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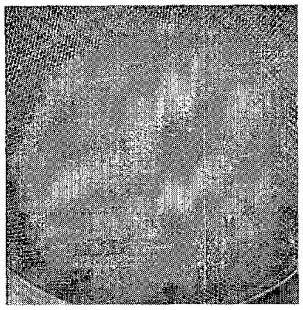

FIGURE 2

(57) Abstract: The present invention relates to use of bovine gelatin (halal) as animal gelatin source for bioencapsule(s) beads. Indeed, the bioencapsule(s) beads having the capability of providing an alternative source for probiotic delivery in pharmaceutical and nutraceutical products preferably consume by Muslim consumers.

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BIOENCAPSULE AND METHOD THEREOF

FIELD OF INVENTION

This present invention generally relates to a biological material and method of manufacturing the material. More specifically, the invention relates to bioencapsules beads for probiotic delivery in mammal. Moreover, the present invention discloses a bioencapsulation process and use of the bioencapsules beads in pharmaceutical and nutraceutical products.

BACKGROUND OF INVENTION

Probiotic are microorganisms which consumes an adequate amount for health benefits. Probiotics are considered to be viable microbial preparations which promote mammalian health by preserving the natural microflora in the intestine. Probiotic/s is thought to attach to the intestinal mucosa, colonize the intestinal tract and thereby prevent attachment of harmful microorganisms thereon. The probitics action resides in that they have to reach the gut's mucosa in a proper and viable form and especially do not get destroyed by the low pH in the stomach.

The use of biopolymer for the bioencapsulation of probiotic has been rapidly developed. This rapid development has occurred for the following reasons: 1) to protect the bacteria cells from damage caused by external environment, 2) guarantee their improved survival during gastro-duodenal transit, 3) enhanced their stability profile.

Among some encapsulation procedures reported, cell encapsulated in gelled biopolymer is commonly used with calcium-alginate as matrices and lactic acid bacteria as probiotic bacteria. However, alginate containing lactic acid bacteria tends to be liquefied by lactic acid (Roy *et al.*, 1987). Indeed, further modification on alginate beads has been carried out in order to reduce mass transfer effects.

Another common biopolymer used is starch. A group of researchers (Talwalkar and Kailasapthy, 2003; Mattila-Sandholm, 2004; Iyer and Kailasapathy, 2005) worked on stabilization of lactic acid bacteria and to formulate new types of food fortified with encapsulate health-promoting bacteria in the starch system. They found that encapsulation prevented cell death from oxygen toxicity and improved the viability of bacteria. However, the limitation of starch-alginate beads is lack of emulsification properties and leads to low cells entrapment.

Gelatin is a thermally reversible gelling agent and useful for encapsulation. Because of its amphoteric nature, it is also an excellent candidate for incorporating with anionic-gel forming polysaccharides, such as alginate. Xiao *et al.* (2009) reported the use of alginate-gelatin to encapsulate *Lactobacillus casei* ATCC 393. Based on their study, it was revealed that the microcapsules had successfully improved the survival of *L.casei* ATCC 393. Annan *et al.* (2008) reported that combination of alginate-coated gelatin-genipin to form microsphere, provided a significant protection for *B.adolescentis* from harsh acidic conditions of simulated gastric juice. However, they also use of porcine gelatin (Type A: 300 bloom strength) (instead of bovine gelatin) with 13% (w/v) as gelatin source in encapsulating *B.adolescentis*. Whereas genipin, a new cross linker derived from *Gardenia jasminidides* plant was used to cross link with gelatin in order to increase the gelatin mechanical and thermal stability. Alginate was used at 1% (w/v) as encapsulation matrix. In this study, the emulsion method was used in the microencapsulation process to form micospheres and variable size of microspheres was obtained. Also, Annan *et al.* (2007) reported that combination of genipin-gelatin to form microspheres to encapsulate *B lactis* Bb-12 was able to protect the cells in simulated gastric juice with higher genipin concentration. The encapsulation yield was reported increased with low gelatin bloom strength. Nevertheless, this scientific research disclose the use of porcine gelatin (Type A: bloom strength range 175-300) (instead of bovine gelatin) at a range of 10%-19% (w/v), genipin at range of 0-10mM. No alginate was reported in this study. Emulsion method was used in microencapsulation process and the size of microspheres was variable.

