



(86) Date de dépôt PCT/PCT Filing Date: 1997/09/03
(87) Date publication PCT/PCT Publication Date: 1998/03/19
(45) Date de délivrance/Issue Date: 2006/11/07
(85) Entrée phase nationale/National Entry: 1999/02/19
(86) N° demande PCT/PCT Application No.: EP 1997/004922
(87) N° publication PCT/PCT Publication No.: 1998/010666
(30) Priorité/Priority: 1996/09/10 (EP96202517.7)

(51) Cl.Int./Int.Cl. *A23L 1/03* (2006.01),
A23C 9/123 (2006.01), *A23C 1/04* (2006.01),
A23C 11/10 (2006.01), *A23P 1/06* (2006.01),
C12N 1/04 (2006.01)

(72) Inventeurs/Inventors:
MEISTER, NIKLAUS, CH;
SUTTER, ANDREAS, CH;
VIKAS, MARTIN, CH

(73) Propriétaire/Owner:
SOCIETE DES PRODUITS NESTLE S.A., CH

(74) Agent: BORDEN LADNER GERVAIS LLP

(54) Titre : ALIMENT DESHYDRATE CONTENANT DES BACTERIES LACTIQUES
(54) Title: DEHYDRATED FOOD CONTAINING LACTIC ACID BACTERIA

(57) **Abrégé/Abstract:**

Process for drying a food composition in which a food composition and a culture of probiotic lactic acid bacteria sensitive to oxygen are sprayed conjointly under a stream of hot air and a dehydrated food composition containing live probiotic lactic acid bacteria is recovered. The probiotic lactic acid bacteria are bacteria which are capable of adhering to the human intestinal cells, of substantially excluding pathogenic bacteria on human intestinal cells, and of immunomodulation in the human body.



**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A23L 1/03, A23C 9/123, C12N 1/04	A1	(11) International Publication Number: WO 98/10666 (43) International Publication Date: 19 March 1998 (19.03.98)
(21) International Application Number: PCT/EP97/04922 (22) International Filing Date: 3 September 1997 (03.09.97) (30) Priority Data: 96202517.7 10 September 1996 (10.09.96) EP (34) Countries for which the regional or international application was filed: CH et al. (71) Applicant (for all designated States except US): SOCIETE DES PRODUITS NESTLE S.A. [CH/CH]; P.O. Box 353, CH-1800 Vevey (CH). (72) Inventors; and (75) Inventors/Applicants (for US only): MEISTER, Niklaus [CH/CH]; Möschbergweg 20, CH-3506 Grosshöchstetten (CH). SUTTER, Andreas [CH/CH]; Chemin des Ormeaux 38, CH-1066 Epalinges (CH). VIKAS, Martin [AT/CH]; Flurweg 4, CH-3510 Konolfingen (CH). (74) Common Representative: SOCIETE DES PRODUITS NESTLE S.A.; Attn.: Florent Gros, P.O. Box 353, CH-1800 Vevey (CH).		(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: DEHYDRATED FOOD CONTAINING LACTIC ACID BACTERIA (57) Abstract <p>Process for drying a food composition in which a food composition and a culture of probiotic lactic acid bacteria sensitive to oxygen are sprayed conjointly under a stream of hot air and a dehydrated food composition containing live probiotic lactic acid bacteria is recovered. The probiotic lactic acid bacteria are bacteria which are capable of adhering to the human intestinal cells, of substantially excluding pathogenic bacteria on human intestinal cells, and of immunomodulation in the human body.</p>		

DEHYDRATED FOOD CONTAINING LACTIC ACID BACTERIA

The subject of the invention is a new process for the preparation of a dehydrated food composition containing live lactic acid bacteria.

5 To dry lactic acid bacteria, industry needs to have available processes which are easy to use and which are economical. Spray-drying generally consists in spraying a suspension of lactic acid bacteria in a vessel and under a stream of hot air, the vessel comprising, to
10 this effect, a hot air inlet, an outlet for discharging the air and an outlet for recovering the powder of dried lactic acid bacteria.

US 3,897,307 (Porubcan et al.) also describes a process for the preparation of a dehydrated milk-containing food composition comprising live lactic acid
15 bacteria. In this process, the milk is fermented by lactic acid bacteria, ascorbic acid and sodium glutamate are added thereto and then the fermented milk is spray-dried under a stream of hot air. Other methods of
20 preparation of spray-dried fermented milk-containing compositions are also described in US 3,985,901 (C. G. Barberan), IE65390 (Charleville Research Ltd), SU724113 (Kiev Bacterial Prep.) and SU 1,097,253 (Protsishin et al.), for example.

