such swelling quickly causes the fibres to grow to such a considerable degree that it gives an unpleasant sensation when the person swallows the food. Possible utilization of fibres in colon cannot be monitored.

Japanese patent application JP 6015163 discloses microcapsules or pearls comprising fibres or drugs released at controllable speed.

To prevent decomposition of the microcapsules while standing in strong saline solutions, the capsules contain alginic acid fibres or an alginic acid salt. The microcapsules are furthermore characterized by good physical stress properties.

Such microcapsules are produced by mixing a sodium alginate solution with another substance, e.g. a fibre. The compound is shaped into pearls and after a fall from a height of 5cm into a calcium chloride solution, it is subjected to subsequent drying.

The microcapsules comprise only two fibres. The capsules are produced in such a way that the previous mixing of the fibres will not cause a complete surrounding of the remaining substance by fibres of alginic acid or alginic acid salt. Hence, it will be liberated gradually to the surroundings as early as at the first contact with a liquid. Furthermore, the design of the microcapsules is stress-proof which make them extremely unpleasant to chew.

The energy content of vegetable fibres is very low and utilization involves some difficulties. However, particularly water-soluble vegetable fibres are more or less fermentable, although such process involves considerable energy consumption.

It has only recently become known within technical science that fibres are combined of various components, the result of which is that different fibres have individual compositions which however only have been identified in very few cases. As an example, the effect of water-soluble dietary fibres of e.g. fruits, oat and legumes on the content of glucose and cholesterol in the blood is known today.
Generated through own experiments, the inventors of this invention have now succeeded in applying this new know-how for the manufacture of a number of various fibre compositions with unique positive effect on health as well as applications so far unknown.

The object of the invention is to provide a particulate fibre composition of the kind mentioned in the opening paragraph, which is applicable for preventive treatment of diseases, for direct treatment of diseases, or for consumption as a beneficial dietary supplement without essential change of eating habits.

A second object of the invention is the making of a particulate fibre composition for adjustment of the retention time of food in one or several sections of the gastrointestinal tract.

A third object of the invention is to provide a multilayer particulate fibre composition, each layer having its own unique effect on or in a previously identified spot in the gastrointestinal tract.

A fourth object of the invention is to provide a fibre composition rich in fibre and low in carbohydrate.

A fifth object of the invention is to provide a method to produce such particulate fibre composition.

The novel and unique features, whereby this is achieved according to the invention, is the insertion of one or several additional layers of at least one other dietary fibre between the at least one first dietary fibre and the coating of the insoluble/ slightly soluble dietary fibre.

Insoluble and soluble dietary fibres will absorb liquid at contact. To prevent dissolution and swelling of fibres e.g. already during storage of the finished product or immediately after initial fibre intake, the fibres may he encapsulated in at least one vegetable fibre coating which has insolubility or slight solubility by nature or through processing.

Production of compositions of fibre combinations with numerous therapeutic and/or healthful possibilities and effects is rendered possible by structuring the particulate fibre composition of layers of different fibres.

The fibre composition is made up of several different fibres, thus according to the invention enabling the design of a particulate fibre composition, partly consisting of fibres whose properties allow the conveyance of one or several fibres to one or several prefixed spots in the gastrointestinal tract, partly consisting of fibres whose purpose is to act in such spots.

By using the fact e.g. that various insoluble fibres or fibres with low solubility are more or less dissolved at various rates and at various pH values, it is possible to apply such fibres for encapsulation of other components such as easily soluble fibres that are fermented in colon, or fibres with low solubility that swell and fill the stomach for a prolonged period of time before passing on in the system.

Other advantageous effects may be e.g. swelling to increase the sense of satiety, to release vitamins and to adjust transit time through the gastrointestinal tract. In addition, there is by fermentation generated acetate supplying energy to the organism (corresponding to approx. 50% carbohydrate), butyrate directly nourishing epithelial cells in colon, and propionate supposedly reducing the content of cholesterol in the blood.

In addition, the soluble fermentable fibres change the bowel flora in colon in such a way that the quantity of bifido bacteria is increased to the detriment of bacteria such as Clostridium, Escherichia coli and Klebsiella. It is a well-known fact that bifido bacteria reduce certain initial stages and markers of colon cancer and reduce the risk of gastrointestinal infections as well as various infections in the bowel, e. g. Crohn's disease and ulcerative colitis.

The particulate fibre composition can advantageously comprise one or several inserted layers of fibres designed to gradually dissolve and/or be released and/or be fermented in the course of conveyance of the
fibre composition through the gastrointestinal tract and its stay in the various sections such as the stomach, the jejunum, the duodenum, the ileum or the colon.

A preferred embodiment of the invention may include at least one first dietary fibre in the fibre composition, accounting for between 65% and 98% of the total fibre content of the fibre composition, and this fibre can advantageously be selected as the fibre required to be conveyed to and utilized in the desired part of the gastrointestinal tract.

The outermost layer of fibre can advantageously be selected as a fibre which does not dissolve until at contact with the gastric juice. When the fibre composition is encapsulated by such a layer of insoluble fibres or fibres of low solubility, the coating will form a protective barrier against absorption and penetration of humidity from the surroundings to the inner layers of fibre.

Consequently, the durability and retention time of the packed fibre composition can advantageously be increased, and the costs of expensive packing techniques and storage conditions be kept down.

At the same time, the advantage is obtained in that the fibre composition will not begin to swell on ingestion, but gradually be dissolved on the desired spot in the stomach or the intestine.

Consequently, it is far more pleasant to eat than e.g. unprocessed fibres of e.g. wheat bran or psyllium. As previously mentioned, such are very difficult to swallow and give an unpleasant, sticky sensation in the mouth.

A particularly advantageous embodiment of the invention offers an outermost coating to the fibre composition, e.g. containing a natural colouring agent to give the composition an attractive appearance. Alternatively, the coating may be a thin natural layer of fibres, sweet to the taste, such as inulin or Raftilose. This makes appearance, taste and texture of the fibre composition attractive to e.g. children for eating on e.g. sour-milk products which children typically eat for breakfast.

Inulin is not hydrolyzed by enzymes in the gastrointestinal tract and therefore presents no metabolisable carbohydrate source.

Consequently, it is excreted in a non-metabolised manner.

In cases where the only sweetener of the fibre composition is a non-decomposable dietary fibre, the fibre composition is also an attractive dietary supplement for diabetes patients.

Alternatively, an outer layer of the fibre composition may be either a glazing or a coating of a monosaccharide such as fructose, glucose or sucrose, adding a pleasantly sweet taste with a prompt taste sensation to the fibre composition and making it even easier to swallow.

Additionally, the fibre composition may have one or several inserted layers or an outermost coating of a protein, such as casein containing all normal amino acids as well as a beneficially large number of the essential amino acids.

The at least one first dietary fibre is preferably selected from the group of psyllium, citrus pulp, apple pulp, black currant pulp, cherry pulp, grape pulp, modified starch, wheat bran, cellulose, acacia gum, alginate and fibres from pulp originating from other vegetables and fruits. However, this invention is not limited to such dietary fibres in as much as other types of fibres will be within the scope of this invention.

Furthermore, similar encapsulation of other nutritive components in fibres will also be possible.

As example of the fibre content in pulp, it can be mentioned that apple pulp from production of apple juice analysed to have a dietary fibre content of approximately 58 per cent, of this approximately 25 per cent is raw
cellulose; the pulp from production of black-currant juice analysed a dietary fibre content of approximately 65 per cent, of this approximately 25 per cent is raw cellulose; and pulp from production of cherry juice analysed a dietary fibre content of approximately 60%, of this approximately 23 per cent is raw cellulose.

Such residual or side products from juice production are a low-priced raw material, containing minerals and vitamins and being particularly well suited for adjustment of the dietary fibre content of food. Frequently, such residual or side products are waste products which are removed either by expensive disposing or by incineration. Consequently, such products are advantageously low-priced and useful ingredients of the fibre composition according to this invention.

When the dietary fibres come from industrial residual products, prior ultrasound processing may be advantageous, e.g. 15-40 kHz, partly to give raw material containing vegetable fibres, such as pulp and pulp fibres from vegetables and fruits, a larger surface, and partly to ensure that the natural germs on pulp and fibres are completely destroyed.

The at least one other dietary fibre can advantageously be selected from the fibre group consisting of pectin, guar gum, acacia gum, dextran, inulin, Raftilose, alginic acid, alginate, mainly K-alginate or Ca-alginate, or combinations of these.