There are several prior art documents disclosing inventions relating to probiotic microorganism, method obtained from the probiotic microorganism and the use probiotic products. The prior art within aquaculture presently shows that probiotic microorganism have been tested in commercial gelatin. For example: US Patent No. 20090263366 (2009) teaches compositions comprised of probiotics and a dried plant powder. Embodiments include probiotic microorganisms such as Pediococcus, Bifidobacterium, Bacteroides, Propionibacterium, Streptococcus, Enterococcus, Lactococcus, Lactobacillus, and Saccharomyces. Embodiments include dried plant powders from vegetables, fruits, cereals and herbs. In embodiments compositions are encapsulated in gelatin capsules. Furthermore, US Patent No. 20090263366 (2009) describes a process of ameliorating effects caused by environmental or biological changes in human or animals comprising the step of: feeding the human or animal in need of such amelioration an encapsulated probiotic composition comprising a viable encapsulated probiotic microbe.

US Patent No. 7282220 (2007) describes a pharmaceutical microsphere comprises a bioactive agent and a biological carrier that encapsulates the bioactive agent, wherein the biological carrier is crosslinked with a crosslinking agent. The crosslinked of porcine gelatin (300 bloom strength) with genipin to encapsulate heparin using emulsion method. However, this document limits its disclosure of the use of bovine gelatin crosslinked to genipin to microencapsulate probiotic live cells using extrusion method.

US Patent No. 5501857 (1996) describes single encapsulation of the mixture of probiotics and vitamins and mineral supplement within commercial gelatin capsule. However, it resulted in the loss of more than 99.79% of viability of the probiotics. Its gelatin source for gelatin capsule was porcine source. Nevertheless, this document limits its teaching to development of bioencapsulation process using porcine gelatin with other cross linker matrix for probiotic live cells delivery.

Based on the previous research and inventions, porcine skin derived gelatin contributes the highest production (46%). However, Islam forbids the consumption of any pork-related products. Therefore, researches are continually searching for an alternative source derived gelatin that might give better properties when use alone or in combination with other

polymers. The problem to be solved by the present invention addresses development of biopolymer composition of bovine gelatin (from halal source), alginate and genipin to microencapsulate *Bifidobacterium* spp as probiotic bacteria. The solution is based on that the present inventors developed biopolymer composition which provides capable effects on bioencapsulation matrix for probiotic delivery.

The present invention responds specifically to the long-felt need heretofore unmet by the prior art, and especially with a view to overcoming the objective of the present invention which relates to use of bovine gelatin (halal) as animal gelatin source for bioencapsule(s) beads. Indeed, the bioencapsule(s) beads (preferably known as beads) having the capability of providing an alternative source for probiotic delivery in pharmaceutical and nutraceutical products preferably consume by Muslim consumers. The foregoing, and other advantages of the present invention, are realized in one aspect thereof in an oral nutritional composition, i.e., a dietary adjunct, useful for manufacturing pharmaceutical composition, cosmetic products or foods products for human consumption.

Yet another objective of the present invention provides the use of lower bloom strength like bovine gelatin (75 to 175 bloom strength) that resulted in higher encapsulation yield. The lower the viscosity of bovine gelatin with lower bloom strength, contributes to higher cell entrapment into the matrix during microencapsulating mixing and consequently higher encapsulation yield than obtained from gelatin of 300 bloom strength (Annan *et al.*, 2007).

