PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P6292PC00	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/EP2018/073217	International filing date *(day/month/year)* 29.08.2018	Priority date *(day/month/year)* 29.08.2017
International Patent Classification (IPC) or national classification and IPC INV. A61K9/48		
Applicant Chr. Hansen A/S		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *(sent to the applicant and to the International Bureau)* a total of 10 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and/or sheets containing rectifications authorized by this Authority, unless those sheets were superseded or cancelled, and any accompanying letters (see Rules 46.5, 66.8, 70.16, 91.2, and Section 607 of the Administrative Instructions).
      ☐ sheets containing rectifications, where the decision was made by this Authority not to take them into account because they were not authorized by or notified to this Authority at the time when this Authority began to draw up this report, and any accompanying letters (Rules 66.4bis, 70.2(e), 70.16 and 91.2).
      ☐ superseded sheets and any accompanying letters, where this Authority either considers that the superseding sheets contain an amendment that goes beyond the disclosure in the international application as filed, or the superseding sheets were not accompanied by a letter indicating the basis for the amendments in the application as filed, as indicated in item 4 of Box No. I and the Supplemental Box (see Rule 70.16(b)).
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing, in the form of an Annex C/ST.25 text file, as indicated in the Supplemental Box Relating to Sequence Listing (see paragraph 3*ter* of Annex C of the Administrative Instructions).

4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☐ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☐ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
28.06.2019	13.12.2019

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2018/073217

Box No. I Basis of the report

1. With regard to the **language**, this report is based on

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3(a) and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4(a))
   - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a) and (b))

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1, 2, 5-15, 17, 19, 20	as originally filed
3, 4, 16, 18	as amended (together with any statement) under Art. 19 PCT

Claims, Numbers

1-15	as originally filed

Drawings, Sheets

1/4-4/4	as originally filed

☐ a sequence listing - see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since either they are considered to go beyond the disclosure as filed, or they were not accompanied by a letter indicating the basis for the amendments in the application as filed, as indicated in the Supplemental Box (Rules 70.2(c) and (c-*bis*)):
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:

5. ☐ This report has been established:
   - ☐ taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rules 66.1(d-*bis*) and 70.2(e)).
   - ☐ without taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91(Rules 66.4*bis* and 70.2(e)).

6. ☒ With regard to top-up searches (Rules 66.1*ter* and 70.2(f)):
   - ☒ A top-up search was carried out by this Authority on 03.09.2019 (all discovered documents are listed in the Supplemental Box Relating to Top-up Search).
     - ☐ Additional relevant documents have been discovered during the top-up search.
   - ☐ No top-up search was carried out by this Authority because it would serve no useful purpose.

7. ☐ Supplementary international search report(s) from Authority(ies) has/have been received and taken into account in establishing this report (Rule 45bis.8(b) and (c)).

* *If item 4 applies, some or all of those sheets may be marked "superseded".*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-7, 13-15
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-15
Industrial applicability (IA)	Yes:	Claims	1-15
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 **DOCUMENTS**

Reference is made to the following documents:

D1 WO 2016/065279

D2 US 2012/039998

D3 GB 1 190 386

D4 US 5 310 555

D5 US 2017/189363

2 **AMENDMENTS**

The claims were not amended and correspond to the claims as originally filed. Amended pages of the description were submitted.

3 **NOVELTY** (Art. 33(2) PCT)

3.1 The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1-7 and 13-15 is not new in the sense of Article 33(2) PCT.

3.2 The examiner is still of the opinion that the wording of claim 1 does not clearly define that both the inner and the outer capsule are hard shell capsules. The claims alone have to be clear and not only in the context of the description.

Furthermore, the term "hard capsule" does not really describe that the capsule is a "hard shell capsule" and is therefore not considered clear.