In a preferred embodiment the at least one first dietary fibre is encapsulated in a coating of K-alginate or a coating of Ca-alginate, the insolubility of such combinations at neutral pH value being well-known to the expert, or that the combinations can easily be processed with a view to becoming insoluble at neutral pH value. (Edvar Onsoyen, Commercial applications of alginates, Carbohydrates in Europe No. 14, May 1996, pp. 26-31). The combinations are very robust against exposure to liquid and do not dissolve until exposure to a liquid with a low pH number, e.g. when exposed to the hydrochloric acid of the stomach. Such coating may also act as an inserted dietary fibre layer.

Alternatively, the encapsulating coating or one or several of the inserted dietary fibre layers may be a combination of K-alginate, Ca-alginate or pectin.

In addition, the fibre composition may include at least one additive, comprising between 0.1 and 5 per cent of the total weight of the finished fibre composition. Such additive has been selected from the fibre group with non-oxidizing properties such as tomatoes and grapes, vitamins, colouring agents, flavouring agents or from the group of sweeteners with low caloric content, mainly from the group of inulin, neohesperidine and stefiolglycosides. Steviolglycosides sweetens up to 300 times more per weight unit and neohesperidine up to 1500 times more per weight unit compared to sucrose which makes this group of sweeteners highly applicable to keep the dietary supplement low in caloric content.

In an alternative embodiment, an additive with anti-oxidizing properties may be added to the particulate fibre composition.

Examples of such additives may be synthetic or natural vitamins such as vitamin C or E. The durability of the finished product will be further ensured by addition of anti-oxidants, or if an essential part of the fibre composition consists of fibres with anti-oxidizing effect.

Furthermore, this produces a good effect on the fibre composition in as much as anti-oxidants inhibit the formation of free oxygen radicals, thus producing an anti-inflammatory effect as well as preventing cardiovascular diseases.

For adjustment of the relation between the fibre content of the fibre and the volume of the finished product, the fibre composition may advantageously additionally include at least one filler, preferably from the group of guar gum, starch, maltodextrin or their breakdown products and/or derivations. In addition, such filler makes for the unification of fibres.
Lucrative applications of this fibre composition may be addition to or coating of corn products, bread, health bars and similar products in as much as addition of the composition will not affect the normal characteristics of the end product.

In addition, the invention relates to a method for producing a particulate fibre composition as described above.

The method may include one or several of the following steps:

- solution/ suspension of the at least one first dietary fibre in water,

- filtration of the solution/ suspension of the at least one first dietary fibre through a filter with a fixed mesh size preferably not exceeding 0.2 mm,

- evaporation of fibres to dryness, -trituration of evaporized fibre paste to a particulate fibre sphere with a spherical particle size preferably not exceeding 0.2 mm,

- coating of the particulate fibre substance with one or several layers of a second dietary fibre, and

- drying of the resultant particulate fibre composition at approximately 60°C.

If the fibres originate from industrial production of e.g. juice, such fibres may produce an undesired microbiological activity. In such cases, the method may advantageously include an initial step where the fibres are ultrasound processed for a period sufficiently long to inactivate microbiological activity. The fibres may further be autoclaved to obtain optimum guarantee for elimination of all germs.

Furthermore, it may be appropriate to treat the evaporized fibres with a solution containing cation, such as CaCl₂, to crosslink and unify the fibres in the fibre composition. To reduce drying time and production time it may be appropriate to apply a CaCl₂ solution with a relatively high concentration, e.g. 5% (5 grams/100 ml).

In an especially preferred embodiment, the method can also include the step of adding sweetener exclusively or together with one or several additives to the nutritional supplement.

When the at least one first dietary fibre and one or several inserted layers of fibre are sprayed and/or coated with a fibre, e.g. an alginate which is insoluble until contact with liquid with a low pH, or pectin which has low solubility at neutral pH, the gelating, coating and stabilising properties of the fibre will cause the fibre to surround the remaining fibres forming a coating, entirely covering the remaining fibre layers.

Furthermore, the invention relates to the application of the fibre composition in food processing, e.g. cereals, cakes, snacks, health bars, healthy candy, drinks as well as health food products in general. Depending on the applied fibres, the energy of such food will be low, as will the optional content of natural or added vitamins, minerals and/or anti-oxidants. The intake of such food products can contribute positively to e.g. adjustment of the cholesterol in the blood stream, adjustment of the uptake of calcium, increase of the number of bifido bacteria in colon as well as adjustment of digestion.

The fibre composition may form part of such food products by way of coating or in the form of added granulates or powders in as much as swelling of the fibre composition is prevented when added to a liquid-containing medium with neutral pH value.

By suspension of the finished particulate fibre composition in an aqueous solution, the suspension can easily be sprayed onto or over any type of cereal, followed by subsequent drying by well-known techniques. In this way, a food product rich in fibre and low in caloric value is produced. Consequently, the fibre composition is extremely suitable for substitution of at least part of the sugar in sugar coatings of generally known cereals.
The fibre present in such a cereal comprises as much as 30 w%.

Particularly beneficial applications of fibre compositions according to this invention is in the form of dietary supplements or pharmaceuticals, e.g. for adjustment of emptying time of the stomach, for adjustment of transit time of food through the intestines, for adjustment of calcium uptake by the gastrointestinal tract, for treatment of insulin resistance, lipaemia, obstipation, overweight or infection in the gastrointestinal tract.

Within the scope of this invention, the fibres for the production of the fibre composition may be fermented or synthesised dietary fibres as well.

Within the scope of this invention, the fibre composition may be applied for numerous purposes in food products, dietary products as well as health food products, and is consequently not limited to the above mentioned applications.

The dietary fibres of the particulate fibre composition may include any combination of various soluble, insoluble, easily fermentable dietary fibres or dietary fibres which are not easily fermentable and particularly beneficial embodiments will be described in the enclosed examples.

In the following examples, the coating unit is a rotary mill, type Mansfield Ltd. UK.

13 C expiratory test was carried out by ingestion of 150mg "C-marked sodium acetate together with the apportioned quantity of dietary fibres or blind test. The 13C content of the expiratory air was measured every third minute for two hours and every fifteenth minute for four hours.

Alternative embodiments of this invention have been produced and tested in the following tests.

EXAMPLES

Test 1:

Production of particulate fibre composition according to the invention with one first dietary fibre and two additional fibre layers.

Transfer of 50 grams psyllium to coater and heating to 60°C.

Crosslinking of fibres by spraying with 10ml of a 1% CACl2, solution for a 60-second period. Drying of fibres by conveyance in coater at 60°C for 5 minutes. Spraying of the dried crosslinked psyllium fibres with 5ml 5% pectin solution for 10 minutes. Subsequent drying of the fibre composition by conveyance in coater for additionally 5 minutes at 600°C. Final spraying of the pectin-coated psyllium fibres with 10ml 10% inulin, and drying of the fibre composition by conveyance in coater for 5 minutes at 60°C.

This fibre composition has a long retention time in the stomach and contains 50 grams of psyllium, 0.1 gram of CACl2 0.25 grams of pectin and 1 gram of inulin, giving an end product with a composition of the substance by percentage as follows: 97.37% psyllium, 0.19% CACl2 2 10.49% pectin and 1.95% inulin, based on the final weight of the finished product.

Test 2:

Production of a-particulate fibre composition according to the invention with one first dietary fibre and two additional fibre layers.

As test 1, however 10ml 5% pectin solution and 20ml 10% inulin suspension are applied.
This fibre composition has long retention time in the stomach and the content of psyllium results in neutralisation and stabilisation of the blood sugar level at ingestion of the fibre composition. The fibre composition contains 50 grams of psyllium, 0.1 gram of CaCl2, 0.5 grams of pectin and 2 grams of inulin. The composition by percentage of the final product is 95.06% psyllium, 0.19% CaCl2, 0.95% pectin and 3.80% inulin, based on the final weight of the finished product.

Test 3:

Production of a particulate fibre composition according to the invention with one first dietary fibre and two additional fibre layers.

Transfer of 100 grams of psyllium to coater and heating to 60°C.

Crosslinking of fibres by spraying with 10ml of a 1% CACl2 solution for a 60-second period. Drying of fibres by conveyance in coater at 60°C for 5 minutes. Spraying of the dried crosslinked psyllium fibres with 25ml 5% pectin solution for 3 minutes. Subsequent drying of the fibre composition by conveyance in coater for additionally 5 minutes at 60°C. Final spraying of the pectin-coated psyllium fibres with 50ml 10% Raftilose, and drying of the fibre composition by conveyance in coater for 5 minutes at 60°C.

The organoleptic experience of the testees was that this fibre composition was pleasant to the taste, it was pleasant to consume as well as gave a long sense of satiety. It contains 100 grams of psyllium, 0.1 gram of CaCl2 1.25 grams of pectin and 5.0 grams of Raftilose, giving an end product with a composition of the substance by percentage as follows: 94.03% psyllium, 0.09% CaCl2, 1.18% pectin and 4.70% Raftilose, based on the final weight of the finished product.