The present invention also provides the use of genipin that increases gelatin strength and stability of a matrix in low pH and higher temperature environment during storage in food matrix. Likewise, the solution is based on that the present inventors identified that the size of the beads are uniform, small and able to be incorporate into food products. Also, the cost to produce alginate-bovine gelatin-genipin beads of probiotic bacteria bioencapsulation shows 60% lower than using porcine gelatin

SUMMARY OF THE INVENTION

Accordingly, the present invention relates novel bioencapsule beads for probiotic delivery in a mammal (preferably human or animal), wherein the beads includes probiotic microorganism (includes selected from the group of *Bifidobacterium pseudocatenulatum* G4, *Bifidobacterium longum* BB536, *Lactobacillus acidophillus* ATCC 4356 and *Lactobacillus plantarum* FTCC 0350), encapsulation matrix and hardening agent (preferably calcium chloride ($CaCl_2$). The encapsulation matrix includes bovine gelatin (10% - 13% w/v), genipin and a coating matrix which is alginate preferably sodium alginate (1% -5% w/v). Indeed, the bovine gelatin is capable of providing a bloom between 75 and 175 bloom and the genipin is between 10mM and 50mM. Also, the encapsulation matrix providing the means of entrapping the probiotic microorganism.

Yet, another aspect of the present invention relates to a method of developing bioencapsule beads for probiotic delivery, wherein the method includes preparing an encapsulation matrix, preparing individual cell culture of *Bifidobacterium pseudocatenulatum* G4,*Bifidobacterium longum* BB536, *Lactobacillus acidophillus* ATCC 4356 and *Lactobacillus plantarum* FTCC 0350, mixing the encapsulation matrix with individual cell cultures, obtaining a mixture comprising cell-alginate-bovine gelatin-genipin, extruding the mixture with a pointer to form globules. Next, introducing the globules into a hardening agent, wherein the hardening agent having a concentration between 0.1 and 0.2 M of $CaCl_2$. Later, allowing the globules and the hardening agent to form beads for at least 2 hours, exposing the beads to Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF), determining the strength of the beads by using a texture analyzer and finally, obtaining beads at strength between 50 and 150g.

Yet, another aspect of the present invention relates to the use of the novel bioencapsule beads and method for the manufacture of a pharmaceutical composition, nutraceutical products food product/s (include beverages, dairy products, confectionaries, chocolates, and any application in food formulation/s as an ingredient or for any functional properties).

Indeed, the use of an effective amount of bioencapsule beads in the preparation of a medicament or probiotic delivery in a mammal (including human).

BRIEF DESCRIPTION OF DRAWINGS

The accompanied drawings constitute part of this specification and include an exemplary or preferred embodiment of the invention, which may be embodied in various forms. It should be understood, however, the disclosed preferred embodiments are merely exemplary of the invention. Therefore, the figures disclosed herein are not to be interpreted as limiting, but merely as the basis for the claims and for teaching one skilled in the art of the invention

Figure 1 shows bioencapsulation unformed beads in 1st trial.

Figure 2 shows bioencapsule beads in 2nd trial.

Figure 3 shows SEM of beads from 2nd trial. a) Before exposed, b) After exposed in simulated gastric fluid and c) After exposed in simulated intestinal fluid.

DETAILED DESCRIPTION OF THE INVENTION

The invention will now be described in more detail by reference to the following Figures and Examples. The following examples are provided for illustrative purposes only and are not intended to limit the invention.

DEFINITION

Prior to a discussion of the detailed embodiments of the invention, a definition of specific terms related to the main aspects of the invention is provided.

The term of "bioencapsulation" can be refer the process of entrapment of microorganisms cells by means of coating them with proper biopolymer(s) in order to segregate the cell from the surrounding environment; in a way that results in appropriate cell release in the intestinal medium (Sultana *et al.*, 2000; Krasackoopt *et al.*, 2003; Picot and Lancroix, 2003a).

The term of "matrix" refer to multicore particle substance used to entrap the probiotic bacteria or probiotic bacteria within.