25 NL 7,413,373 (DSO Pharmachim) describes the preparation of a soya bean-based food composition comprising live lactic acid bacteria, the said composition being spray-dried under a stream of hot air.

Likewise, J73008830 (Tokyo Yakult Seizo)
30 describes the preparation of food compositions based on tomato or soya bean comprising live bacteria or yeasts, the said compositions being spray-dried under a stream of hot air.

When a culture of lactic acid bacteria is spray-
35 dried, the sprayed culture is generally subjected to a stream of hot air having a temperature of the order of 100°C to 180°C, depending on the devices. The drying temperature poses, nonetheless, a few problems. US 3,985,901 (C. G. Barberan) shows indeed that a drying

13 10 1996

temperature of the order of 180°C to 300°C is capable of killing all the live organisms. These observations were also confirmed in EP298605 (Unilever: page 2, lines 43-48), and EP63438 (Scottish Milk Marke: page 1, lines 14-21).

5

To remedy the destructive influence of the drying temperature, the culture of lactic acid bacteria is generally mixed with protective agents such as vitamins, amino acids, proteins, sugars and/or fats, for example.

10 Unfortunately, the influence of temperature still remains predominant. Only the lactic acid bacteria which are naturally resistant to high temperatures survive a spray-drying sufficiently to make the process economically attractive.

15

In all the preceding documents one stream of product is subjected to the spray-drying. There is another way to proceed, which consists in spray-drying conjointly two streams; on one side, the food composition stream and on
20 the other side the probiotic lactic acid stream. This is the case for following patents : FR 712 791, CH 527568, GB 2127524. In these documents, the air inlet temperature never exceeds 100°C, so that there is no problem of survival of the present bacteria.

25

The invention aims to overcome the disadvantages of the prior art, by providing a process for drying lactic acid bacteria which is particularly suited to the survival of probiotic lactic acid bacteria, which are moreover known to
30 be particularly sensitive to oxygen and/or heat.

To this effect, the present invention relates to a process for drying a food composition in which a food composition and a culture of probiotic lactic acid bacteria are sprayed
35 conjointly under a stream of hot air and a dehydrated food composition containing live probiotic lactic acid bacteria

- 2bis -

is recovered, wherein the culture of lactic acid bacteria and the food composition are sprayed in a spray-drying device with at least two nozzles having a heated air inlet temperature of between 100°C and 400°C and an air outlet
5 temperature of between 40°C and 90°C, the residence time of the lactic acid bacteria in the device being adjusted so as to obtain at least 1% survival of the lactic acid bacteria after drying.

10 It is found, surprisingly, that the survival of the lactic acid bacteria is substantially improved when a culture of lactic acid bacteria and another food composition are spray-dried at the same time and in the same vessel.

15 Furthermore, it has been observed that acceptable survival of lactic acid bacteria can be obtained when a culture of lactic acid bacteria and a food composition are dried conjointly in a spray-drying device having an air inlet temperature greater than 200-300°C. It has indeed been
20 observed that depending on the residence time of the droplets in the drying device, the internal temperature of the droplets may not exceed about 40-70°C,

25

30

35

because of the cooling caused by the evaporation of water.

To carry out the present process, a culture of one or more species of probiotic lactic acid bacteria is prepared. Persons skilled in the art are capable of selecting the culture medium which is best suited to the growth of these lactic acid bacteria.

These probiotic lactic acid bacteria may be chosen from the species *Lactococcus lactis*, in particular *L. lactis* subsp. *cremoris* and *L. lactis* subsp. *lactis* biovar *diacetylactis*; *Streptococcus thermophilus*; the group of acidophilic bacteria consisting of *Lactobacillus acidophilus*, *Lactobacillus crispatus*, *Lactobacillus amylovorus*, *Lactobacillus gallinarum*, *Lactobacillus gasserii* and *Lactobacillus johnsonii*; *Lactobacillus brevis*; *Lactobacillus fermentum*; *Lactobacillus plantarum*; *Lactobacillus helveticus*; *Lactobacillus casei* in particular *L. casei* subsp. *casei* and *L. casei* subsp. *rhamnosus*; *Lactobacillus delbruckii* in particular *L. delbruckii* subsp. *lactis*, and *L. delbruckii* subsp. *bulgaricus*; the bifidobacteria in particular *Bifidobacterium infantis*, *Bifidobacterium breve*, *Bifidobacterium longum*; and finally *Leuconostoc mesenteroides* in particular *L. mesenteroides* subsp. *cremoris*, for example (Bergey's Manual of Systematic Bacteriology, Vol. 2, 1986; Fujisawa et al., Int. Syst. Bact, 42, 487-491, 1992).