Test 4:

Production of a particulate fibre composition according to the invention with one first dietary fibre of citrus pulp and two additional fibre layers.

Transfer of 500 grams of citrus pulp to 100ml water and ultrasound-processing for a 15-minute period. Filtration of the fibre composition and autoclave treatment at 112°C for 10 minutes and drying overnight. Microbiological control in the form of cultivation on nutrient substrate showed no occurrence of germs.

Transfer of 250 grams of citrus pulp to coater and heating to 60°C.

Crosslinking of fibres by spraying with 25ml of a 1% CaCl, solution for a 120-second period. Drying of fibres by conveyance in coater at 60°C for 5 minutes. Spraying of the dried crosslinked citrus pulp with 50ml 5% pectin solution for 3 minutes. Subsequent drying of the fibre composition by conveyance in coater for additionally minutes at 60°C. Final spraying of the pectin-coated citrus pulp with 50ml 10% Raftilose, and drying of the fibre composition by additional conveyance in coater for 5 minutes at 60°C.

This fibre composition contains 250 grams of psyllium, 0.25 grams of CaCl2, 2.5 grams of pectin and 5.0 grams of Raftilose, giving an end product with a composition of the substance by percentage as follows: 96.99% psyllium, 0.10% CaCl2, 0.97% pectin and 1.94% Raftilose, based on the final weight of the finished product.

For control of the ability of the particulate fibre composition to prevent liquid absorption, 5 grams of unprocessed fibres and 5 grams of the particulate fibre composition according to the invention respectively were transferred to centrifugal bottle containing 50 ml water and agitated for 60 seconds. Subsequent centrifugation at 1000 rpm for 120 seconds and measurement of supernatant. The test was repeated 5 times and showed significantly less absorption of liquid by the particulate fibre composition-up to 77%-compared with unprocessed fibres.
In addition, this fibre composition was applied for the stomach emptying test, shown in Fig. 3. A testee was given 15 grams of water and 15 grams of unprocessed citrus pulp respectively as well as 15 grams of the fibre composition according to the invention for comparison. The rate of stomach emptying was determined by means of 13C expiration test and the content of glucose in the blood was monitored.

The test shows that coating of citrus pulp with a pectin layer results in an increase of the half life period for stomach emptying (T/2) by approximately 60%. This fibre composition is rich in vitamins and well suited for prolongation of the sense of satiety. Furthermore, it will contribute to a reduction of the content of cholesterol and triglyceride in the blood.

Consequently, it is particularly suitable for reduction of the risk of arteriosclerosis, development of arteriosclerotic heart disease and cerebral haemorrhage.

Test 5:

Production of a particulate fibre composition according to the invention with one first dietary fibre of apple pulp and two additional fibre layers.

Conducted as Test 4, however with apple pulp in stead of citrus pulp.

This fibre composition was applied for the stomach emptying test, shown in Fig. 4. A testee was given 30 grams of water and 30 grams of unprocessed apple pulp respectively and for comparison 30 grams of the fibre composition according to the invention. The rate of stomach emptying was determined by means of 13C expiration test and the content of glucose in the blood was monitored.

This test reveals surprising new information and shows that apple pulp single-handedly reduces the rate of stomach emptying, and that coating of apple pulp with a pectin layer results in a reduction in the half life period for stomach emptying (T/2) by approximately 33%. Consequently, this fibre composition is suitable for treatment of persons with problems of too long passage time through the gastrointestinal tract as well as persons suffering from obstipation. Apple pulp is rich in C vitamins. In addition, it is easily fermentable and thus has a positive effect in colon due to stimulation of the generation of bifido bacteria.

Test 6:

Production of a particulate fibre composition according to the invention with one first dietary fibre and two additional fibre layers.

Conducted as Test 4, however with wheat bran in stead of citrus pulp.

This fibre composition was applied for the stomach emptying test, shown in Fig. 5. A testee was given 30 grams of water and 30 grams of unprocessed wheat bran respectively, and for comparison 30 grams of the fibre composition according to the invention. The rate of stomach emptying was determined by means of 13C expiration test and the content of glucose in the blood was monitored.

This fibre composition enables increase of the half life period for stomach emptying by approximately 50%. Consequently, this fibre composition is suitable for prolongation of e.g. the sense of satiety and is well suited as a dietary product as well as a health product.

Test 7:

Production of a particulate fibre composition according to the invention with two first dietary fibres and three additional fibre layers.
Dissolution of 1000 grams of psyllium in 1000ml boiling water and filtration through a filter with mesh size 0.05mm. Pouring of this compound over 1000 grams of oat bran and evaporation and grinding to particles of less than 0.02mm. Transfer to heated coater (rotary mill). Crosslinking of fibres by spraying with 50ml of a 5% CaCl2 solution for a 60-second period. Drying of fibres by conveyance in coater at 60°C for 5 minutes. Spraying of the dried crosslinked fibre composition with 100ml 3% pectin solution for 10 minutes. Subsequent drying of the fibre composition by conveyance in coater for additionally 5 minutes at 600C. Spraying of the pectin-coated fibres with 100ml 5% alginate solution which is insoluble at neutral pH value, and drying of the fibre composition by conveyance in coater for 5 minutes at 60°C. Final coating of the fibre composition with 20 grams of inulin in a 10% solution and drying of the fibre composition by conveyance in coater at 60°C for minutes.

This fibre composition contains 1000 grams of psyllium, 1000 grams of oat bran, 2.5 grams of CaCl2, 3.0 grams of pectin, 5.0 grams of alginate and 20 grams of inulin, giving an end product with a composition of the substance by percentage as follows: 49.25% psyllium, 49.25% oat bran, 0.12% CaCl2, 0.15% pectin, 0.25% alginate and 0.98% inulin, based on the final weight of the finished product.

The final product is sweet to the taste, is partially soluble in the stomach and contributes to prevention of the rate of stomach emptying by approximately 50%. Psyllium and oat bran is fermented in colon, and ingestion of the fibre composition results in reduced insulin response.

Test 8:

Production of a particulate fibre composition according to the invention with two first dietary fibres and three additional fibre layers as well as an outer coating of glucose.

Production of fibre composition as in Test 7, however with a glucose coating.

This fibre composition has a higher energy content of easily metabolisable monosaccharids, and the outer sweet-tasting layer is pleasant to the taste promptly.

Test 9:

Production of a simple fibre composition according to the invention with one first dietary fibre and one additional fibre layer.

Transfer of 100 grams of psyllium to coater and heating to 60°C.

Crosslinking of fibres by spraying with 10ml of a 1% CaC1, solution for a 60-second period. Drying of fibres by conveyance in coater at 600C for 5 minutes. Spraying of the dried crosslinked psyllium fibres with 25ml 5% pectin solution for 3 minutes. Subsequent drying of the fibre composition by conveyance in coater for additionally 5 minutes at 600C.

This fibre composition contains 100 grams of psyllium, 0.1 gram of CaC1, and 1.25 grams of pectin, giving an end product with a composition of the substance by percentage as follows: 98.67% psyllium, 0.10% CaCl2 and 1.23% pectin, based on the final weight of the finished product.

CLAIMS (ENGLISH)

Patent claims

1. A particulate fibre composition of the kind that comprises at least one first dietary fibre, coated by an insoluble dietary fibre or a dietary fibre with low solubility serving to prevent dissolution of the fibre composition in the oral cavity and during passage through the oesophagus, characterised by the insertion of
one or several layers of at least one second dietary fibre between the at least one first dietary fibre and the coating of the insoluble dietary fibre/dietary fibre of low solubility.

2. A particulate fibre composition according to claim 1, characterised by the first primary dietary fibre accounting for between 65 and 98% of the weight of the finished fibre composition.

3. A particulate fibre composition according to claim 1 or 2, characterised by at least one fibre of the particulate fibre composition having a therapeutic effect.

4. A particulate fibre composition according to claim 1, 2 or 3, characterised by the at least one first dietary fibre being a soluble, easily fermentable fibre.

5. A particulate fibre composition according to any of claims 1-4, characterised by the selection of the at least one first dietary fibre from the group of psyllium, citrus pulp, apple pulp, grape pulp, modified starch, wheat bran, oat bran, cellulose, gum arabic, alginate and pulp fibres from vegetables and fruits.

6. A particulate fibre composition according to any of claims 1-5, characterised by the selection of the at least one second dietary fibre from the group of pectin, guar gum, acacia gum, dextran, inulin, Raftilose, alginic acid, alginate, preferably K alginate or Ca alginate, or combinations of these.

7. A particulate fibre composition according to any of claims 1-6, characterised by the inclusion of additionally at least one additive to the fibre composition, selected from the group of fibres with anti-oxidizing properties, vitamins, colouring agents, flavouring agents or from the group of sweeteners with low calorie value, preferably from the group of inulin, neohesperidine and steviolglycosides.