The term of "probiotic bacteria" can be refer to any material which has a functional or nutritive activity and which typically exhibits low stability, and/or a reduction or loss of bio-effectiveness when exposed to unfavorable conditions. The unfavorable conditions include moisture, elevated temperature, oxygen, and acidic or basic pH. When the probiotic bacteria is exposed to such conditions, the probiotic bacteria can decompose, deactivate and/or loss viability. Example of probiotic bacteria are *Bifidobacterium* spp.

The term of probiotic refers to bacterial genera that have a beneficial effect in animal organs, such as the human gastrointestinal. The bacterial genera used most often as probiotics are lactobacilli and bifidobacteria. After passage through the stomach and small intestine, some probiotics survive and become established transiently in the large bowel, where the colon's fermentation capacity is positively modified.

Alginates are natural anionic polysaccharides made up of D-mannuronic and L-guluronic acids residues joined linearly. The variable proportional and sequential arrangement of the D-mannuronic and L-guluronic units results in distribution of negative charges along the backbone in aqueous solution (Thu *et al.*, 1996), potentially allowing for polyion complexation with positively charged gelatin polymers. An alginate gels are stable low pH solutions but swell in weakly basic solutions, alginate coating of gelatin capsules and microspheres can be used to protect drugs from acidity of gastric juice while allowing subsequent release in the basic environment of intestinal fluids (Rao and Rao, 1997).

An object of the invention is the use of bioencapsule(s) beads for the manufacturing of a food product, feed product, nutraceutical product(s) (such as dietary supplement), natural remedy, pharmaceutical active formulation and medicinal product The term "nutraceutical" as used herein denotes usefulness in both the nutritional and pharmaceutical field of application.

Thus, the novel bioencapsule(s) beads is use as supplement to food, and as pharmaceutical formulations for enteral or parenteral application which may be solid formulations such as capsules or tablets, or liquid formulations, such as solutions or suspensions.

The invention relates to bioencapsule(s) beads for delivery of probiotic bacteria particularly *Bifidobacteria* spp. to gastrointestinal tract and in particularly to colon in a human. The

composition includes bovine gelatin with genipin and alginate combination to provide protection to the cells upon delivery to the targeted area. The composition provides a matrix that is preferably in the form of bioencapsule where the probiotic bacteria are well protected in an acidic environment. In accordance with the subject invention; encapsulated sensitive probiotic bacteria could provide full viability and retained growth ability. The term sensitive probiotic bacteria are referring to sensitivity in low pH environment mainly in the stomach area (pH range: 1.5 – 3.5). Most of living cells are destroyed at this pH including probiotic bacteria. Therefore, the cells need to be encapsulated in order to maintain the viability upon reaching the target area.

BEST MODE TO CARRY OUT THE INVENTION

Before the present invention is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims. When a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. When the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

EXAMPLES

Method of preparing bioencapsulation beads

a) Encapsulating Matrix

Obtain Bovine gelatin (Type B, range 75 to 175 Bloom strength) and sodium alginate (medium viscosity) from Sigma-Aldrich Co. (Oakville, ON, Canada). While, Genipin is purchased from Challenge Bioproducts Co. (Taiwan, PRC). A 0.1M dihydrated calcium chloride solution was used as hardening agent.

b) Culture Preparation

Obtaining *Bifidobacteria pseudocatenulatum* G4 (probiotic bacteria) from Probiotic Laboratory, Faculty of Food Science and Technology, Universiti Putra Malaysia. Using Trypticase Phytone Yeast extract media (TPY) (Scharlau-Chemie, Barcelona, Spain) to maintain and propagated the strain. Cultures were incubated anaerobically at 37°C in an anaerobic jar. Anaerocult A (Merck, Darmstadt, Germany) was used to maintained anaerobic condition in the jar. Harvesting of cells was done by centrifugation at 8000 rpm for 10 min at 4°C and after discarding the supernatant of spent culture broth, the cell pellet was resuspended in peptone saline (1 gL^{-1} peptone, 8.5gL^{-1} NaCl) and centrifuged again under the same conditions. Washed cells were then suspended in a total of 10 mL peptone saline and stored at 4°C until usage. Prior to bioencapsulation, the culture was further transferred in 150mL skim milk supplemented with yeast extract (as an inoculum medium: 6.1% skim milk, 1.2% yeast extract) before further cultivate in 2L bioreactor (fermentation medium: 2.8% skim milk, 2.2% yeast extract) in order to obtained 10^{10} cfumL^{-1} cell densities. Fresh cell suspension was prepared for each experiment.

