The present invention is about a pre-fermented symbiotic matrix based on a cereal suspension, preferably oat, containing encapsulated probiotics and prebiotics, the manufacturing process and the corresponding utilization. The present invention’s object is the development of a cereal symbiotic matrix, preferably from oat, pre-fermented with encapsulated probiotics and free and/or encapsulated prebiotics, with the aim of complementing the actual functional food market and solving problems inherent to the reduced shelf-life period of such foods due to loss of probiotic viability to values below the minimum limits needed in order to promote biological activity. Furthermore, the present invention’s object is to improve the fermentative process conditions at different levels, namely fermentation time reduction in order to reduce energy consumption during the process and the risk of contamination reduction as well as promote long term microbial stability maintenance. The pre-fermented symbiotic matrix is designed, in particular, for those cases where intolerance and/or allergy to dairy products occur, yet it is further applicable to the pharmaceutical, cosmetic and preferably food industries, including pet food.
PRE-FERMENTED SYMBIOTIC MATRIX
BASED ON A CEREAL SUSPENSION WITH
ENCAPSULATED PROBIOTICS,
MANUFACTURE PROCESS AND
CORRESPONDING UTILIZATION

INVENTION FIELD
[0001] The present invention is related with the manufacture of a pre-fermented symbiotic matrix, containing probiotics and/or prebiotics, and it is applicable to the pharmaceutical, cosmetic and preferably food industries, including pet food. The pre-fermented symbiotic matrix, free or not of dairy ingredients, applies to all populations, in particular to those elements with intolerance and/or allergy to dairy products.

SUMMARY
[0002] The present invention’s object is the development of a cereal symbiotic matrix, out preferably, pre-fermented with encapsulated probiotics and free and/or encapsulated prebiotics, with the purpose of complementing the actual functional food market and solving problems inherent to the reduced shelf-life period of these foods. Moreover, the present invention’s object is to improve the fermentative process conditions at different levels, namely reduction of fermentation time as a means to economize energy during the manufacturing process, to reduce the risk of contamination and to maintain long term microbial stability.

INVENTION STATE OF THE ART
[0003] Over the last few decades, detailed knowledge on the influence of diet on human health has increased greatly, and populations across the world have become conscious of the need for a so-called ‘healthy diet’, justified by the life expectancy increases as well as representing an important public health issue. With the increasing popularity of probiotic products among consumers, food companies need to face the call for the manufacture of such products in order to appropriately meet constant market requests. All food is functional, in the general meaning of the term, insofar as they supply energy and nutrients necessary toward growth and maintenance. A food ingredient is considered as functional if it has been clearly demonstrated and scientifically validated, in an efficient scientific way that it beneficially affects health, beyond the classical nutritional effect associated therewith.

[0004] This market is characterized by being dynamic and innovative with a market quota of 10 to 15% and a growth rate of 20 to 30% per year, at world level.

[0005] Probiotics can be defined as viable microorganisms that affect the host beneficially in as much as they promote the balance of its intestinal bacterial ecosystem. Lactobacillus, Bifidobacterium and Enterococcus, genera considered potentially probiotic, offer a protection to the host against infections, considering that they prevent the attack, setting, response and/or virulence of specific enteropathogens (anti-microbial activity). These probiotics also have a beneficial effect in the control of diarrhoeas, as well as in the reduction of the risk of development of some forms of cancer (anticarcinogenic activity). An effect on reduction levels of blood cholesterol (hypercholesterolaemic activity) is also described. Another possible effect, which has been scientifically validated, is the effect on the digestion of lactose, through the production of lactase (β-galactosidase) which facilitates the digestion of this sugar, and offers solutions for individuals intolerant to lactose. The probiotic products’ beneficial effect is secured when these contain a minimum of 10^5 CFU/ml, which is in agreement with the assumption of a minimum therapeutic dose per day suggested to be 10^5 to 10^6 viable cells, which may be realised through an intake of approximately 100 grams of product containing 10^5-10^6 viable cells per millilitre or gram. Probiotics present natural limitations to their health benefits, due to their susceptibility to certain technological and functional factors, for instance high levels of oxygen, acid environments, freezing and the passage through the gastrointestinal tract.

[0006] Methods of encapsulation have begun to be applied, as a means to increase the survival rate of probiotics, through their protection from abovementioned adverse conditions. Microencapsulation is the technology of packing solids, liquids or gases in very small capsules, capable of releasing their content at controlled rates and under specific conditions.