In view of the above mentioned objections, the following novelty objection concerning document D1 is maintained:

3.3 D1 discloses fecal bacteria which are encapsulated by extrusion (microcapsules). The wall contains sodium alginate and glycerol (example 1). The capsules are suspended with a hydrogenated oil and are filled into HPMC

capsules, which are considered "hard capsules". The oil prevents contact of the microcapsules with the inner surface of the capsule shell (example 1). Consequently, the subject-matter of claims 1-7 and 13-15 is not novel in view of D1.

4 **INVENTIVE STEP** (Art. 33(3) PCT)

4.1 Being not novel, the subject-matter of claims 1-7 and 13-15 does not involve an inventive step in the sense of Article 33(3) PCT.

4.2 Even if novelty is given, an inventive step could not be acknowledged for the following reasons:

4.3 The applicant considered D1 as closest prior art and defined the difference between the claims and D1 as the inner capsule as claimed is a hard shell capsule.

4.4 The problem was defined as provision of a capsule which can be used for delivering a large amount of uncoated microorganisms to a host in need thereof and can be stored at a conventional freezer at -18°C for a considerable time. The applicant referred to examples 1-4 of the application which show capsules being neither sticky nor leaking after 3 months of storage at -18°C. The applicant further argued that the coating step used in D1 might lead to cell loss or to a lower concentration of microorganisms.

4.5 However, the alleged technical effects were not proven by experimental data and it is questionable whether any capsule in combination with any hydrophobic liquid falling under the scope of claim 1 shows the claimed effects. Therefore, the problem to be solved would be considered as provision of an alternative capsule.

4.6 The solution according to claim 1 would be considered not to involve an inventive step, because a hard shell capsule comprising a hydrophobic liquid does not really seem to behave differently than a soft gel capsules which also comprises such a hydrophobic liquid. The way to interact with the microorganisms seems to be comparable and it rather appears that the system of a hard capsule incorporated into a hard shell capsule is an arbitrary modification of D1. The encapsulation of a capsule into a capsule is already known from D4 (see claim 1). Consequently, even if it is assumed that claim 1 refers to two hard shell capsules, the subject-matter of claim 1 would lack an inventive step in view of D1 in combination with D4.

5 **INDUSTRIAL APPLICABILITY** (Art. 33(4) PCT)

The subject-matter of claims 1-15 is industrial applicable according to Art. 33(4) PCT.

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VAT no.: 12516479

June 28, 2019

Our ref: P6292PC00
International Patent Application PCT/EP2018/073217

Response to Written Opinion

Dear Madam/Sir,

We hereby file a Demand for International Preliminary Examination of the above PCT application, together with a Fee Calculation sheet authorizing the debiting of the requisite fees from our deposit account No. 28030081. If the amount indicated is incorrect, you are authorized to debit the correct amount from our deposit account.

We intend to file a detailed response to the issues raised in the Written Opinion of the International Searching Authority (WO-ISA). However, a **two month extension of time is hereby requested** to the period set for filing a reply to the WO-ISA, together with any amendments under Article 34 PCT which may be deemed appropriate in connection with that reply.

In the event that no extension of time is available we request that you contact the undersigned by telephone as a matter of urgency.

Yours sincerely,

Chr. Hansen A/S
IPR & Licensing

Anne Schouboe
European Patent Attorney

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August 16, 2019
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Our ref: P6292PC00
International Patent Application PCT/EP2018/073217

Response to objections raised in Written Opinion

In response to the Written Opinion we hereby provide our argumentation to the objections raised. We further provide substitute pages 3, 4, 16, and 18 where clerical errors have been corrected. The clerical errors are explained in detail below.

Amended description pages

The present patent application provides a summary of the teaching of D1, WO2016/065279, on page 3, lines 4-10, and a further discussion of this reference on page 4, lines 35-37. In connection with the preparation of this response we have noted two clerical errors on page 3, line 4, and page 4, line 37, in that reference is made to WO2016/065479 rather than WO2016/065279.

Further, we have noted an enumeration error in that Table 2 on page 16 proceeds Table 1 on page 18. We have amended the numbering so that Table 1 comes first.