c) Bioencapsulation Preparation

Preparing bovine gelatin (10% to 13% w/v), by dissolving the gelatin powder in distilled de-ionized water (DDW) at 50°C. One milliliter of washed cell suspension was added to sodium alginate (range 1% to 5% w/v) to obtain a cell to sodium alginate ratio 1:10. Aqueous gelatin solution was prepared separately. An internal coating alginate was obtained by add the cell-

alginate mixture into aqueous gelatin and leave it stir for 30 min. Genipin (giving a final concentration range from 10mM to 50mM in cells-alginate- gelatin mixture) was subsequently added into the mixture. The cells-alginate- gelatin-genipin mixture was then leave stir for 60 min. The mixture then extrude through a 23G pointer (preferably needle) is size in the form of globules or droplets to free-fall into a hardening solution containing 0.1M $CaCl_2$.

d) Beads gel strength determination

The strength of gel beads was determined using Stable Microsystem (Texture Exponent 32) equipped with 36mm diameter probe (P/36R). The gel strength measured at trigger force (5g) for 5 second and strain (75%).

The gel strength of bioencapsulation beads was taken before and after exposed to simulated gastric fluid for 3 h and simulated intestinal fluid for 2 h. Experiments are carried out in triplicates.

Engineering Trials

1st Trial

Table 1: 1st trial using 13% w/v halal bovine gelatin with different concentration of genipin (0mM- 20 mM) and 1% w/v sodium alginate and 0.1M $CaCl_2$

Gelatin (% w/v)	Genipin (mM)	Sodium alginate (% w/v)	$CaCl_2$
13	0	1	0.1
13	5	1	0.1
13	10	1	0.1
13	15	1	0.1
13	20	1	0.1

1st Result: Beads not well form

1st trial: 13% (w/v) of bovine gelatin with 75 bloom strength (the maximum in range) and different genipin concentration was used. Initially, the cells (1%) were mixed to 1% alginate for 15 min before bovine gelatin (13% w/v) added into the mixture. After cells-alginate-bovine gelatin mixed well for 30 min, different concentration of genipin (0 mM -20 mM) was added and leaved mix for 60 min. The mixture was then dropped into 0.1M $CaCl_2$ (hardening agent) to form bioencapsules. However, the beads are not well form in all genipin concentration.

2nd Trial

Table 2a: 2nd using 13% (w/v) bovine gelatin, 50 mM genipin, different concentration of sodium alginate (1%-5% w/v) and 0.1M $CaCl_2$

Gelatin (% w/v)	Genipin (mM)	Sodium alginate (% w/v)	$CaCl_2$
13	50	1	0.1
13	50	2	0.1
13	50	3	0.1
13	50	4	0.1
13	50	5	0.1

Result 2nd Trial: Beads are well form

2nd trial: 13% (w/v) of bovine gelatin with 75 bloom strength (the maximum in range) and genipin with 50mM concentration (maximum in range) were used. Initially, the cells (1%) were mixed different % of alginate (1%-5% w/v) for 15 min before bovine gelatin added into the mixture. After cells-alginate-bovine gelatin mixed well for 30 min, genipin was added and leaved mix for 60 min. The mixture was then dropped into 0.1M $CaCl_2$ (hardening agent) to

form bioencapsules. The result was showed better in beads formation in this trial. Further parameters like encapsulation yield (%) and bead strength (g) were carried out.