8. A particulate fibre composition according to claim 8, characterised by the at least one additive being a layer of monosaccharides, oligosaccharides or proteins.

9. A particulate fibre composition according to claim 7 or 8, characterised by the at least one additive being added in a quantity of between 0.1% to 5% of the weight of the finished fibre composition.

10. A method for producing a particulate fibre composition of the kind that comprises at least one first dietary fibre surrounded by a coating of an insoluble dietary fibre or a dietary fibre with low solubility, serving to prevent dissolution of the fibre composition in the oral cavity and during passage through oesophagus, characterised by the method comprising one or several of the following steps:

dissolution/suspension of the at least one first dietary fibre in water, filtration of the fibre solution/suspension through a filter with fixed mesh size, evaporation of fibres, trituration of evaporated fibre mass to a particulate fibre matter, encapsulation or coating of the particulate fibre matter in a rotary mill by one or several layers of a second dietary fibre, and drying of the resultant particulate fibre composition.

11. A method according to claim 10, characterised by the inclusion of an initial step, namely ultrasound processing of the dietary fibres for a sufficient period to inactivate microbiological activity.

12. A method according to claim 10 or 11, characterised by the additional inclusion of a step for treatment of the evaporated fibres with a CaCl2 solution.

13. A method according to claim 10, 11 or 12, characterised by the additional inclusion of a step for adding of at least one sweetener exclusively or together with one or several additives.

14. Application of a particulate fibre composition according to any of claims 1-8, characterised by the application of the fibre composition for coating of cereals or for coating of food products produced of or containing cereals.
15. Application of a particulate fibre composition according to any of claims 1-8, characterised by the application of such quantity of the fibre composition, applied for coating of cereals or for coating of food products produced of or containing cereals, which will give the finished product a fibre content above 30% and especially above 40% of the weight of the finished particulate fibre composition.

16. Application of a particulate fibre composition according to claims 1-8, characterised by the application of the fibre composition as a dietary supplement.

17. A particulate fibre composition according to any of claims 1-8, characterised by the application of the fibre composition as a pharmaceutical.

18. A particulate fibre composition according to any of claims 1-8, characterised by the application of the fibre composition for adjustment of rate of stomach emptying.

19. Application of a particulate fibre composition according to claims 1-8 for the production of a pharmaceutical for treatment of insulin resistance, lipaemia, obstipation, overweight or infection in the gastrointestinal tract.

20. Application of a particulate fibre composition according to claims 1-8 for the production of a pharmaceutical for adjustment of uptake of calcium by the gastrointestinal tract or for adjustment of the intestinal transit time of food.

* * * * *
Part 3: Case B: Infringement (40% of marks)

Your client is active in the field of tissue bio-engineering. They have now found a production process for tissue that has the function of heart valves, which can be used to replace any malfunctioning heart valve in situ. The production process starts by seeding smooth muscle fibroblast cells that are derived from the patient (which is referred to as being ‘homologous’) on a scaffold, after which homologous endothelial cells are seeded thereupon. This scaffold is made of a biocompatible and biodegradable polymer such as PGA, PLA, polycaprolactone and/or the polyhydroxyalkanoate, P4HB, wherein the structure of the scaffold is obtained by electrospinning these materials. The tissue is then grown under steady flow-rate and pressure conditions in a bioreactor (pulsatile flow chamber), that makes use of bovine plasma as a source of nutrients, and the necessary growth factors. When the tissue has grown sufficiently, it is harvested and implanted into the patient. Once implanted, the extracellular matrix will continue to form as the scaffold gradually degrades.

Your client would like to sell the heart valves, as described above, in the United States and in various countries in Europe. The client has asked you to conduct a patent infringement/patent clearance search in order to identify any potential patent infringement risks. The results of your search are Documents 1-9. A copy of each has been attached. Which of these documents would you cite in a search report as potentially relevant from a patent infringement perspective and for what reason(s)?

There is no need to use Espacenet (or any other external database) to obtain status information for these documents. You may assume that all annuity fees for the patents and patent applications have been paid. All the information you need to answer the question is included herewith.

List of Documents:
Document 1- US20080233162
Document 2- US5192312
Document 3- US5855610
Document 4- EP1077072
Document 5- US6514515
Document 6- Extract from "Artificial Heart Valves"
Document 7- EP1339356
Document 8- WO2006/099334
Document 9- EP1878451
FIBROUS 3-DIMENSIONAL SCAFFOLD VIA ELECTROSPANNING FOR TISSUE REGENERATION AND METHOD FOR PREPARING THE SAME

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Appl. No.: 12/064,801
PCT Filed: Aug. 28, 2006
PCT No.: PCT/KR2006/003390
§ 371 (c)(1), (2), (4) Date: Feb. 25, 2008

Foreign Application Priority Data

Publication Classification
Int. Cl.
A61K 8/00 (2006.01)
C12N 5/06 (2006.01)
A61K 35/12 (2006.01)
A61P 45/00 (2006.01)

ABSTRACT
The present invention relates to a fibrous 3-dimensional porous scaffold via electrospinning for tissue regeneration and a method for preparing the same. The fibrous porous scaffold for tissue regeneration of the present invention characteristically has a biomimetic structure established by using electrospinning which is efficient without wasting materials and simple in handling techniques. The fibrous porous scaffold for tissue regeneration of the present invention has the size of between nanofiber and microfiber and regular form and strength, so that it facilitates 3-dimensional tissue regeneration and improves porosity at the same time with making the surface area contacting to a cell large. Therefore, the scaffold of the invention can be effectively used as a support for the cell adhesion, growth and regeneration.
1. A fibrous porous 3-dimensional scaffold for tissue regeneration comprising a polymer and/or a low molecular fiber, which is formed in a 3-dimensional network structure by electrospinning.

2. The fibrous porous 3-dimensional scaffold for tissue regeneration according to claim 1, wherein the polymer is one or more synthetic polymers selected from a group consisting of representative bio-degradable aliphatic polyesters such as polylactic acid (PLA), polyglycolic acid (PGA), poly(D,L-lactide-co-glycolide) (PLGA), poly(caprolactone), diol/diacid aliphatic polyester, polyester-amide/polyester-urethane, poly(valerolactone), poly(hydroxyl butyrate) and poly(hydroxyl valerate) or one or more natural polymers selected from a group consisting of chitosan, chitin, alginic acid, collagen, gelatin and hyaluronic acid.

3. The fibrous porous 3-dimensional scaffold for tissue regeneration according to claim 2, wherein the polylactic acid (PLA) is a low molecular and/or a polymer poly-L-lactic acid (PLLA).

4. The fibrous porous 3-dimensional scaffold for tissue regeneration according to claim 1, wherein the fiber is 1-15 < in diameter.

5. A method for preparing the fibrous porous 3-dimensional scaffold for tissue regeneration of claim 1 by using electrospinning.

6. The method for preparing the fibrous porous 3-dimensional scaffold for tissue regeneration using electrospinning according to claim 5, which comprises the following steps:
   (i) preparing a spinning solution by dissolving a polymer and/or a low-molecular compound singly or together in an organic solvent; and
   (ii) spinning the polymer solution by using an electro-spinner and volatilizing the organic solvent at the same time to form a 3-dimensional network structure.

7. The method for preparing the scaffold for tissue regeneration according to claim 5, which additionally includes the step of molding the fiber to fit defective area.

8. The method for preparing the scaffold for tissue regeneration according to claim 5, wherein the polymer and/or low molecular compound is PLLA.

9. The method for preparing scaffold for tissue regeneration according to claim 5, wherein the organic solvent is one or more compounds selected from a group consisting of chloroform, dichloromethane, dimethylformamide, dioxane, acetone, tetrahydrofurane, trifluoroethane and hexafluoroisopropylpropanol.

10. The method for preparing the scaffold for tissue regeneration according to claim 9, wherein the organic solvent is a mixture of dichloromethane and propylpropanol or a mixture of dichloromethane and acetone.

11. The method for preparing the scaffold for tissue regeneration according to claim 5, wherein the organic solvent has a boiling point of 0-40[deg.] C. and a viscosity of 25-35 cps.

12. The method for preparing the scaffold for tissue regeneration according to claim 5, wherein the polymer and low molecular compounds are dissolved in 5-20 weight % organic solvent to prepare a spinning solution.

13. The method for preparing the scaffold for tissue regeneration according to claim 5, wherein the step (ii) is carried out under the following conditions; temperature: 15-25[deg.] C., humidity: 10 40%, spinning distance: 10-20 cm, voltage: 10-20 kV, releasing speed: 0.050 < 0.150 ml/min and the internal diameter of the syringe: 0.5-1.2 mm.