[0007] Several microencapsulating techniques are available, viz. emulsion and spray-drying. Emulsion encapsulation consists on adding a small volume of solution containing microbial cells and polymers (discontinuous phase) to a greater volume of vegetable oil (continuous phase). This mixture is then homogenized forming a water-in-oil emulsion. Once obtained, the water soluble polymer must be insolubilized via a saline solution with the objective of creating small gel particles in the oil phase. The size of the capsules can be controlled by varying the type of stirring and its speed as well as the saline solution addition mechanism. The process of emulsion encapsulation is easily scaled-up and leads great survival rates of microorganisms (80 to 95%). The resulting capsules present various sizes, which range from 25 µm to 50 µm.

[0008] On the other hand, the spray-drying method consists on drying an aqueous encapsulating agent mixture with viable microbial cells, using an atomizer. The drying occurs when the solution, after being vaporized, comes in contact with a hot air flow (entry temperature), and is subsequently, with the aid of a vacuum, gathered in the appropriate recipient. This technology has as greatest advantages the low cost of the procedure, the easiness of the operation, the possibility of using thermo-sensitive functional ingredients, the high quality/stability of the capsules obtained and the easy production in large quantities. The obtained capsules can vary in size between 5 and 75 µm.

[0009] A prebiotic is by definition a non-digestible food ingredient which positively affects the host, stimulating selectively the growth and/or activity of one or a limited number of bacteria in the colon.

[0010] The term symbiotic refers to a synergistic association of pre- and probiotic agents with physiological activity in the same food.

[0011] Presently, prebiotics and their combination with probiotics in an encapsulated form consist in an investment for food industry, with the intention of maintaining their long term stability and optimizing the nutritional qualities of the associated product.

[0012] The document US2001/0016220 lists components of food products, which contain biologic active ingredients that may be encapsulated, as well as the process for their production and utilization. The components mentioned in this document comprise plant fibres including the ones proceeding from oats, soluble and insoluble polysaccharides, pectins, lehizens and gums. The biologically active components in the mentioned plant fibres may be probiotic microorganisms,
prebiotics, enzymes, nutrients, secondary metabolites, natural or synthetic, substances with antioxidant activity, etc. The substances of encapsulation may be constituted by polysaccharides (of plant or microbial origin), emulsifiers, peptides, proteins and prebiotic substances.

[0013] In terms of the process of attainment of these components, the document in question foresees the introduction of biological active components into an environment that contains substrates that form the capsules. Even though this document describes products based on cereals containing biologically active components, such as probiotics and/or prebiotics, encapsulated in a matrix formed by the plant fibres of the cereals, it differs considerably from the present invention, since this describes an aqueous suspension of pre-fermented cereals, with posterior encapsulation of microorganisms, by emulsion, fluidized bed, or by drying and subsequent addition of prebiotic components. In other words, in the previous document, all the components, probiotics or prebiotics, are encapsulated and are introduced in the matrix at the same phase, while this invention describes a process of attainment of the components in several phases, with protection of microbial activity through the encapsulation of microorganisms.

[0014] The document WO2005/002367 reports products and therapeutical compositions made of oats free of probiotic microorganisms, including proteins, hydrolysed proteins and emulsifying lipids. Besides that, the products and compositions may still include β-glucans and plant sterols. The corresponding production process is carried out via an enzymatic treatment of the oats fraction for removal of the carbohydrates (preferentially by hydrolysis).

[0015] The document W002.065855 mentions non dairy products, made of cereal dispersions, containing β-glucans, proteins, natural sugars and proteins. The process to obtain such products uses enzymes, particularly hydrolyses, as well as isomerases, applied to cereal suspensions.

[0016] The document WO02/37984 reports products leavened by microbial cultures based on oat suspensions, free of soy and milk, as well as the corresponding manufacture. This document foresees the use of Lactobacillus and Streptococcus strains in the fermentation of the oat suspension, as well as the inclusion of several components, such as calcium hydroxide-phosphate and/or calcium phosphate, β-glucan, maltose, maltodextrin, proteins, etc in an aqueous oat suspension which is later incubated for fermentation.

[0017] The document W000/65930 reports products made of cereals, particularly oats, for further utilization as raw material in the food industry. The process of attainment of these products includes the preparation of a suspension, from bran, flakes or flour of cereals. This suspension is later homogenized, at a predetermined temperature and pressure, in order to obtain an emulsion. Afterwards, the emulsion can be leavened by microorganisms, such as Lactobacillus and Bifidobacterium, among others, acidified and finally pasteurized, (or even presented as a powder).