Table 2b: Bovine gelatin beads strength (hardness in g) before and after exposed to simulated gastric fluid (3 h) and simulated intestinal fluid (2 h) and *Bifidobacteria pseudocatenulatum* G4 encapsulation yield (%) using different formula of bioencapsulation matrix

Bovine gelatin

Sodium alginate (% w/v)	Hardness (g)			Encapsulation yield (%)[3]
	Before	After SGF[1] exposed	After SIF[2] exposed	
1	313.89	525.11	154.21	45.06 ± 1.83 [a]
2	848.62	1031.09	520.92	45.72 ± 0.87 [a]
3	3118.66	5912.28	484.75	54.25 ± 3.54 [b]
4	3748.18	5575.34	627.83	57.53 ± 2.50 [b]
5	4299.80	7214.19	749.25	57.66 ± 2.68 [b]

[1] Simulated Gastric Fluid
[2] Simulated Intestinal Fluid
[3] Encapsulation Yield (N/N_0 x 100) :N= Number of viable cells entrapped, N_0 = Number of viable free cells loaded into the encapsulate matrix
Different letter within column is significantly different ($p<0.05$)

At 13% (w/v) bovine gelatin and 50mM genipin concentration: Cells were mixed with alginate with range from 1% - 5% (w/v) before proceed further bioencapsulation process. Result showed that after exposed to Simulated Gastric Fluid (SGF) for 2 h, the beads become stronger before being exposed. However the strength of beads change to become weak after exposed to Simulated Intestinal Fluid (SIF) for 3 h. Gelatin is amphoteric nature; therefore range of isoelectric point of bovine gelatin type B is between pH 4.7-5.4. Within this range, gelatin is positive isoelectric charge. The combination between gelatin and alginate is a good combination because alginate is anionic polysaccharides, meaning that it a negative charge agent. In lower pH environment (lower than pH 4.7) like in stomach area, the interaction of these two polymers is become stronger. Due to that, the cells will not be release into the stomach area and well protected in the matrix. However, when the pH change to more alkaline

environment like in the intestine (higher than pH 5.4), gelatin slowly turn to negative charge. As a result, the interaction between gelatin and alginate change weak and start to repel each other. Therefore, the release of cells will take place in the intestine.

Table 3: Porcine gelatin beads strength (hardness in g) before and after exposed to simulated gastric fluid (3 h) and simulated intestinal fluid (2 h) and *Bifidobacteria pseudocatenulatum* G4 encapsulation yield (%) using composition suggested by Annan *et al.*, 2008.

Hardness (g)			Encapsulation yield (%)
Before	After SGF1 exposed	After SIF2 exposed	
2477.55	5039.21	391.72	58.94 ± 3.16

As for comparison, porcine gelatin with 300 bloom strength (13% w/v) was used to encapsulate cells- alginate (1% w/v) and then cross linked with genipin (10mM). Result showed that the beads strength was significantly different ~~almost similar range~~ to the 2nd trial of the experiment using bovine gelatin (13% w/v) with genipin (50mM) and various concentrations of alginate (Table 4). Tables 5, 6 and 7 show the optimization processes using RSM of genipin and alginate carried out in order to obtain the optimum composition for beads strength that comparable to that of porcine before and after exposed to SGF and SIF, as well as encapsulation yield in order high viability of probiotic bacteria able to deliver to the target site is achieved. The strength of beads ranges is between 50 to 100g (before exposed), 100g to 150g (after SGF exposure) and 10 to 50g (after SIF exposure).

Table 4 : Comparison of beads strength (hardness in g) before and after exposed to simulated gastric fluid (2 h) and simulated intestinal fluid (3 h) and *Bifidobacterium pseudocatenulatum* G4 encapsulation yield (%) between bovine gelatin-genipin-alginate (from 2nd trial based on

3% to 4% (w/v) alginate) and porcine gelatin gelatin-genipin-alginate beads (using composition suggested by Annan *et al.*, 2008).