Claims

When updating the priority-founding European patent application before filing the application as a PCT patent application the words "hard" and "one" (highlighted in the below claim) were added to claim 1, i.e. the claim was amended to read

> A **hard** capsule comprising an outer and **one** inner capsule, wherein the outer capsule comprises a hydrophobic liquid and the inner capsule which comprises a composition comprising uncoated microorganisms and optionally at least one desiccant.

Novelty

It is held in the Written Opinion that the capsules mentioned in claim 1 are not further defined, apart from being hard capsules and according to the wording of this claim the term seems to apply to the outer shell of the whole composition, so that D1 which discloses fecal bacteria which are encapsulated by extrusion (microcapsules) are relevant for novelty.

We respectfully disagree for the reasons explained in detail in the following.

The present invention provides on page 5, lines 20-26, emphasis on inner capsules added:

"As used herein the term "capsule" refers to a conventional hard capsule intended for oral administration to a human or animal being. The capsules of the present invention do not structurally depart from the conventional definition of hard capsules. When reference is made herein to "capsule" it refers to the outer **or inner capsule** or the outer capsule comprising the inner capsule unless the context indicates otherwise. Generally, the term "capsule" refers to both empty and filled capsules whereas "shell" specifically refers to an empty capsule".

It is thus evident from the patent application as filed that the term "hard capsule" also applies to the inner capsule and there is no basis for the assumption by the Examiner that the term should apply only to the outer shell of the whole composition. In order to further distinguish the present invention from D1 claim 1 was amended to provide that the hard capsule of the present invention comprises an outer and **one** inner capsule.

D1 discloses fecal bacteria which are encapsulated by extrusion (microcapsules). Each microcapsule comprises a hydrophilic matrix formed from an aqueous medium, stabilized into a discrete structure by a colloidal polymer which contains the fecal bacteria.

The disclosure of D1 is clearly different from the present invention which relates to **uncoated** bacteria (see claim 1 above). The present patent application provides on page 4, lines 28 to 29, that the term "uncoated" used in the present specification and claims means that the probiotic bacteria are not coated or microencapsulated to form microcapsules.

In summary, the invention as presently claimed is thus clearly novel over D1.

Inventive step

D1 can be considered the closest prior art.

D1 provides a therapeutic capsule for the oral administration of bacteria to the gastrointestinal system, comprising a capsule shell enveloping a lipophilic matrix per-

meated with discrete microcapsules, wherein each microcapsule is a lipophilic matrix comprising an aqueous medium, stabilized into a discrete structure by a colloidal polymer, and containing the bacteria.

The problem underlying the present patent application is to provide a capsule which can be used for delivering a large amount of uncoated microorganisms to a host in need thereof and can be stored at a conventional freezer at -18˚C for a considerable time.

As a solution to this problem the present patent application proposes a **hard** capsule comprising an outer and **one** inner capsule, wherein the outer capsule comprises a hydrophobic liquid and the inner capsule which comprises a composition comprising **uncoated** microorganisms and optionally at least one desiccant.

Example 1 of the present patent application provides data that the capsules of the present invention are neither sticky nor leaking after 3 months of storage at -18˚C (Table 1).

Examples 3 and 4 of the present patent application describe capsules with lactic acid bacteria mixed with glycerol in the proportion 2:1 and provide data that the capsules are neither sticky nor leaking when measured after 3 to 4 weeks.

In contrast, D1 does not provide any stability data for storage at -18˚C longer than 48 hours (Table C).

A microencapsulation or coating step may well lead to a cell loss during the microencapsulation or coating process and will also lead to a lower concentration of microorganisms through the addition of coating material to the formulation.

Example 1 of D1 describes that a suspension of 8% weight/volume (w/v) of purified bacteria was used to form microcapsules encapsulating the bacteria and that five mL of microcapsules were suspended with 5 mL of hydrogenated oil to form a slurry which was filled into HPMC size 0 capsules, i.e. the maximum load of bacteria is 4% (w/v).

