



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A23C 9/123, 9/15, 9/12	A1	(11) International Publication Number: WO 98/41103 (43) International Publication Date: 24 September 1998 (24.09.98)
(21) International Application Number: PCT/BE98/00036 (22) International Filing Date: 13 March 1998 (13.03.98) (30) Priority Data: 9705456.3 17 March 1997 (17.03.97) GB (71) Applicant (for all designated States except US): UCB, S.A. [BE/BE]; Allée de la Recherche 60, B-1070 Bruxelles (BE). (72) Inventors; and (75) Inventors/Applicants (for US only): MARSHALL, Colin [GB/GB]; Sebastian Plaskets Lane, Wigton, Cumbria CA7 9ES (GB). SEALBY, Lesley [GB/GB]; 11 Gable Avenue, Cockermouth, Cumbria CA13 9BU (GB). TAYLOR, Lewis [GB/GB]; 4 Gleneagle Close, Chippenham, Wiltshire SN15 3XL (GB). (74) Agent: DEBLED, Thierry; Rue d'Anderlecht 33, B-1620 Drogenbos (BE).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, 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>
(54) Title: FERMENTED MILK PRODUCT PRODUCTION PROCESS		
(57) Abstract <p>The present invention is directed to a fermented milk production process in self-rehydrating container comprising a carbohydrate, a fermented milk product forming starter culture, milk powder and eventually other edible solutes, and to a self-rehydrating container adapted thereto. The container contains a semi-permeable membrane which allows the preparation of substantially sterile water solutions using osmotically driven filtration.</p>		

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Fermented Milk Product Production Process.**Field of invention.**

The invention relates to a self-rehydrating container adapted for the production of fermented milk products, to a process for the production of fermented milk product using a self-rehydrating container, to a container adapted thereto and to the fermented milk product
5 produced as such.

Background of the invention.

The use of yoghurts or fermented milk products in general as part of the human diet is perceived extremely beneficial for many reasons including:
10 major bacterial contamination in the upper intestine are prevented by beneficial organisms colonisation;
problems with lactose intolerance are reduced, due to lactose conversion;
antibiotics are produced locally by lactobacillus type bacteria;
certain minerals are potentially more available within the foodstuff for digestion;
15 fermented milk products have a better stability than equivalent milk products.
However, to prepare yoghurts or fermented milk products in general on the field is often difficult in many locations around the world. This is mostly due to contamination (poor hygiene and sanitation), and a lack of necessary heat stable organisms. Moreover, it is essential that the fermented milk product forming organisms can grow in the correct
20 environment.
A secondary benefit of fermented milk product forming organisms is their probiotic property. This is where growth of potentially pathogenic organisms present in the food product is suppressed or severely lowered by the presence of large numbers of beneficial organisms due to their rapid growth, which is known as a colonisation effect. It has been explained that it is
25 the low pH resulting from lactic acid production which prevents growth of harmful bacteria.
The classic fermented milk product production process is known to the man skilled in the art. As much as the starter culture needs to be free of contaminating micro-organisms, it is indicated that the vessels wherein the fermented milk product is produced are as clean and disinfected as possible. Notwithstanding the fermented milk product producing bacteria have
30 a colonising effect, other pathogenic micro-organisms might equally develop. This is certainly so when the temperature at which the fermented milk product is prepared is lower than 40-45°C.

Aims of the invention.

35 Taken into account the beneficial effects of a fermented milk product and the need to provide convenient and non-infected fermented milk product preparation vessels, especially in countries where pure water is not easily available and when the need for healthy food is very important, the present invention aims at providing a fermented milk product which allows yoghurt to be prepared on the field, even in a heavily biologically contaminated environment.

It is another aim of the invention to exploit the colonising property of the beneficial fermented milk product cultures when the shelf life of rehydrated food products, potentially contaminated, need to be increased.

5 **Description of the invention.**

According to the invention, a fermented milk product (such as yoghurt or buttermilk) is produced within a self-rehydrating container comprising, as at least a portion of its walls, a semi-permeable membrane.