However, these probiotic lactic acid bacteria should have the following properties, namely the capacity to adhere to human intestinal cells, to exclude pathogenic bacteria on human intestinal cells, and/or to act on the human immune system by allowing it react more strongly to external aggression (immunomodulatory capacity), for example by increasing the phagocytosis capacities of the granulocytes derived from human blood (J. of Dairy Science, 78, 491-197, 1995; immunomodulatory capacity of the strain La-1

which has been deposited under the treaty of Budapest at the Collection Nationale de Culture de Microorganisme

(CNCM), 25 rue due docteur Roux, 75724 Paris, where it was attributed the deposit number CNCM I-1225).

By way of example, the probiotic strain *Lactobacillus acidophilus* CNCM I-1225 described in EP577904 can be used. This strain was recently reclassified among the *Lactobacillus johnsonii* strain, following the new taxonomy, proposed by Fujisawa et al., which is now authoritative as regards the taxonomy of acidophilic lactobacilli (Int. J. Syst. Bact., 42, 487-791, 1992). Other probiotic bacteria are also available, such as those described in EP199535 (Gorbach et al.), US 5,296,221 (Mitsuoka et al.), US556785 (Institut Pasteur), or US 5,591,428 (Probi AB), for example.

The culture of lactic acid bacteria may comprise, before or after fermentation, at least one protective chemical agent known to improve the survival of lactic acid bacteria during drying and/or during preservation of the powder. Persons skilled in the art have available an abundant literature on these protective agents. To this effect, the protective agents described in patents US 3,897,307, US 4,332,790, J73008830, J57047443, J02086766, J02086767, J02086768, J02086769, J02086770, SU724113, SU 1,097,253, SU 1,227,145, SU 1,292,706 and SU 1,581,257 are incorporated by reference into the description of the present invention. As a guide, these protective agents may be vitamins such as ascorbic acid, amino acids or their salts such as lysine, cysteine, glycine and sodium glutamate, proteins or protein hydrolysates which may be obtained from milk or soya bean, sugars such as lactose, trehalose, sucrose, dextrin and maltodextrin, fats in particular butter fat (butter oil), palm fat, groundnut fat, cocoa fat, rapeseed fat or soya bean fat, for example. Finally, these protective agents may be added to the culture in an amount of 0.1 to 80% by weight, for example.

At least 80% by weight of one of the food compositions described hereinafter may be added to the culture of lactic acid bacteria. For the sake of simplicity, it will be considered that this mixture always designates

the culture of lactic acid bacteria. It should however be noted that the best survivals obtained after drying are generally linked to the presence of a high cell titre in the culture. The culture of lactic acid bacteria thus preferably contains at least 10^7 live cell colonies per gram of cfu/g (cfu is the abbreviation for "Colony forming unit"). The choice may also be made to concentrate this culture, for example by centrifugation, so as to increase its live cell titre up to at least 10^8 cfu/g, preferably 10^8 - 10^{11} cfu/g.

Preferably, the food composition which is sprayed conjointly with the culture of lactic acid bacteria is a liquid composition of which at least one of the components is chosen from the group consisting of milk, meat, fish, a fruit and a vegetable, for example. Preferably, the food composition is concentrated, before it is sprayed, to a water content of up to 70% by weight.

This food composition may therefore comprise a finely divided part, cooked or raw obtained from an edible vegetable, whether this is a seed, root, tuber, stem, leaf, flower or fruit, for example. Among the preferred vegetables, there may be distinguished more particularly leaves, in particular leek, apparatus, fennel and cabbage; stems, in particular rhubarb and broccoli; seeds such as cocoa, pea, soya bean or obtained from cereals; some roots, in particular carrot, onion, radish, celery and beet; tubers, in particular cassava and potato; and fruits, in particular tomato, courgette, aubergine, banana, apple, apricot, melon, water melon, pear, plum, peach, cherry, kiwi fruit, sea buckthorn.

berry, medlar and mirabelle plum, for example. There may also be used, as plants, higher edible mushrooms, in particular *Agaricus bisporus*, *Pleurotus ostreatus*, *Boletus edulis* or *Lentinus edodes*, for example.