In contrast, Examples 3 and 4 of the present patent application describe capsules having a load of microorganisms of 67%.

In conclusion, the hard capsule of the present invention can have a load of microorganisms which is much higher than the maximum of 20% weight/volume contemplated in WO2016/065279 [00111].

A further advantage compared to D1 is that the capsules of the present invention do not contain coating substances such as sodium alginate which are not necessarily beneficial for the subject to which the capsules are to be administered.

Another advantage of the present invention compared to D1 is that there are fewer steps which particularly for anerobic bacteria minimizes the risk of loss of viable bacteria.

Table F of D1 provides viability of fecal-derived bacteria stored at -20˚C for a week showing 45% of viable bacteria in microcapsules with lipid matrix and 60% viable bacteria in aqueous matrix without microencapsulation, i.e. the embodiment used in the capsules of the present invention.

Summing up, the present invention provides a **hard** capsule comprising an outer and **one** inner capsule, wherein the outer capsule comprises a hydrophobic liquid and the inner capsule which comprises a composition comprising **uncoated** microorganisms and optionally at least one desiccant.

The advantages of the capsules of the present invention over D1 is that the one inner capsule comprises a large amount of uncoated microorganisms and that the capsules can be stored at a conventional freezer at -18˚C for a considerable time and that the bacteria remain viable.

It is respectfully submitted that a person of ordinary skill in the art would find that D1 points away from two of the essential features of the invention of claim 1 as presently claimed, namely **one** inner capsule and **uncoated** microorganisms.

D2

US 2012/0039998 describes a process of manufacturing a soft gel capsule containing microencapsulated probiotic bacteria which have been coated with at least one vegetable lipid having a melting point of between 35˚C and 75˚C.

As also recognized by the Examiner, D2 does not disclose a hard capsule.

D2 describes on page 3, left column, lines 4 to 5, that "the term microencapsulated, as used herein, means coated with a composition." D2 does not provide any disclosure of "uncoated" bacteria. The advantages of capsules with "uncoated" bacteria have been described in detail with regard to D1 above.

A further advantage of the present invention compared to D2 is that the production of the capsules of the invention does not require drying which can lead to a significant loss of viable bacteria.

It is respectfully submitted that a person of ordinary skill in the art would not read D2 either alone or combined with D1 as providing any incentive to the invention of claim 1.

D3

GB 1 190 386 describes a lactic acid bacteria drug resistant to antibiotics which comprises an enteric-coated gelatin capsule containing living lactic acid bacteria absorbed in sterilised starch.

As also recognized by the Examiner, there is no inner and outer capsule in D3.

It is respectfully submitted that a person of ordinary skill in the art would not read D3 either alone or combined with any of D1 or D2 as providing any incentive to the invention of claim 1.

D4

US 5,310,555 discloses a dietary adjunct for livestock which includes incompatible live microbial cultures, and vitamin and mineral supplements, each separated from the other via multiple encapsulation.

In order to solve the problem of providing incompatible supplements such as vitamin and minerals and bacteria to cattle, sheep and goats *in vivo* nearly simultaneously, D4 provides a double capsule which includes live cultures of rumen bacteria in a first capsule which is enclosed with vitamin and mineral supplements, incompatible with the bacteria, in a second capsule.

As also recognized by the Examiner, D4 does not disclose the presence of a hydrophobic liquid which is an essential feature of the capsules of the present invention as evidenced by the data provided in the examples, see e.g. Tables 1 to 5.

It is respectfully submitted that a person of ordinary skill in the art would not read D4 either alone or combined with any of D1, D2, or D3 as providing any incentive to the invention of claim 1.

In summary, it is respectfully submitted that the person of ordinary skill in the art would have no reason to combine the disclosure of D1 with any of D2, D3, or D4 to reach something falling within the scope of claim 1.