10 In a preferred embodiment, the self-rehydrating container has two compartments as disclosed in our copending British patent application No. 9705455.5. In this compartmented container, a compartment has water- and optionally oxygen-tight walls and contains a material to be rehydrated. Another compartment which is osmotically active has at least a portion of its walls which is semi-permeable allowing obtention of sterile water through the process of osmotically driven filtration as described in EP 360,612 or in USP 4,920,105, and contains
15 osmotically active solutes. These compartments are separated by a non-permanent seal. The container which is thus used in the fermented milk product production process of the present invention contains fermented milk product forming organisms and milk powder in admixture with a carbohydrate such as e.g. sugar which can function as the osmotically active solute.

20 In the variant using the compartmented container, the fermented milk product forming organisms and the milk powder are retained in the watertight compartment and the osmotically active solute in the osmotically active compartment.

A "fermented milk product" according to the present invention may contain at least one of the following organisms: *Lactococcus lactis* (different sub-species), *Streptococcus salivarius*,
25 *Streptococcus cremoris*, *Streptococcus lactis*, *Streptococcus diacetylactis* or *Streptococcus thermophilus* (different sub-species), *Lactobacillus bulgaricus* (different sub-species), *Leuconostoc cremoris* or *Leuconostoc lactis*. but other fermented milk product forming organisms are known from those skilled in the art. These organisms can be tailored to provide products of different consistency, i.e. liquid fermented milk product, thick fermented milk
30 product, yoghurt, kefir, buttermilk, cheese-like products, etc.

Organism grades have been identified that have excellent shelf stability if kept dry and free from oxygen. The compartmented container allows to fulfill this requirement as the watertight compartment may be further provided with excellent oxygen barrier and can be gas flushed to remove oxygen before closing. This container can further be contained itself within a
35 hermetically sealed pack.

The self-rehydrating container is an indicated fermented milk product production vessel because the present applicant has found that using these organisms in conjunction with carbohydrate and dried skimmed milk, conversion of lactose into lactic acid and water is possible within the container. As a consequence, a reduction in pH is achieved along with a

significant growth of said organisms. A combination of these effects helps suppress potential growth of pathogenic organisms within the pack and generates fermented milk products, substantially free from microbiological contaminants.

5 The carbohydrate which is needed for osmotically driven filtration to occur is favorably any carbohydrate which can sustain the yoghurt producing organisms in converting lactose into lactic acid and favour their growth. Preferably, the carbohydrate is lactose, sucrose, dextrose, and the like; most preferably one uses lactose.

10 Either solid or liquid fermented milk products which are perceived to have extreme consumption benefits can be produced within the self-rehydrating container using known cultures.

The milk powder contained in the container can be any dried milk type. Best results are obtained though with skimmed milk powder. A milk powder useful for specific applications such as High Energy Therapeutic Milk may equally be used.

15 The fermented milk product preparation process according to the invention comprises immersing a self-rehydrating container in water and leaving the container immersed until the filtered water has reached a sufficient level.

20 The osmotically active carbohydrate, the starter culture and the milk powder are contained altogether in the container. Once the container is immersed in water, rehydration of the different solutes starts and the starter cultures is activated. Using fermented milk product organisms in conjunction with a carbohydrate and dried milk powder results in a pH reduction and a significant growth of said organisms. The combination of these effects helps therefore to suppress potential growth of contaminating organisms within the bag and generates a fermented milk product.

25 In a variant, the fermented milk product production process according to the present invention comprises immersing a comparted self-rehydrating container in water, wherein the watertight compartment contains the starter culture and the milk powder, while the osmotically active compartment contains an osmotically active carbohydrate, leaving the container immersed until the filtered water has reached a sufficient level, breaking a non-permanent seal separating the two compartments, leaving the waterproof compartment to be
30 filled with the sterile water of the other compartment, allowing the starter culture and the milk powder to dissolve, keeping the container at a temperature above 25°C, preferably between 30°C and 45°C depending on the thermal resistance of the fermented milk product forming organism and allowing for the fermented milk product to be formed.

35 When the starter culture is sufficiently resistant to be kept without particular measures to improve its shelf-stability and/or when the starter culture has a sufficiently high colonising potential (thus preventing the growth of contaminating micro-organisms), the single-compartment container is particularly suitable.

On the other hand, when it is necessary to keep the starter culture dry and free from oxygen to have an acceptable shelf life or when the colonising potential of the culture is not high enough, it is preferable to use the compartmented container.