Encapsulation matrix	Harness (g)			Encapsulation Yield (%)
	Before	After SGF	After SIF	
13% (w/v) Bovine gelatin- 50mM genipin- 3% (w/v) alginate	3118.66[a]	5912.28[a]	484.75[a]	54.25 ± 3.54[a]
13% (w/v) Bovine gelatin- 50mM genipin- 4% (w/v) alginate	3748.18[b]	5575.34[a]	627.83[b]	57.13 ± 2.50[b]
13% (w/v) Porcine gelatin- 10Mm genipin – 1% (w/v) alginate	2477.55[c]	5039.21[b]	391.72[c]	58.94 ± 3.16[b]

Table 5: Further optimization of bovine gelatin, genipin and sodium alginate as bioencapsulation matrix of *Bifidobacterium* spp.

Bioencapsulation Matrix	**Range**
Bovine Gelatin	10% - 13% (w/v)
Genipin	10mM – 50Mm
Sodium alginate	1% - 5% (w/v)

Table 6: Optimized point for bovine gelatin, genipin and alginate using response surface methodology based on four responses: beads strength before, beads strength after SGF, beads strength after SIF and encapsulation yield.

	Bovine gelatin (% w/v)	Genipin (mM)	Sodium alginate (% w/v)
Optimized point	12.05	10.00	3.69

Table 7: Comparison of beads strength (hardness in g) before and after exposed to simulated gastric fluid (2 h) and simulated intestinal fluid (3 h) and *Bifidobacterium pseudocatenulatum* G4 encapsulation yield (%) between optimized bovine gelatin-genipin-alginate (using RSM) and porcine gelatin gelatin-genipin-alginate beads (using composition suggested by Annan *et al.*, 2008).

	Harness (g)			Encapsulation Yield (%)
	Before	After SGF	After SIF	
Optimized encapsulation matrix : Bovine gelatin (12.05% w/v), genipin (10.00mM) and alginate (3.69% w/v)	2154.91[a]	4577.27[a]	203.22[a]	57.22 ± 1.98[a]
Encapsulation matrix based on literature: Porcine gelatin (13% w/v), genipin (10mM) and alginate (1% w/v)	2477.55[a]	5039.21[b]	391.72[b]	58.94 ± 3.16[a]

Different letter in a column are significantly different ($P<0.05$)

CLAIMS

1. Novel bioencapsule beads for probiotic delivery in a mammal, wherein the beads includes:

   a) probiotic microorganism; and

   b) encapsulation matrix.

2. The bioencapsule beads as claimed in claim 1(b),wherein the encapsulation matrix includes:

   a) bovine gelatin;

   b) genipin and;

   c) alginate

3. The bioencapsule beads as claimed in claim 1 and 2 further includes a hardening agent, wherein the hardening agent comprises calcium chloride ($CaCl_2$).

4. The bioencapsule beads as claimed in claim 1, wherein the probiotic microorganism is selected from the group of *Bifidobacterium pseudocatenulatum* G4, *Bifidobacterium longum* BB536, *Lactobacillus acidophillus* ATCC 4356 and *Lactobacillus plantarum* FTCC 0350.

5. The bioencapsule beads as claimed in claim 2, wherein bovine gelatin is between 10% and 13% w/v.

6. The bioencapsule beads as claimed in claim 2, wherein bovine gelatin is capable of providing a bloom between 75 and 175 bloom.

7. The bioencapsule beads as claimed in claim 2, wherein the genipin is between 10mM and 50mM.

8. The bioencapsule beads as claimed in claim 2, wherein the alginate is sodium alginate between 1% and 5% w/v.

9. The bioencapsule beads as claimed in claim 8, wherein the alginate is a coating matrix.

10. The bioencapsule beads as claimed in claim 1, wherein the encapsulation matrix providing the means of entrapping the probiotic microorganism.