5 This food composition may also comprise a finely divided part, cooked or raw, obtained from an animal, whether it is milk, egg, meat, fish and/or a fraction thereof, in particular a protein fraction and/or a hydrolysate of these proteins, for example. This food
10 composition may thus be a hydrolysed and hypoallergenic cows milk conforming to European Directive 96/4/EC (Official Journal of the European Communities, No. OJ L49/12, 1996), for example.

 To carry out this process, there may be dried
15 conjointly 1 part of a culture of lactic acid bacteria and at least 1 part of a food composition, in particular 1-1000 parts, the said parts being calculated in the dry state, for example.

 The spray-drying devices traditionally used for
20 the industrial manufacture of a milk or coffee powder may be particularly well suited to the needs of the present invention (see Jensen J.D., Food technology, June, 60-71, 1975). By way of example, the spray-drying devices described in IE65390 (Charleville Res. LTD) and US
25 4,702,799 (Nestlé) may be easily adapted.

 Preferably, these devices have, in operation, a zone at very high temperature (100-400°C) at the end of at least one of the spray nozzles, it being possible for the said zone to represent up to 50% of the volume of the
30 vessel, preferably 0.1% to 20%, the remainder of the device having a lower temperature which may reach the air outlet temperature, for example. The device described in US 3,065,076 (Nestlé) particularly fulfils these needs.

 Preferably, if the heated air inlet temperature
35 is greater than 200°C, these devices also have, in operation, a secondary air inlet. The secondary air inlet temperature is chosen so as to adjust the air temperature at the outlet of the device. The secondary air inlet may be situated near the heated air inlet defined above, for

example.

To carry out the present invention, at least one spray nozzle should be provided per composition. In operation, the position of the spray nozzles is not critical. It is thus possible to spray the culture and the food composition in the zone at very high temperature, for example. It is also possible to spray the food composition in the zone at very high temperature, and at the same time to spray the culture in a zone having a lower temperature, for example.

The invention in fact also consists in the appropriate selection of the residence time of the lactic acid bacteria in the drying device. Preferably, the sprayed droplets arrive in a dry form towards the outlet of the device, that is to say at the point where the outlet temperature is 40-90°C, for example. This residence time may be adjusted with the aid of the various parameters regulating a spray-drying device, such as the pressure for spraying the droplets, the pressure of the stream of hot air, and/or the distance which the droplets have to cover in the drying chamber, for example. It is not possible to provide precise values for each parameter involved in adjusting the residence time since these parameters and their associated values depend on the type of spray-drying device used. As a guide, the pressure applied at the end of the nozzles spraying the culture or the food composition may be between 5-250 bar and the hot air pressure at the inlet of the device may be between 100 and 200 mbar. Thus, to simplify the definition of this adjustment of the residence time of the culture according to the invention, it will be accepted that this time conforms to the present invention if the rate of survival of the bacteria which have just been dried is at least 1%, persons skilled in the art being indeed capable of selecting the appropriate operating parameters to achieve this result.

Preferably, the residence time of the culture in the drying device is adjusted so as to also obtain a powder having a water activity (A_w) at 25°C of between

WO 98/10666

PCT/EP97/04922

- 8 -

0.05 and 0.5. Indeed, the best rates of survival after drying and during preservation are obtained for a powder having this range of water activity.

Likewise, the best rates of survival after drying and during preservation are obtained when the drying device has at least one of the following conditions, namely, an inlet temperature of 250-400°C, an outlet temperature of 50-75°C, and a culture residence time adjusted so as to obtain at least 10% survival after drying.

Other parameters may also influence the survival of the lactic acid bacteria. Thus, the relative humidity of the air at the outlet of the drying device may be of the order of 10-40%, preferably 20-40%. Furthermore, there may be introduced into the culture of lactic acid bacteria, before the spray nozzle, an inert gas capable of being used in food processes, in particular CO₂, nitrogen, argon, helium, alone or in a mixture, for example.