[0018] The document CA2383021 describes symbiotic compositions departing from β-glucans produced from cereals, obtained from flours or extracts of cereals, inoculated by bacteria for fermentation. Lactobacillus, Streptococcus and/or Bifidobacterium are inoculated in aqueous suspensions of cereals, treated with α-amylases, and added with a stabilizing agent.

[0019] The document WO2004/037191 describes symbiotic products, in liquid or frozen form, derived from soy or dairy products, composed of a mixture of probiotic components (e.g. Lactobacillus and Bifidobacterium) and prebiotics, in which these may be constituted by polymers, particularly, inulin or oligofructose. The process of manufacture thereof uses a mixture of prebiotic and probiotic components in a liquid phase; fermentation of this mixture occurs until pH reaches 4.5; and final blend leads to the final product. At this final stage, it is, still possible to include a percentage of carbon dioxide.

[0020] The content described in the six aforementioned documents, differs substantially from the content of our invention, since they describe the fermentation of oat suspensions, with the addition of free microorganisms or enzymes, in just one phase, and may eventually include other additional components, while the invention under analysis describes suspensions of cereals pre-fermented by immobilized microorganisms, to which encapsulated probiotics and free or encapsulated prebiotics are subsequently added.

[0021] When the microorganisms are used in the free form the shelf-life of the final product is reduced and the stability/viability of microorganisms over the storage period as well as in their passage through the gastro-intestinal tract is diminished, in comparison to the symbiotic pre-fermented matrix with encapsulated probiotics (increase of 40 to 60%), object of the present invention.

[0022] This present solution also solves the problem associated with reduced shelf-life mentioned in patents WO02.37984, W000/65930, CA2383021, WO2004/037191 (an increase of 40 to 60% compared to the existing products with free microorganisms is reported) and maintains long term microbial stability.

[0023] The document EP 0 862 863 A2 has for object of invention the development of dried extruded cereals with surface and/or enclosed microorganisms, and with soluble fibre sources listing as examples of application breakfast cereals and animal feed. The object of invention foresees the development of a cereal symbiotic matrix, preferably in oatmeal, pre-fermented with encapsulated probiotics and free and/or encapsulated prebiotics, which, when applied together, will confer a stabilizing effect on the microorganisms present in the final product and favour the passage through the gastrointestinal tract. Furthermore, an additional object of this invention is the health claim of cholesterol reduction associated to p-glucan, as a source of non-digestible prebiotic soluble fibres. The cereal suspension, preferentially oatmeal, is presented in fresh, lyophilized, and frozen forms, adapted to the needs of the intervening parts in the food chain, and hence with several applications in the food industry.

[0024] The document WO2004.070262 discloses continuous processes concerning yeast immobilization in κ-carrageenan or alginate gel spheres, e.g. in beer production, through formation of an emulsion e.g. with the continuous non-aqueous phase (plant oil) and the disperse aqueous phase (inoculated κ-carrageenan with yeast), using static stirrers. This subject differs from that disclosed in the present invention, because the immobilization process described, although pertaining to an emulsion between a plant oil and a microorganism-inoculated polymer, encompasses a yeast, whereas those in the present invention are all of probiotic microorganisms.

[0025] The conceptualization of a phased process, to obtain symbiotic products, from pre-fermented cereal suspensions with added encapsulated probiotics, and subsequent incorporation of prebiotic compounds in the cereal matrix leads to a
superior product, not only from a nutritional point of view, due to long term microbiological stability maintenance, but also in what concerns.

GENERAL DESCRIPTION OF INVENTION

[0026] The present invention reports a cereal symbiotic matrix, preferentially oatmeal, pre-fermented with encapsulated probiotic and prebiotic compounds, its process of manufacture and its use in several applications, especially in the food industry but also in the pharmaceutical industry or similar counterparts.

[0027] The products obtained possess organoleptic characteristics that are identical to those produced by traditional fermentation processes.

[0028] When encapsulated microorganisms are included, these products also have the advantage of increasing their viability/stability, either as a long shelf-life or during passage through the gastro-intestinal tract following ingestion.

[0029] The matrices also present, as an additional advantage, an extended expiration date up to 40% to 60% higher than those presented by available products on the market.

[0030] Through the use of this technology one obtains a pre-fermented product with residual quantities of free microorganisms and with the same organoleptic characteristics as those of a traditionally fermented product, being therefore a more valued product.