11. A method of developing bioencapsule beads for probiotic delivery, wherein the method comprising the steps of

a) preparing an encapsulation matrix;

b) preparing individual cell culture of *Bifidobacterium pseudocatenulatum* G4,*Bifidobacterium longum* BB536, *Lactobacillus acidophillus* ATCC 4356 and *Lactobacillus plantarum* FTCC 0350;

c) obtaining individual cell culture containing *Bifidobacterium pseudocatenulatum* G4, *Bifidobacterium longum* BB536, *Lactobacillus acidophillus* ATCC 4356 and *Lactobacillus plantarum* FTCC 0350; from step (c);

d) mixing the encapsulation matrix from step (a) with the cell culture from step (c);

e) obtaining a mixture comprising cell-alginate-bovine gelatin-genipin from step (d);

f) extruding the mixture from step (e) with a pointer to form globules;

g) obtaining globules from step (f) and introducing the globules into a hardening agent, wherein the hardening agent having a concentration between 0.1 and 0.2 M of $CaCl_2$;

h) allowing the globules from step (g) and the hardening agent to form beads for at least 2 hours;

i) exposing the beads from step (h) to Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF);

j) determining strength of the beads from step (h) using a texture analyzer;

k) obtaining beads at a strength between 50 and 150g.

12. Use of the novel bioencapsule beads as claimed in any of preceding product claims 1 to 10 and method claim 11 for the manufacture of a pharmaceutical composition, nutraceutical product or food product/s.

13. Use of the novel bioencapsule beads as claimed in 12, wherein the food product/s include beverages, dairy products, confectionaries, chocolates, and any application in food formulation/s as an ingredient or for any functional properties.

14. Use of an effective amount of bioencapsule beads of claim 1to 10 in the preparation of a medicament or probiotic delivery in a mammal, wherein the mammal is human.

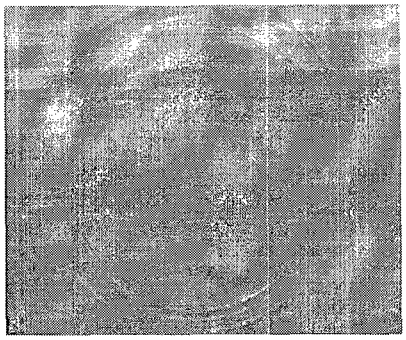

FIGURE 1

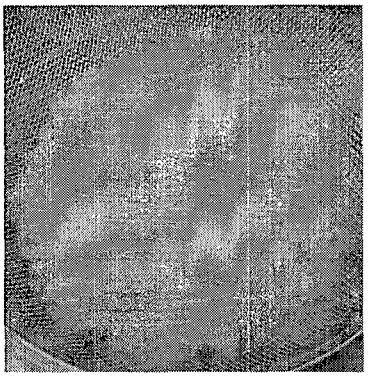

FIGURE 2

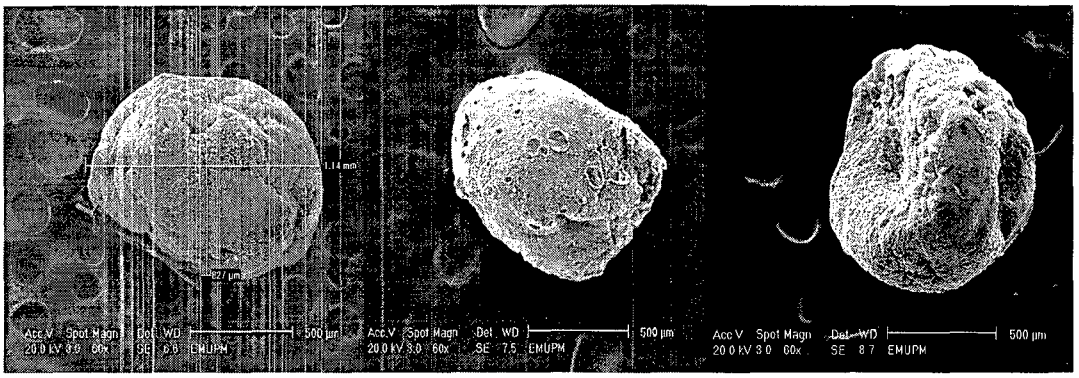

FIGURE 3