The present process may thus provide a food powder which is easily dispersible, having a density of the order of 200-1000 g/l, having an Aw at 25°C of the order of 0.05-0.5, having 1 to 10⁹ cfu/g, and exhibiting at least 10% survival of probiotic lactic acid bacteria per year at 20°C. This food powder may be preserved at a temperature of between -20°C to 40°C for several months. It can be sold as food which can be rehydrated and consumed directly. It can also be used as ingredient in a more complex food composition, for example.

The present invention is described in greater detail below with the aid of the following additional description which refers to examples of drying of cultures of lactic acid bacteria and yeasts. The percentages are given by weight unless otherwise stated. It goes without saying, however, that these examples are given by way of illustration of the subject of the invention and do not in any way constitute a limitation thereto.

Comparative example

This example is designed to show that the spraying of a food composition comprising at least 25% by weight of a culture of probiotic lactic acid bacteria gives less satisfactory survival rates than those obtained in Examples 1 to 3 when a culture of probiotic bacteria and a food composition are co-sprayed.

For that, 3% of a fresh preculture, in an MRS medium, of the *Lactobacillus johnsonii* CNCM I-1225 strain is mixed with sterile MSK medium comprising 10% reconstituted powdered skimmed milk, 0.1% commercial yeast extract, 0.5% peptone and 0.1% Tween 80, then it is fermented for 8 hours at 40°C, without mixing.

A large-scale culture of this strain is then prepared by fermenting a medium comprising 3% lactose, 0.5% of a commercial yeast extract, 0.1% hydrolysed whey, 0.5% peptone and 0.1% Tween 80, with 3% of the above fermented mixture, at 40°C, until a pH of 5.5 is obtained, with mixing at 30 revolutions per min. and under a CO₂ atmosphere. The fermentation of the mixture at pH 5.5 is continued for a few hours by controlled additions of an alkaline base. The culture is cooled to 15-20°C, it is concentrated by centrifugation so as to obtain about 10% dry matter and 10¹⁰ cfu/g, then it is supplemented with 2% by weight of ascorbic acid, 1.25% by weight of sodium glutamate, and 300% by weight of concentrated milk having 50% by weight of dry matter.

The culture is spray-dried with the operating conditions described in Table 1 below and in a device adapted from that described in Figure 1.c of US 3,065,076, the only difference being that no agglomerating device is used; the powder which went into the dust recovering device attached to the dryer is recycled into the vessel; secondary air having a temperature of 18-30°C (depending on the room temperature) is injected near the heated air inlet by means of a mere opening of the vessel to the external medium, and CO₂ and/or nitrogen is injected into the culture just before it is sprayed. After spraying, the powder is recovered on a

fluidized bed passing through 3 compartments, the first two compartments serving to further dry the powder at temperatures of 60-90°C, and the last compartment serving to cool the powder to about 30°C. Finally, the number of colonies of lactic acid bacteria which survived the drying is determined. The results are presented in Table 1 below.

Examples 1-3

Milk and a culture of the *Lactobacillus johnsonii* CNCM I-1225 strain are spray-dried conjointly. For that, a bacterial culture is prepared as described in the comparative example, protective agents are added thereto and 1 part of this culture of bacteria is continuously co-sprayed with about 40 to 100 parts of concentrated milk having 50% dry matter, the said spraying being carried out conjointly in devices adapted from that described in Figure 1.c of US 3,065,076.

After spraying, the powder is recovered on a fluidized bed passing through 3 compartments, the first two compartments serving to further dry the powder at temperatures of 60-90°C, and the last compartment serving to cool the powder to about 30°C. The number of surviving bacteria in the dehydrated food powder is then counted, taking into account the dilution made with milk. The results are presented in Table 2 below. The various powders exhibit, in addition, very good stabilities over time, more than 10% of the lactic acid bacteria surviving indeed after storing at 20°C for 1 year under a carbon dioxide atmosphere. It can also be noted that the A_w at 25°C of these food powders is always between 0.05 and 0.5.

In Example 1, two sprayings are carried out conjointly in the device represented in Figure 1.c of US 3,065,076, the only difference being that no agglomerating device is used. The powder which went into the dust recovering device is recycled into the vessel. The secondary air having a temperature of 18-30°C (depending on the room temperature) is injected near the heated air inlet by means of a mere opening of the vessel to the

external medium. CO₂ is injected into the culture just before it is sprayed, and the culture and the milk are sprayed conjointly with the aid of two nozzles whose ends are placed, in the vessel, at the level of the heated air inlet (same position as nozzle 14 of Figure 1.c of US 3,065,076). The operating conditions are described in Table 1 below.