[0031] The immobilization technique of microbial cells confers advantages in comparison to free cell systems, such as: (i) reduction in fermentation time up to 50 to 60%; (ii) increase of the microbial metabolism and stability; (iii) reduced risk of contamination; (iv) higher cell density; (v) stable product quality associated with a decrease of post-acidification risk due to probiotic action, for example; and (vi) improved substrate use and (vii) long time cell reutilization due to constant cellular regeneration.

[0032] The process of obtaining these products reveals a method for improvement of the fermentative process conditions at several levels such as, (i) continuous reutilization of the immobilized cells; (ii) fermentation time reduction contributes to energy saving throughout the process, (iii) reduction of contaminating risks and (iv) long term maintenance of microbial stability.

DETAILED INVENTION DESCRIPTION

1. Preparation Process of Oatmeal Suspension

[0033] 1.1. Preparation of an oatmeal concentrate 5-20% (w/w) from flakes, bran and/or flour and subsequent mixture in water;

[0034] 1.2. Heating of this mixture for 5 to 20 minutes in a temperature range of 80 to 110°C, with continuous stirring;

[0035] 1.3. Grinding of the resulting preparation;

[0036] 1.4. Filtration of the obtained suspension; and

[0037] 1.5. Cooling of the mixture until a range of temperatures between 25 and 45°C.

2. Process of Pre-Fermentation in Fluidized Bed Reactor Associated with Cell Encapsulation by Emulsion

[0038] 2.1. Process of pre-fermentation

[0039] The process of pre-fermentation is performed in a fluidized bed reactor with immobilized microorganisms by cells obtained in steps 2.1.1. through 2.4.3.

[0040] The capsules are introduced in a column, with porosity smaller than the diameter of the capsules to induce the microorganisms-matrix interaction, inside the pressurized reactor with constant and controlled bi-directional nitrogen flow. The immobilized cells inside the column are reutilized in the fermentation process until they lose their metabolic properties.

[0041] 2.1.1. Cell Culture Preparation

[0042] 2.1.1.1. Preparation of the inocula from frozen cultures and consequent activation by two consecutive transfers in MRS Broth supplemented with L-cysteine-HCl 0.05% (w/v).

[0043] 2.1.1.2. Inoculation of 1 to 20% (v/v) in 1000 mL of MRS Broth (Man Rogosa and Sharpe) supplemented with L-cysteine-HCl 0.05% (m/v) and subsequent incubation for 24 h at 37°C, under anaerobic conditions, for Lactobacillus acidophilus Ki and 48 h at 37°C, under anaerobic conditions for Bifidobacterium animalis Bo and Bb12, for example.

[0044] 2.1.1.3. Centrifugation of the resulting cultures at 4000 rpm for 15 minutes, at 4°C, subsequent washing of pellet with, for example, NaCl 0.9% (w/v) solution, and reuspension in 100 mL of the same solution.

[0045] 2.2. Polymer Solution Preparation

[0046] 2.2.1. Preparation of a polymer solution, for example, k-carrageenan 1 to 5% (w/v), with continuous stirring, variable duration between 1 to 4 hours, temperatures between 60 and 80°C, followed by cooling down to a temperature range of 35 to 45°C.

[0047] 2.3. Oil Solution Preparation

[0048] 2.3.1. Mixture of vegetable oil with one of the following compounds: Tween 80 0.2% (v/v) and/or a protective agent, as for a non limiting example, laurel sodium sulphate 0.5% (v/v).

[0049] 2.4. Capsule Preparation

[0050] 2.4.1. Mixture of cellular suspension 1 to 20% (v/v) (see 2.1.) with the polymer solution 1 to 5% (w/v) (see 2.2.).

[0051] 2.4.2. Addition of the resulting mixture to 75 to 98% of the prepared oil solution (see 2.3.). The obtained solution is homogenised forming a water-in-oil emulsion.

[0052] 2.4.3. The capsule formation occurs after the addition of a solution of KCl 10 mM, for example, to the mixture at a temperature range of 4 to 8°C.

[0053] 2.5. Pre-fermentation process operation conditions

[0054] After attainment of the capsules with microorganisms for utilization in a fluidized bed reactor, the process of pre-fermentation is performed at a temperature between 20°C to 52°C, during 4 to 8 hours, under sterile and anaerobic conditions (circulating nitrogen flux), resulting in a fermented matrix. This suspension is drained into the reactor where the incorporation of the remaining food ingredients occurs.