For the above reasons it is submitted that the solution of the objective problem underlying the present invention is not obvious to the skilled person. Consequently, the invention according to the amended claims involves an inventive step.

It is believed that in view of the argumentation provided above and the described amendments of the application, all objections raised in the Written Opinion have been met and we look forward to receiving a favourable International Preliminary Examination Report on Patentability.

Yours sincerely,

Chr. Hansen A/S
IPR & Licensing

Anne Schouboe

wherein the aqueous sample and the emulsifying agent form a water-in-oil emulsion, and the pharmaceutical is stable at room temperature for at least about 30 minutes.

WO2016/065279 describes a therapeutic capsule for the oral administration of bacteria to the gastrointestinal system, comprising a capsule shell enveloping a lipophilic matrix permeated with discrete microcapsules, wherein each microcapsule is a lipophilic matrix comprising an aqueous medium, stabilized into a discrete structure by a colloidal polymer, and containing the bacteria. The microparticles have a particle size about 0.1 micrometers to 3,000 micrometers. A capsule may have about 5% to about 20% weight/volume of live bacteria.

US 2012/0039998 describes a process of manufacturing a soft gel capsule containing microencapsulated probiotic bacteria which have been coated with at least one vegetable lipid having a melting point of between 35°C and 75°C.

GB 1 190 386 describes a lactic acid bacteria drug resistant to antibiotics which comprises an enteric-coated gelatin capsule containing living lactic acid bacteria absorbed in sterilised starch.

US 5,310,555 discloses a dietary adjunct for livestock which includes incompatible live microbial cultures, and vitamin and mineral supplements, each separated from the other via multiple encapsulation.

US 2017/189363 discloses an oral pharmaceutical composition for oral administration of a therapeutic protein or peptide, comprising (a) a gastric acid modulator in an amount effective to decrease gastric acids levels in the stomach, and (b) a therapeutically effective amount of a protein or peptide which may be provided in a capsule-in-capsule dosage form, i.e. wherein the protein or peptide is contained in an inner capsule and the gastric acid modulator is contained in an outer capsule. The aim is to increase the oral bioavailability of the protein or peptide.

SUMMARY OF THE INVENTION

If fecal microbiota transplantation could be provided using capsules having an appropriate stability, the treatment could be done by the recipient at home, avoiding transportation to a medical clinic. Accordingly, the problem to be solved is to provide a capsule of fecal microbiota with sufficient stability to avoid leakage prior to the consumption by the recipient and which can be stored at a conventional freezer at -18°C

rather than at -65°C or at -80°C meaning that the capsules can be stored in a conventional freezer in the home of the recipient.

The present inventors have surprisingly discovered that by using a hard capsule having an outer and one inner capsule comprising the fecal microbiota and adding a desiccant to the composition comprising microorganisms and/or a hydrophobic liquid between the inner and outer capsule, the stability of the capsule system is increased dramatically, and the capsule can be stored at -18°C for months without being sticky or leaking. As evident to a person of ordinary skill in the art, it is a significant advantage to avoid the procedure of freezing the fecal microbiota to -65°C or at -80°C as this low temperature will inevitably kill a number of species of microorganisms present in the microbiota which might have been useful for the recipient. The hard capsules of the present invention have the advantage that the recipient can be provided with a sufficient amount of capsules to be taken for months thus avoiding numerous visits for the recipient at a medical clinic and the advantage for the medical clinic that there is no need to assign staff to prepare for and provide capsules to the recipient at the numerous visits.

DETAILED DISCLOSURE OF THE INVENTION

In one embodiment the present invention relates to a hard capsule comprising an outer capsule and an inner capsule, wherein the outer capsule comprises a hydrophobic liquid and an inner capsule which comprises a composition comprising uncoated microorganisms.

US2012/0039998 describes "microencapsulated" probiotic bacteria and that the term microencapsulated means coated with a composition. The term "microcapsule" used in WO2016/065279 is defined in a similar manner. Consistent herewith, the term "uncoated" used in the present specification and claims means that the probiotic bacteria are not coated or microencapsulated to form microcapsules.