In examples 2-3, the two sprayings are carried out conjointly in the device represented in Figure 1.c of US 3,065,076, the only difference being that no agglomerating device is used; the powder which went into the dust recovering device is recycled in the vessel, the entry of the recycled powder taking place at the half-way height of the vessel; the secondary air having a temperature of 18-30°C (depending on the room temperature), is injected near the heated air inlet by means of a mere opening of the vessel to the external medium; the milk is sprayed with the aid of a nozzle whose end is placed, in the vessel, at the level of the axis and of the end of the heated air inlet (same position as nozzle 14 of Figure 1.c of US 3,065,076). Simultaneously, the bacterial culture is sprayed with the aid of a nozzle whose end is placed, in the vessel, at the level of the axis and of the end of the recycled powder inlet. The operating conditions are described in Table 1 below.

Table 1

Operating conditions	Comparative example	Example 1	Example 2	Example 3
<u>Bacterial culture</u>				
Protective agents	*Milk+A+SG	*M+A+T	*M+A+SG	*M+A+T
% Dry matter	41.82	31.08	28.79	31.08
pH	6.3	6.15	6.48	6.15
Gas (l/min)	6.5(CO ₂)	2.5(CO ₂)	-	-
Flow rate (l/h)	496.3	78	30	53
Spraying pressure (bar)	59	70	8(nozzles with two phases: N ₂)	8(nozzles with two phases: N ₂)
<u>Milk</u>				
% Dry matter	-	46.88	46.88	46.88
Flow rate (kg/h)	-	378	556	420
Spraying pressure (bar)	-	30	48	38
Air at inlet (°C)	310	309	310	305
Hot air pressure (mbar)	190	164	190	160
Air at outlet (°C)	65	65	64	65
Humidity of the air at outlet (%)	20.7	20	24.2	20.6
Humidity of the powder (%)	3.3	3.5	3.8	4.0
Powder yield (kg/h)	215	209	280	220
Density of the powder (g/l)	440	535	335	320
Cfu/ml before spraying	1.2×10^{10}	4.45×10^9	9.63×10^9	5.81×10^9
Cfu/g after spraying	5.3×10^6	6×10^7	6.5×10^7	8.2×10^7
Loss of viability (log cfu/g)	3.72	1.42	1.19	1.23
Viability after drying (%)	<0.1	3.8	6.45	5.88

*Milk + A + SG: 300% concentrated milk having 50% dry matter
+ 2% ascorbic acid + 1.25% sodium glutamate

*M + A + SG: 100% concentrated milk having 50% dry matter
+ 2% ascorbic acid + 1.25% sodium glutamate

*M + A + T: 100% concentrated milk having 50% dry matter
+ 5% ascorbic acid + 5% trehalose

Example 4

A culture of lactic acid bacteria CNCM I-1225 comprising 5% ascorbic acid and 5% trehalose, and a finely divided concentrated tomato juice having 50% dry matter are sprayed conjointly under the conditions described in Example 2.

Example 5

A culture of lactic acid bacteria CNCM I-1225 comprising 5% ascorbic acid and 5% trehalose, and a soya bean-based vegetable milk having 50% dry matter are sprayed conjointly under the conditions described in Example 2.

WO 98/10666

PCT/EP97/04922

-14-

TRAITE DE BUDAPEST SUR LA RECONNAISSANCE
INTERNATIONALE DU DEPOT DES MICRO-ORGANISMES
AUX FINS DE LA PROCEDURE EN MATIERE DE BREVETS