3. Microorganisms Encapsulation Process by Emulsion and/or Spray-Drying

[0055] The microorganisms' encapsulation is done using the encapsulation techniques:

[0056] 3.1. Emulsion, as described in point 2;

[0057] 3.2. Spray-drying;

[0058] 3.2.1. Preparation of a cellular suspension with polymers (see points 2.2; 2.2.1; and 2.4.1);
3.2.2. Drying of 250 ml of the previous mixture under the constant conditions of inlet and outlet temperatures of 150-175°C and 50-85°C, respectively;

3.2.3. Addition of the resulting powder into the pre-fermented oat suspension (point 2.5) in a proportion of 2-5% (w/v), in a way to guarantee 10^2-10^3 CFU in the matrix, per 100 g or 100 ml.

4. Food Ingredients Incorporation

Addition of ingredients to the matrix obtained in the previous process (point 3.2.3.), having as an example inulin, at a concentration range between 1-3%, maintaining, as a non-limitative example sea-salt, among others.

5. Presentation Forms of the Matrix

The pre-fermented symbiotic matrix based on an oat suspension with encapsulated prebiotics can be presented either in a fresh form, lyophilized and/or frozen. The fresh matrix can be further presented either in gel or extruded form.

1. A pre-fermented symbiotic matrix comprising a suspension of cereals with encapsulated microorganisms including at least one of the following compounds:
   - free and/or encapsulated prebiotics;
   - free microorganisms;
   - other food ingredients.

2. A pre-fermented symbiotic matrix according to claim 1, wherein the cereals include flakes, flour or bran.

3. A pre-fermented symbiotic matrix according to claim 2, wherein the cereals include oats.

4. A pre-fermented symbiotic matrix according to claim 2, wherein the cereals are combined with one or more other cereals and/or legumes usually applied in food industry.

5. A pre-fermented symbiotic matrix according to claim 3, wherein the cereals include oats combined with cereals and/or legumes, including barley and soy.

6. A pre-fermented symbiotic matrix according to claim 1, wherein a prebiotic source includes β-glucan soluble fibres in biologically active quantities.

7. A pre-fermented symbiotic matrix according to claim 6 wherein β-glucan soluble fibres are extracted from cereals and/or legumes.

8. (canceled)

9. A pre-fermented symbiotic matrix according to claim 7, having a minimum of 0.75% (w/w) of β-glucan soluble fibres.

10. A pre-fermented symbiotic matrix according to claim 6, further comprising other prebiotic compounds including inulin, fructooligosacharides (FOS) and chitosans.

11. A pre-fermented symbiotic matrix, according to claim 1, including integration of other food ingredients, that apart from the prebiotic function can still allow for other functions, such as, organoleptic functions such as sweeteners, flavours and/or fruit pulp and texture such as enzymes.

12. A pre-fermented symbiotic matrix, according to claim 1, including extra antioxidant sources, fatty acids such as omega 3, omega 6 and its derivatives, vitamins and minerals beyond the already existent in cereals and/or legumes, in a free or encapsulated form.

13. A pre-fermented symbiotic matrix, according to claim 1, wherein the microorganisms includes “GRAS”, probiotic and non-probiotic and wherein an encapsulate includes at least one of proteins, polysaccharides, lipids and hydrocolloids.

14. A pre-fermented symbiotic matrix, according to claim 13, including inoculated microorganisms in a quantity not inferior to 10^2-10^3 CFU/g, ensuring that the final product, when consumed, contains between 10^2-10^3 CFU/g.

15. A pre-fermented symbiotic matrix, according to claim 1, wherein the matrix is in a fresh, lyophilised and/or frozen form.

16. A pre-fermented symbiotic matrix, according to claim 15, wherein the fresh matrix includes a gel or extruded form.

17. A process of obtaining a pre-fermented prebiotic matrix according to claim 1, comprising:
   - a pre-fermentation including placing a cereal suspension in a reactor with immobilized microbial cells in macrocapsules, coated by proteins, polysaccharides, lipids and hydrocolloids;
   - a separation wherein the microorganisms are separated from the matrix;
   - an encapsulation, including drying, atomization, emulsion or coacervation; and
   - incorporating food ingredients.

18. (canceled)

19. (canceled)

20. (canceled)

21. (canceled)

22. (canceled)

23. (canceled)

24. (canceled)

25. The pre-fermented matrix according to claim 12, wherein the microorganisms include the Bifidobacterium and Lactobacillus genus.

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