14. An implantation material for cell adhesion, growth and regeneration comprising the fibrous porous 3-dimensional scaffold for tissue regeneration of claim 1.

15. The implantation material for cell adhesion, growth and regeneration according to claim 14, wherein the cell is cartilage cell, endothelial cell, skin cell, osteocyte, bone cell or stem cell.
Mock Certification Exam for Patent Information Professionals 2011

Document 2 (for Part 3 of exam)

**United States Patent [19]**

**Orton**

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**TREATED TISSUE FOR IMPLANTATION AND METHODS OF TREATMENT AND USE**

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**Inventor:** E. Christopher Orton, Fort Collins, Colo.

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**Assignment:** Colorado State University Research Foundation, Fort Collins, Colo.

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**Appl. No.:** 664,902

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**Filed:** Mar. 5, 1990

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**Int. Cl.** A61F 2/24; A61F 2/02; A61F 2/54; A01N 1/02

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**U.S. Cl.** 623/2; 623/11; 623/66; 627/2

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**Field of Search** 623/66, 623/1, 627/2

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**Attorney, Agent, or Firm—Kenyon & Kenyon**

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**ABSTRACT**

Tissue which is suitable for implantation for treatment with a growth factor and cells which populate the tissue and native cells must be removed, they cannot be “mashed” reduce immunogenicity; this increases the longevity of the tissue upon transplant. The preferred growth factor is basic fibroblast growth factor, and the preferred cells are fibroblasts. The tissue can be an allograft or xenograft taken from a cow, pig or other mammal.

24 Claims, 2 Drawing Sheets
Document 2 (for Part 3 of exam)
Claims for US 5,192,312 (only independent claims are shown)

1. An implantable human heart valve treated with growth factor effective on fibroblast cells and populated with fibroblast cells in an amount and for a time period effective for rendering the heart valve substantially non-immunogenic upon implant into a mammal.

2. An implantable non-human mammalian heart valve treated with growth factor effective on fibroblast cells and populated with fibroblast cells in an amount and for a time period effective for rendering the heart valve substantially non-immunogenic upon implant into a mammal.

11. A method of reducing the immunogenicity or improving the longevity of an implantable mammalian heart valve comprising: treating the heart valve with growth factor effective on fibroblast cells, and populating the heart valve with fibroblasts in an amount effective for reducing the immunogenicity of the heart valve upon implant into a patient.

18. A method of reducing transplant tissue rejection of an implantable mammalian heart valve comprising: treating the heart valve with growth factor effective on fibroblast cells, and populating the treated heart valve with fibroblasts to reduce tissue rejection upon transplant into a patient.
Mock Certification Exam for Patent Information Professionals 2011

United States Patent

Vacanti et al.

INVENTORS: Joseph P. Vacanti, Winchester; Christopher K. Breuer, Brighton; Beverly E. Chiu, Tufts; Shin-Isah, both of Brookline, all of Mass.

ASSIGNEE: Children's Medical Center Corporation, Boston, Mass.

Filed: May 19, 1995

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Primary Examiner—David J. Isabella

Attorneys, Agent, or Firm—Amick, Gokas & Gregory, LLP

ABSTRACT

It has been discovered that improved yields of engineered tissue following implantation, and engineered tissue having enhanced mechanical strength and flexibility or pliability, can be obtained by implantation, preferably subcutaneously, of a fibrous polymeric matrix for a period of time sufficient to obtain ingrowth of fibrous tissue and/or blood vessels, which is then removed after completion of the site where the implant is desired. The matrix is optionally seeded prior to the first implantation, after ingrowth of the fibrous tissue, or at the time of reimplantation. The time required for fibrous ingrowth typically ranges from days to weeks. The method is particularly useful in making valves and tubular structures, especially heart valves and blood vessels.

9 Claims, No Drawings
Document 3 (for Part 3 of the exam)
Claims for US 5,855,610

1. A cell-matrix structure comprising a fibrous matrix formed of a biocompatible, biodegradable synthetic polymer, and seeded with dissociated human cells, wherein the matrix is configured to form a tissue structure having mechanical strength and flexibility or pliability, wherein the cell-matrix structure is formed by seeding the matrix, implanting the seeded matrix into a recipient human or animal for a period of time sufficient to form extracellular matrix; and harvesting of the resulting cell-matrix structure.

2. The cell-matrix structure of claim 1 wherein the matrix is configured to form a tube.

3. The cell-matrix structure of claim 1 wherein the matrix is configured to form a valve in a blood vessel, intestine, or heart.

4. The cell-matrix structure of claim 3 wherein the matrix is configured to form a heart valve.

5. The cell-matrix structure of claim 1 wherein the cells are selected from the group of consisting of parenchymal and connective tissue cells.

6. A tissue-engineered heart valve formed of a porous polymeric matrix seeded with dissociated endothelial and fibroblast cells, wherein the cells form extracellular matrix following implantation into a human or animal recipient, and wherein the extracellular matrix is shaped to form a heart valve.

7. The heart valve of claim 6 wherein the matrix is formed of a polymer selected from the group consisting of poly(lactic acid), poly(glycolic acid), and combinations thereof.

8. The heart valve of claim 7 wherein the matrix is formed of polymer fibers having an interstitial spacing of between 100 and 300 microns and having pore sizes and structure to control the pattern and extent of fibroblastic tissue ingrowth following implantation.

9. The heart valve of claim 7 wherein the matrix was seeded with dissociated cells selected from the group consisting of fibroblasts, myofibroblasts, and endothelial cells and includes elastin fibers.
Mock Certification Exam for Patent Information Professionals 2011

Document 4 (for Part 3 of the exam)

EUROPEISCHES PATENTAMT
European Patent Office
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KORRIGIERTE EUROPÄISCHE PATENTSCHRIFT

Hinweis: Bibliographie entspricht dem neuesten Stand

Korrigierte Ausgabe Nr. 1 (W1 B1)

INID code(s): 72

Corrigendum ausgegeben am:
14.06.2004 Patentblatt 2004/16

Veröffentlichungstag und Bekanntmachung des
Hinweises auf die Patenterteilung:
12.11.2003 Patentblatt 2003/46

Anmeldenummer: 00108986.1

Anmeldestag: 27.04.2000

(54) In vitro-Verfahren zum Herstellen einer homologen Herzklappen- oder Gefäßprothese
Procédé de préparation in vitro de prothèse de valve cardiaque ou de vaisseaux

(84) Benannte Vertragsstaaten:
AT BE CH CY DE DK ES FI FR GB GR IE IT LU LU MC NL PT SE

(50) Priorität: 29.04.1999 DE 19919625

(43) Veröffentlichungstag der Anmeldung:
21.02.2001 Patentblatt 2001/08

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XP000993059


Bemerkungen:
Die Akte enthält technische Angaben, die nach dem Eingang der Anmeldung eingereicht wurden und die nicht in dieser Patentschrift enthalten sind.

Document 4 (for Part 3 of the exam)
Claims for EP 1 077 072

In vitro process for the production of a homologous heart valve prosthesis or vessel prosthesis for implantation in human patients, comprising the following steps:

- provision of a biodegradable carrier,
- colonization of the carrier with homologous fibroblasts and/or myofibroblasts to develop a connective tissue matrix,
- colonization of the carrier/matrix with endothelial cells,
- introduction of the carrier/matrix into a pulsatile flow chamber in which it can be exposed to increasing flow rates and/or pressure, and
- continuous or discontinuous increasing of the flow rate from an initial value of 50-100 ml/min up to a final value of 2,000-5,000 ml/min and/or of the pressure to 120-240 mm/Hg.

2. Process according to claim 1, characterized in that the biodegradable carrier is a biodegradable polymer matrix or an acellular biological matrix.

3. Process according to one of claims 1 or 2, characterized in that the carrier comprises a polyglycolic acid (PGA), polylactic acid (PLA), polyhydroxyalkanoate (PHA), poly-4-hydroxybutyrate (P4HB) or a mixture of two or more of these polymers.

4. Process according to one of claims 1 to 3, characterized in that the biodegradable carrier is preformed in the form of a heart valve.

5. Process according to one of claims 1 to 3, characterized in that the biodegradable carrier is preformed in the form of a blood vessel.

6. Process according to one of claims 1 to 4, characterized in that the carrier has a polymer density of 40 to 120 mg/cm³, preferably 50 to 80 mg/cm³.

7. Process according to one of claims 1 to 6, characterized in that the carrier is a porous polymer with a pore size of 80 to 240 µm.

8. Process according to one of claims 1 to 7, characterized in that the fibres of the carrier have a diameter of 6 to 20 µm, preferably 10 to 18 µm.

9. Process according to one of claims 1 or 2, characterized in that the carrier is a connective tissue skeleton of an animal heart valve.