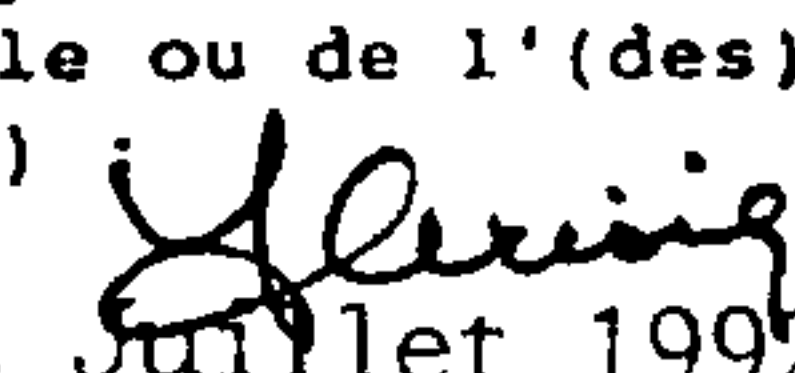
FORMULE INTERNATIONALE

DESTINATAIRE :
Messieurs ARCHAMBAULT et WAVRE
NESTEC S.A.
Service des Brevets
Avenue Nestlé 55 - CH-1800 VEVEY - SUISSE

RECEPISSE EN CAS DE DEPOT INITIAL,
délivré en vertu de la règle 7.1 par
l'AUTORITE DE DEPOT INTERNATIONALE
identifiée au bas de cette page

NOM ET ADRESSE
DU DEPOSANT

NESTEC S.A. - Service des Brevets - Avenue Nestlé 55 -
CH-1800 VEVEY - SUISSE

I. IDENTIFICATION DU MICRO-ORGANISME	
Référence d'identification donnée par le DEPOSANT : <div style="text-align: center; font-size: 1.2em;">La 1</div>	Numéro d'ordre attribué par l'AUTORITE DE DEPOT INTERNATIONALE : <div style="text-align: center; font-size: 1.2em;">I - 1225</div>
II. DESCRIPTION SCIENTIFIQUE ET/OU DESIGNATION TAXONOMIQUE PROPOSEE	
Le micro-organisme identifié sous chiffre I était accompagné : <input checked="" type="checkbox"/> d'une description scientifique <input checked="" type="checkbox"/> d'une désignation taxonomique proposée (Cocher ce qui convient)	
III. RECEPTION ET ACCEPTATION	
La présente autorité de dépôt internationale accepte le micro-organisme identifié sous chiffre I, qu'elle a reçu le 30.06.1992 (date du dépôt initial) ¹	
IV. RECEPTION D'UNE REQUETE EN CONVERSION	
La présente autorité de dépôt internationale a reçu le micro-organisme identifié sous chiffre I le _____ (date du dépôt initial) et a reçu une requête en conversion du dépôt initial en dépôt conforme au Traité de Budapest le _____ (date de réception de la requête en conversion)	
V. AUTORITE DE DEPOT INTERNATIONALE	
Nom : Collection Nationale de Cultures de Microorganismes Institut Pasteur 25, Rue du Docteur Roux Adresse : 75724 PARIS CEDEX 15	Signature(s) de la (des) personne(s) compétente(s) pour représenter l'autorité de dépôt internationale ou de l'(des) employé(s) autorisé(s) :  Date : Paris le 02 Juillet 1992 Mme Y. CERISIER

¹ En cas d'application de la règle 6.4.d), cette date est la date à laquelle le statut d'autorité de dépôt internationale a été acquis.

-14-

CLAIMS:

1. Process for drying a food composition which comprises spraying conjointly under a stream of hot air a food composition and a culture of probiotic lactic acid bacteria sensitive to oxygen, said probiotic lactic acid bacteria having one or more properties selected from the capacity to adhere to human intestinal cells, the capacity to exclude pathogenic bacteria on human intestinal cells, and the capacity to act on the human immune system by allowing it to react more strongly to external aggression, and recovering a dehydrated food composition containing live probiotic lactic acid bacteria, wherein the culture of lactic acid bacteria and the food composition are sprayed through separate nozzles into a common vessel of a spray-drying device having a heated air inlet temperature of between 100°C and 400°C and an air outlet temperature of between 40°C and 90°C, the residence time of the lactic acid bacteria in the device being adjusted so as to obtain at least 1% survival of the lactic acid bacteria after drying.
2. Process according to Claim 1, in which 1 part of the culture of lactic acid bacteria and at least 1 part of the food composition are sprayed conjointly, the said parts being calculated in the dry state.
3. Process according to Claim 1, in which the culture is concentrated, before it is sprayed, to at least 10^8 cfu/g.

-15-

4. Process according to Claim 1, in which the food composition is concentrated, before it is sprayed, to a water content of up to 70% by weight.

5. Process according to Claim 1, in which the residence time of the composition in the spray-drying device is adjusted so as to obtain a powder having an A_w at 25°C of between 0.05 and 0.5.