A microencapsulation or coating step may well lead to a cell loss during the microencapsulation or coating process and will also lead to a lower concentration of microorganisms through the addition of coating material to the formulation.

A major advantage of the present invention is that the hard capsule of the invention can have a load of microorganisms which is much higher than the maximum of 20% weight/volume contemplated in WO2016/065279, such at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, or about 80%, such as about 77% to 82%, the actual

Results from trials with feces extract

Table 1

Capsules with feces extract Trial	Feces extract + additives	Ingredient between the capsules	Description of the capsules after storage at –18°C
1	None	None	Sticky after few days, leakage after storage for 1–2 weeks
2	None	Glycerol (99.5%) (130 µL)	Sticky after 10 days – trial stopped
3	None	Olive oil (130 µL)	Sticky but no leakage after storage for 2½ months
4	None	Tuna fish oil (130 µL)	Sticky but no leakage after storage for at least 1½ months
5	None	Rapeseed oil (130 µL)	Sticky but no leakage after storage for 2½ months
6	2% calcium silicate	None	Sticky after 1 month
7	2% calcium silicate	Rapeseed oil (130 µL)	Neither stickiness nor leakage after storage for 3 months
8	10% potato starch	Olive oil (130 µL)	Neither stickiness nor leakage after storage for more than 3 months
9	10% corn starch	Olive oil (130 µL)	Neither stickiness nor leakage after storage for more than 3 months

Conclusion

The capsules of trial 1 where nothing was added to the cavity were sticky and leaking after storage at –18°C for 1 week (Figure 1). The result is not surprising due to the fact that it is expected that water (liquid phase) from the feces extract will leak to the surface of the capsules leading to slow dissolution (swelling) of the capsule material making this permeable to water.

The capsules of trial 2 to which 130 µL of 99.5% glycerol has been added to the cavity were sticky after 10 days (Figure 2). This means that the stickiness appeared a little later than without any addition. A possible explanation is that water from the feces extract which has migrated through the inner capsule is diluted in the glycerol thereby

was followed. The results are provided in Table 2.

Results

Table 2

Capsules with water and glycerol (2:1)

Trial	Capsules	Storage at -18°C
10	None	Sticky after 1 day
11	130 µL rapeseed oil	Slightly sticky after 4 weeks
12	130 µL olive oil	Sticky after 2½ weeks

Conclusion

The conclusion was that oil between the inner and outer capsules prolonged the time before the capsules became sticky.

EXAMPLE 3

PREPARATION OF CAPSULES WITH LACTIC ACID BACTERIA

Preparation of capsules with *L. fermentum*

Glycerol was added to a concentrate of *L. fermentum* containing a dry matter of 15% in the proportion concentrate:glycerol 2:1. The mixture of culture and glycerol was filled in size 0 capsules and as soon as possible the size 0 capsules were placed in size 00 capsules. To some of the size 00 capsules nothing was added, to other capsules 130 µL of rapeseed oil or olive oil was added. The capsules were placed at -18°C and the stickiness was followed. The results are provided in Table 3.

Results

Table 3

Capsules with concentrate of *L. fermentum* and glycerol (2:1)

Trial	Capsules	Storage at -18°C
13	None	Sticky after 4 day
14	130 µL rapeseed oil	Not sticky after 4 weeks
15	130 µL olive oil	Not sticky after 4 weeks

Preparation of capsules with *Bifidobacterium longum* subsp. *infantis* BB-02 containing a dry matter of 12.9%

Glycerol was added to a concentrate of *Bifidobacterium longum* subsp. *infantis* BB-02 containing a dry matter of 12.9% in the proportion concentrate:glycerol 2:1. The mixture of concentrate and glycerol was filled in size 0 capsules and as soon as possible the size 0 capsules were placed in size 00 capsules. To some of the size 00 capsules nothing was