10. Process according to one of claims 1 or 2, characterized in that the carrier is a connective tissue skeleton of an animal vessel.

11. Process according to one of claims 1 to 10, characterized in that the step of colonization with fibroblasts and/or myofibroblasts is repeated 3 to 14 times, preferably 5 to 10 times.

12. Process according to one of claims 1 to 11, characterized in that approx. 10⁵ to 5 x 10⁸, preferably 4-5 x 10⁸ fibroblasts and/or myofibroblasts are employed per square centimetre of carrier/matrix and colonization step.

13. Process according to one of claims 1 to 12, characterized in that the step of colonization with endothelial cells is repeated 3 to 14 times, preferably 5 to 10 times.
Document 4 (for Part 3 of the exam)
Claims for EP 1 077 072

14. Process according to one of claims 1 to 11, characterized in that approx. $10^5$ to $5 \times 10^6$, preferably $4 \times 5 \times 10^6$ endothelial cells are employed per square centimetre of carrier/matrix and colonization step.

15. Process according to one of claims 1 to 14, characterized in that the fibroblasts and/or myofibroblasts and/or endothelial cells are human cells.

16. Process according to one of claims 1 to 15, characterized in that the fibroblasts and/or myofibroblasts and/or endothelial cells are autologous cells.

17. Process according to one of claims 1 to 16, characterized in that flow rates of 50 ml/min to 5,000 ml/min, preferably 50 to 2,000 ml/min, are established in the pulsatile flow chamber.

18. Process according to one of claims 1 to 17, characterized in that the flow rate is increased over a period of time of 1 week to 12 weeks.

19. Process according to one of claims 1 to 18, characterized in that the initial flow rate is 50 to 100 ml/min.

20. Process according to one of claims 1 to 19, characterized in that the initial pulse frequency is 5 to 10 pulses/min.

21. Process according to one of claims 1 to 19, characterized in that the flow rate is increased up to max. 5,000 ml/min for vessel prostheses and for heart valve prostheses.

22. Process according to one of claims 1 to 20, characterized in that the pulse frequency is increased up to max. 180 pulses/min for vessel prostheses and for heart valve prostheses.

23. Process according to one of claims 1 to 17, characterized in that systemic pressures of 10 to 240 mm Hg are established in the pulsatile flow chamber.

24. Homologous heart valve which can be produced by the process according to one of claims 1 to 23.

25. Homologous heart valve according to claim 24 for implantation in a human patient, wherein the homologous heart valve has a connective tissue inner core surrounded by an endothelial cell layer, and a collagen density of 43 to 55% exists in the connective tissue inner core.

26. Homologous heart valve according to claim 24, characterized in that it withstands the flow conditions in the human heart.

27. Homologous vessel which can be produced with the process according to one of claims 1 to 23.
Mock Certification Exam for Patent Information Professionals 2011

Document 5 (for Part 3 of the exam)

(12) United States Patent

Williams

(10) Patent No.: US 6,514,515 B1

(45) Date of Patent: Feb. 4, 2003

(54) BIOABSORBABLE, BIOCOMPATIBLE POLYMERS FOR TISSUE ENGINEERING

(75) Inventor: Simon F. Williams, Sherborn, MA (US)

(73) Assignee: Topha, Inc., Cambridge, MA (US)

( ) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: 09/518,123

(22) Filed: Mar. 3, 2000

Related U.S. Application Data

(60) Provisional application No. 60/122,827, filed on Mar. 4, 1999

(51) Int. Cl.7 ... A61K 2/02, A61K 8/00

(52) U.S. Cl. ... 424/434, 424/429, 424/500, 424/502

(58) Field of Search ... 424/424, 425, 424/500, 502

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(74) Attorney, Agent, or Firm—Holland & Knight LLP

(57) ABSTRACT

Bioabsorbable biocompatible polymers which provide a good match between their properties and those of certain tissue structures are provided. The bioabsorbable biocompatible polymers can be prepared with tensile strengths, elongation to breaks and/or tensile moduli (Young's moduli) values of the tissues of the cardiovascular, gastrointestinal, kidney and genitourinary, musculoskeletal, and nervous systems, as well as those of the oral, dental, periodontal, and skin tissues. Methods for processing the bioabsorbable biocompatible polymers into tissue engineering devices are also provided.

39 Claims, 2 Drawing Sheets

Tensile Strength, psi:

<table>
<thead>
<tr>
<th>TENDON</th>
<th>CARTILAGE</th>
<th>SKIN/ENAMEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>5000</td>
<td>10000</td>
</tr>
<tr>
<td>STRONG</td>
<td>ELASTIC</td>
<td>SKIN/ENAMEL</td>
</tr>
</tbody>
</table>

Elongation, %:

<table>
<thead>
<tr>
<th>TENDON</th>
<th>CARTILAGE</th>
<th>MUSCLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>ELASTIC</td>
<td>CARTILAGE</td>
<td>MUSCLE</td>
</tr>
</tbody>
</table>

(List continued on next page.)
Claims for US 6,514,515

1. A composition or device for use in tissue engineering comprising a bioabsorbable biocompatible polymer comprising polyhydroxyalkanoate, wherein the composition or device has one or more mechanical properties selected from the group consisting of stress, strain, stress-strain, stress-strain hysteresis, stress-strain relaxation, viscoelasticity, contraction stress, resting stress, Young's modulus, tensile strength, durability, yield point, failure strength, toughness, ductility, softness, hardness, creep, elastic deformation, wear resistance, shear failure, roughness, compressive strength, load capacity, modulus of elasticity, ultimate compressive strength, yield strength, stress-strain relationship, scratch resistance, abrasion resistance, flexural modulus, shear modulus, contact angle, surface tension, adhesive strength, surface free energy, bending strength, shear strength, bonding strength, bending strength, bending stiffness, compressive modulus, bending modulus, fracture toughness, elongation, fiber strength, fiber modulus, fiber elongation, thermal expansion coefficient, fracture toughness, static and dynamic elasticity, longitudinal stretch, radial stretch, stress and strain, circumferential stretch, ultimate elongation, viscosity, expansion, static and kinetic coefficients of friction, plasticity, axial tension, shock absorbance, bearing strength, formability, rigidity, stress rupture, bend radius, impact strength, and fatigue strength, equivalent to the same properties of a differentiated tissue or tissue structure.

2. The composition of claim 1 wherein the polymer degrades in vivo in less than one year.

3. The composition of claim 1 wherein the polymer has an extension to break of over 25%.

4. The composition of claim 3 wherein the polymer is in the form of a fiber and the extension to break is over 45%.

5. The composition of claim 1 wherein the polymer has a tensile strength less than 10,000 psi.

6. The polymer of claim 5 wherein the polymer is in the form of a fiber and the tensile strength is less than 50,000 psi.

7. The composition of claim 1 wherein the polymer has a Young's modulus of less than 100,000 psi.

8. The polymer of claim 7 wherein the polymer is in the form of a fiber and the Young's modulus is less than 200,000 psi.

9. The composition of claim 1 wherein the polymer has a melting temperature less than 190[deg.] C.

10. The composition of claim 1 wherein the polymer has a glass transition temperature less than 20[deg.] C.

11. The composition of claim 1 wherein the polymer has two or more properties selected from the group consisting of extension to break over 25%, tensile strength less than 10,000 psi, Young's modulus less than 100,000 psi, glass transition less than 20[deg.] C., and melting temperature less than 190[deg.] C.

12. The composition of claim 1 wherein the tissue is selected from the group consisting of cardiovascular, gastrointestinal, kidney, genitourinary, musculoskeletal, nervous, oral, breast, periodontal, and skin.

13. The composition of claim 1 wherein the mechanical property is selected from the group consisting of tensile strength, Young's modulus, elongation to break, hardness, compressive strength, burst strength, toughness, and impact strength.

14. The composition of claim 1 wherein the tissue is cartilage and the polymer has a tensile strength of 435 psi+25%.

15. The composition of claim 1 wherein the tissue is skin and the polymer has a tensile strength of 1,100 psi+25%.
Document 5 (for Part 3 of the exam)
Claims for US 6,514,515

16. The composition of claim 1 wherein the tissue is tendon and the polymer has a tensile strength of 7,700 psi+25%.

17. The composition of claim 1 wherein the tissue is aorta and the polymer has a tensile strength of 160 psi+25%.

18. The composition of claim 1 wherein the tissue is cardiac muscle and the polymer has a tensile strength of 16 psi+25%.

19. The composition of claim 1 wherein the tissue is bone and a polymer has a tensile strength of 10,000 psi+25%.

20. The composition of claim 1 wherein the tissue is enamel and the polymer has a tensile strength of 1,600 psi+25%.

21. The composition of claim 1 wherein the tissue is skin and the polymer has an ultimate elongation of 78%+25%.

22. The composition of claim 1 wherein the tissue is tendon and the polymer has an ultimate elongation of 10%+25%.

23. The composition of claim 1 wherein the tissue is cartilage and polymer has an ultimate elongation of 30%+25%.

24. The composition of claim 1 wherein the tissue is heart and the polymer has an ultimate elongation of 10-15%+25%.

25. The composition of claim 1 wherein the tissue is aorta and the polymer has an ultimate elongation in the transverse and longitudinal directions of 77-81%+25%.

26. The composition of claim 1 wherein the tissue is skin and the polymer has a Young's modulus of 2,000-18,000 psi+25%.

27. A device comprising a bioabsorbable biocompatible polyhydroxyalkanoate polymer, wherein the device has one or more mechanical properties equivalent to a specific tissue or tissue structure, wherein the device is selected from the group consisting of a tissue engineering scaffold, guided tissue repair material, wound dressing, drug delivery vehicle, anti-adhesion material, cell encapsulation material, coating, implant, stent, orthopaedic device, prosthetic, adhesive, diagnostic, sutures, surgical meshes, staples, meniscus repair and regeneration devices, screws (interference screws and meniscal screws), bone plates and plating systems, cardiovascular patches, pericardial patches, slings, pins, anti-adhesion barriers, articular cartilage repair devices, nerve guides, tendon and ligament repair devices, atrial septal defect patches, bulking and filling agents, vein valves, bone marrow scaffolds, bone graft scaffolds, skin substitutes, dural substitutes, ocular implants, spinal fusion cages, and muscular implants (cardiac and skeletal), wherein the mechanical properties are selected from the group consisting of stress, strain, stress-strain, stress-strain hysteresis, stress-strain relaxation, viscoelasticity, contraction stress, resting stress, Young's modulus, tensile strength, durability, yield point, failure strength, toughness, ductility, softness, hardness, creep, elastic deformation, wear resistance, shear failure, roughness, compressive strength, load capacity, modulus of elasticity, ultimate compressive strength, yield strength, stress-strain relationship, scratch resistance, abrasion resistance, flexural modulus, shear modulus, contact angles, surface tension, adhesive strength, surface free energy, bending strength, shear strength, bonding strength, bending strength, bonding stiffness, compressive modulus, bending modulus, fracture toughness, elongation, fiber strength, fiber modulus, fiber elongation, thermal expansion coefficient, fracture toughness, static and dynamic elasticity, longitudinal stretch, radial
Document 5 (for Part 3 of the exam)
Claims for US 6,514,515

stretch, stress and strain, circumferential stretch, ultimate elongation, viscosity, expansion, static and kinetic coefficients of friction, plasticity, axial tension, shock absorbance, bearing strength, formability, rigidity, stress rupture, bend radius, impact strength, and fatigue strength.

28. The device of claim 27 wherein the device is a tissue engineering scaffold or matrix.

29. The device of claim 28 wherein the polymer degrades in vivo in less than two years.

30. The device of claim 28 wherein the tissue engineering scaffold which has different properties in different regions.

31. The device of claim 28 wherein the scaffold or matrix is flexible.

32. The device of claim 28 wherein the tissue is heart valve or blood vessel.

33. The device of claim 28 wherein the tissue engineering scaffold or matrix is for tissue engineering of musculoskeletal tissue.

34. The device of claim 28 wherein the tissue is selected from the group consisting of cartilage, tendon, ligament, and bone.

35. The device of claim 28 wherein the tissue engineering scaffold or matrix is for tissue engineering of genitourinary tissue.

36. The device of claim 28 wherein the tissue forms a structure selected from the group consisting of bladder, ureter, and urethra.

37. The device of claim 28 for tissue engineering of gingiva.

38. The device of claim 28 seeded with cells for implantation.

39. The device of claim 28 further comprising materials selected from the group consisting of other polymers, compounds, additives, biologically active substances, growth factors, cell attachment factors, and drugs.
7. REGENERATIVE MEDICINE APPROACHES TO HEART VALVE REPLACEMENT

The field of regenerative medicine is based on the innovative and visionary principle of using the patient's own cells and extracellular matrix components to restore or replace tissues and organs that have failed. This regenerative approach is a derivative of reconstructive surgery, where surgeons use the patient's tissues to rebuild injured or aging body parts. Modern approaches to heart valve regenerative medicine include several research methodologies, collectively known as tissue engineering. The most intensely researched approaches are (1) the use of decellularized tissues as scaffolds for in situ regeneration, (2) construction of tissue equivalents in the laboratory before implantation, and (3) use of scaffolds preseeded with stem cells.

A widespread approach is to use native (uncrosslinked) decellularized valves obtained from processed human or animal tissues. Once implanted, decellularized tissues are expected to provide proper environment and sufficient stimuli for host cells to infiltrate, remodel, and eventually regenerate the valvular tissue. As antigens, determinants are mainly concentrated on the surface of cells, removal of the original cells (decellularization) is necessary for avoiding complications related to immune rejection, which can be satisfactorily attained using combinations of detergents and enzymes, leaving behind 3-D scaffolds comprising apparently intact matrix molecules. Experimental data obtained with decellularized porcine valves yielded spectacular results (35). In vitro hydrodynamic performance was excellent, and valves performed well after implantation in sheep for 5 months as replacement of pulmonary valves. Explanted valves showed good repopulation of porcine scaffolds with fibroblast-like cells as well as an almost complete re-endothelialization of valve surfaces.

These encouraging studies prompted several small scale studies in humans using a commercially available decellularized porcine heart valve. However, despite the initial enthusiasm, the results of a small study in children were catastrophic. In a breakthrough and intrepid study, a group of cardiac surgeons from Austria reported that three out of four children implanted with decellularized porcine heart valves had died within 1 year after implantation because of tissue rupture, degeneration, and calcification of the implant (36). Analysis of explanted decellularized porcine heart valves revealed lack of cell repopulation or endothelialization. The collagen matrix induced a severe inflammatory response and encapsulation of the graft and also served as a substrate for development of numerous calcification sites. The same group of researchers also recently identified the presence of the porcine cell-specific disaccharide galactose-alpha-1-3-galactose (alpha-Gal epitope) in decellularized porcine heart valves as the possible source of immunogenicity (37). Similarly, decellularized blood vessels also elicited inflammatory responses (38) and failed to maintain patency (39).

Evidently, more basic studies are required for further development of these products as well as to reevaluate the relevance of animal models. Several academic groups, as well as a group of medical device companies, continue to pursue research and development of decellularized cardiovascular tissues (40–42). A second approach involves construction of tissue equivalents in the laboratory prior to implantation, with the expectation that assembling the tissue-engineered valvular constructs from appropriate cells, and synthetic or natural matrix components, would create mechanically competent, nonthrombogenic, living tissues capable of adaptation and growth. Compared with decellularized tissues, this approach provides better control of the device properties before implantation. However, because of the enormity of this task, and because of possible early clinical failures of decellularized tissues, researchers in the field of heart valve tissue regeneration have taken a cautious, stepwise approach. The in vitro assembly of heart valve tissue from its individual components was pioneered by I. Vesely et al. (43,44). In this ingenious approach, a chemically crosslinked nonbiodegradable glycosaminoglycan gel was seeded with vascular cells and cultured in vitro, which resulted in the formation of a thin sheet of elastin at the interface between cells and the glycosaminoglycan gel. The collagen structural component was created by fibroblast-mediated compaction of soluble collagen, with the expectation that in vitro assembly of these building blocks would, at some point, create a valve-like structure. For more details, please refer to the chapter on Tissue Engineering of Heart Valves in this Encyclopedia.
In an exemplary collaborative effort, clinicians, basic scientists, polymer chemists, and biomedical engineers focused on creating functional tissue-engineered heart valves in vitro using biodegradable scaffolds (45). These efforts included scaffold design and characterization, optimization of cell sources, finding adequate mechanisms for cell delivery, and optimizing in vitro culture of the seeded scaffolds, culminating with the surgical replacement of heart valves in sheep. A wide variety of scaffolding models created from biodegradable polymers have been tested for heart valve tissue engineering, including polyglycolic acid, polylactic acid, polycaprolactone, and biodegradable elastomers, which are manufactured into nonwoven textiles in shapes and conformations that mimic the natural architecture of the heart valve. These scaffolds are then seeded with cells that can be obtained from (1) fully differentiated cells such as myofibroblasts and endothelial cells derived from systemic arteries, or (2) pluripotent stem cells derived from adipose tissue, bone marrow, or peripheral blood (46). As mature cells have a limited lifespan, an attractive cell source was stem cells for heart valve tissue engineering. Recently, in a landmark experiment, mesenchymal stem cells obtained from ovine bone marrow were seeded onto biodegradable scaffolds and the constructs were cultured in vitro before implantation as a valve in the pulmonary position of sheep (47) for up to 8 months. Tissue-engineered valves performed well hemodynamically, with signs of slow degradation of the scaffolds, and concomitant deposition of new extracellular matrix. Moreover, cell types resembled those present in natural heart valves. Overall, these exciting results hold great promise for truly effective regenerative approaches to treatment of heart valve disease.
EUROPEAN PATENT SPECIFICATION

EP 1 339 356 B1

19 European Patent Office
21 Application number: 01992527.0
22 Date of filing: 30.10.2001

C17 A61F 2/06, A61F 2/36, A61L 27/00
51 Int Cl7:

56 References cited:
WO-A-99/15237

54 TISSUE-ENGINEERED VASCULAR STRUCTURES
AUS GEWEBE HERGESTELLTE GEFÄSSSTRUKTUREN
STRUCTURES VASCULAIRES DE SYNTHÈSE

84 Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE TR

85 Priority: 30.10.2000 US 244277 P
86 International application number:
PCT/US2001/048946

87 International publication number:
WO 2002/030592 (10.06.2002 Gazette 2002/19)

30 Priority: 30.10.2000 US 244277 P
36 Date of publication of application:
03.09.2003 Bulletin 2003/36

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1. A tissue-engineered vascular construct comprising a scaffold configured to form said construct and seeded with endothelial progenitor cells and collagen cells capable of forming vascular tissue, said scaffold comprising a biodegradable material.

2. The tissue-engineered vascular construct of claim 1, wherein the scaffold is configured to form a tube.

3. The tissue-engineered vascular construct of claim 1, wherein the scaffold is configured to form a trileaflet heart valve.

4. A method for making a tissue-engineered vascular construct comprising the steps of: (a) providing a substrate shaped to form the vascular construct, said substrate comprising a biodegradable material; (b) contacting said substrate with endothelial progenitor cells and collagen cells capable of adhering thereto and forming vascular tissue, thereby forming a primary cell-seeded construct; (c) maintaining said primary cell-seeded construct for a first growth period in a fluid media suitable for growth of said cells and imparting stresses in the construct during said first growth phase to stimulate the physiological conditions to be encountered by the construct once implanted to form the vascular construct.

5. The method of claim 4, wherein the step of imparting stresses comprises cyclical increases in pressure and flow within said construct.

6. The method of claim 4, wherein step of imparting stresses comprises a gradual increase in shear stress.

7. The method of claim 4, wherein said biodegradable material is a polymer.

8. A tissue-engineered trileaflet heart valve produced by the method of claim 4, 5, or 6.


10. A tissue-engineered two-dimensional construct produced by the method of claim 4, 5, or 6.
Title: PRODUCTION OF TISSUE ENGINEERED HEART VALVES

Abstract: The invention is directed to methods for preparing artificial heart valves by preconditioning a matrix seeded with endothelial cells and smooth muscle cells differentiated from induced progenitor cells. These cell seeded matrices are exposed to fluid conditions that mimic blood flow through the heart to produce tissue engineered heart valves that are analogous to native heart valves.
Document 8 (for Part 3 of the Exam)
Claims for WO2006/099334 (only independent claims are shown)

1. A method for producing a preconditioned heart valve, comprising: obtaining a population of endothelial cells differentiated from progenitor cells; seeding a matrix having a heart valve shape with the endothelial cells such the cells attach to the matrix to form an endothelial cell layer; obtaining a population of smooth muscle cells differentiated from progenitor cells; depositing the smooth muscle cells onto the endothelial cell layer such that the smooth muscle cells form a smooth muscle cell layer that joins to the endothelial cell layer; attaching seeded matrix to an attachment element in a preconditioning chamber, wherein the attachment element has a channel that is fluidly coupled to fluid flow system; and preconditioning the seeded matrix with the flow system in which a biological fluid is moved through the seeded matrix, wherein the flow-rate and pulse-rate of the biological fluid is controlled such that a preconditioned heart valve is produced.

25. A preconditioned tissue engineered heart valve, comprising: a biocompatible matrix having a heart valve shape seeded with a population of endothelial cells differentiated from progenitor cells, wherein the endothelial cells attach to the matrix to form an endothelial cell layer; a population of smooth muscle cells differentiated from progenitor cells seeded onto the endothelial cell layer, wherein the smooth muscle cells form a smooth muscle cell layer that attaches to the endothelial cell layer; and wherein the seeded cells have been preconditioned in a preconditioning chamber that exposes the seeded cells to a biological fluid that is passed through the seeded matrix at a flow-rate and pulse-rate equivalent to normal blood flow through the heart.
(54) Bioabsorbable, biocompatible polymers for tissue engineering

Bioabsorbable biocompatible polymers which provide a good match between their properties and those of certain tissue structures are provided. The bioabsorbable biocompatible polymers can be prepared with tensile strength, elongation to breaks, and/or tensile modulus (Young’s modulus) values of the tissues of the cardiovascular, gastrointestinal, kidney and genitourinary, musculoskeletal, and nervous systems, as well as those of the oral, dental, periodontal, and skin tissues. Methods for processing the bioabsorbable biocompatible polymers into tissues engineering devices are also provided.

**FIG. 1**

![Tensile Strength, psi](image)

<table>
<thead>
<tr>
<th>Tensile Strength, psi</th>
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<tbody>
<tr>
<td>AORTA</td>
</tr>
<tr>
<td>Weak</td>
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<table>
<thead>
<tr>
<th>Elongation, %</th>
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<tbody>
<tr>
<td>ELASTIC</td>
</tr>
<tr>
<td>TENDON</td>
</tr>
<tr>
<td>PLAG (PGA)</td>
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</table>

**(11)** EP 1 878 451 A1

**European Patent Application**

**Document 9 (for Part 3 of the exam)***
Claims

1. A device for use in tissue engineering selected from the group consisting of a meniscus repair and regeneration device, an articular cartilage repair device, a tendon or ligament repair device and a bone graft scaffold, the device comprising a bioabsorbable biocompatible polymer comprising polyhydroxyalkanoate, wherein the polymer has two or more mechanical properties selected from the group consisting of an extension to break of over 25%, a tensile strength of less than 10,000 psi (or, where the device is a bone graft scaffold, a tensile strength of 10,000 psi ± 25%), and a Young's modulus of less than 100,000 psi, and wherein the two or more of the extension to break, the tensile strength and the Young's modulus of the device and/or polymer are equivalent to the corresponding mechanical properties of cartilage where the device is a meniscus repair and regeneration device or an articular cartilage repair device, of tendon where the device is a tendon repair device, of ligament where the device is a ligament repair device, or of bone where the device is a bone graft scaffold.

2. The device of any preceding claim wherein the polymer has an extension to break of over 25%.

3. The device of Claim 2 wherein the polymer is in the form of a fibre and the extension to break is over 45%.

4. The device of any preceding claim wherein the polymer has a tensile strength of less than 10,000 psi.

5. The device of Claim 4 wherein the polymer is in the form of a fibre and the tensile strength is less than 50,000 psi.

6. The device of any preceding claim wherein the polymer has a Young's modulus of less than 100,000 psi.

7. The device of Claim 6 wherein the polymer is in the form of a fibre and the Young's modulus is less than 200,000 psi.

8. The device of Claim 1 wherein the device is a meniscus repair and regeneration device.

9. The device of Claim 1 wherein the device is an articular cartilage repair device.

10. The device of Claims 8 or 9 wherein the polymer has a tensile strength of 435 psi ± 25% and/or an ultimate elongation of 30% ± 25%.

11. The device of Claim 1 wherein the device is a tendon repair device.

12. The device of Claim 11 wherein the polymer has a tensile strength of 7,700 psi ± 25% and/or an ultimate elongation of 10% ± 25%.

13. The device of Claim 1 wherein the device is a ligament repair device.

14. The device of Claim 1 wherein the device is a bone graft scaffold.
Document 9 (for Part 3 of the exam)

Claims for EP 1 878 451 as currently pending:

15. The device of Claim 14 wherein the polymer has a tensile strength of 10,000 psi ± 25%.

16. The device of any preceding claim wherein the polymer degrades in vivo in less than two years.

17. The device of any preceding claim wherein the polymer degrades in vivo in less than one year.

18. The device of any preceding claim wherein the polymer has a melting temperature of less than 190°C.

19. The device of any preceding claim wherein the polymer has a glass transition temperature less than 20 °C.

20. The device of any preceding claim which is seeded with cells for implantation.

21. The device of any preceding claim further comprising materials selected from the group consisting of other polymers, compounds, additives, biologically active substances, growth factors, cell attachment factors, and drugs.

***** END of Exam*****