Normally, 6 to 7 hours are needed for a mixture of milk and starter culture to change into a fermented milk product. It is thus necessary to foresee a starter culture which can overgrow the contaminating micro-organisms potentially present in the other solutes during rehydration time, the milk powder/water/starter culture mixture is not at its full growing capacity.

A preferred "single compartment" self-rehydrating container comprises a composite semi-permeable membrane comprising a support layer with a relatively high molecular weight cut-off selected from the group consisting of cellulose, regenerated cellulose (CELLOPHANE® or cuprophane), benzoylated cellulose and collagen, and a thin layer with low molecular weight cut-off as disclosed in our copending British patent application No. 9705454.8. Preferably the thin layer consists of an hydrophilic polyurethane such as those which are conventionally used for covering textile with a protective coating which is waterproof but water vapour permeable. Lowering the molecular weight cut-off of the overall membrane structure allows for more solute compounds to be maintained within the milk solutions of the present invention, thus resulting in a more nutritious yoghurt especially when High Energy Therapeutic Milk is used for example.

It is well within the possibilities of the man in the art to develop more sophisticated fermented milk products by using for example additional beneficial micro-organisms (such as bifidus), additional solute powders (like fruit juice powder, thickeners, etc.) colouring agents, etc. Again the colonising effect (pH and growth rate) of the fermented milk product organisms will prevent the contaminating organisms possibly present in such solutes to develop.

Preferably, fermented milk product organisms with pH lowering properties ensuring a pH decrease to pH 4 - 4.5 within 6 hours of rehydration are used.

The examples which follow illustrate the invention without any intention to limit its scope in any way. Yoghurt has been embodied as fermented milk product, but other kinds of fermented milk products are well within the scope of invention.

Examples.

Example 1

A container is prepared with a composite membrane consisting of a support membrane of regenerated cellulose film with a molecular weight cut-off of 1800 and of a thin hydrophilic polyurethane layer applied by direct gravure coating at 10 g/m² (wet weight).

The hydrophilic polyurethane is prepared as follows:

A solution of 133.02 g of polyethyleneglycol (PEG 600 (HOECHST)) having a molecular weight of about 600 and 79.00 g of 1,1'-methylenebis(4-isocyanatocyclohexane) in 342.00 g of toluene is introduced into a 2-liter four necked round bottomed flask equipped with a mechanical stirrer, a thermometer, an air condenser, a nitrogen inlet and a dropping funnel. The mixture

is heated at 90°C while stirring and 25 mg of dibutyltinlaurate (DABCO T12 (AIR PRODUCTS)) as catalyst, is introduced. The reaction mixture is maintained at 90°C for 6 hours and then cooled.

A solution of 13.68 g of isophorone diamine in 350.00 g of isopropyl alcohol is introduced in a second 2-liter four necked round bottomed flask equipped with a mechanical stirrer, a thermometer, an air condenser, a nitrogen inlet and a dropping funnel. The content of the first flask is cooled at room temperature and it is then added slowly to the mixture alcohol/amine (second flask). Chain extension is complete after about 3 hours.

44.2 g of fumed silica (TS100 DEGUSA) and 50 g of toluene are added to the mixture.

10

Example 2

Self-rehydrating containers containing Infant Formula Milk (IFM) 30.7 g or Therapeutic milk (TM) 36.5 g and different yoghurt forming organisms are placed in water. When the internal volume has reached 200 ml, 7.5 hours for Infant Formula Milk and 8.5 hours for Therapeutic milk, the bags are gently removed from water.

15

The bags are then stored at different temperatures:

in the fridge (at about + 4°C);

at ambient temperature (at 25°C, with a relative humidity of 75%);

in tropical oven (at 38°C, with a relative humidity of 90% C).

Hourly (over 24 hours) a bag is open and the pH of its content is measured.

20

The TABLE I shows the measured values of the pH.

The Blank samples do not contain any yoghurt forming organisms,

the strain 1 samples contain 6 mg of RA024 (from TEXEL) as yoghurt forming organisms;

the strain 2 samples contain 6 mg of MY087 (from TEXEL) as yoghurt forming organisms;

25

the strain 3 samples contain a mixture of 3 mg of RA024 (from TEXEL) and of 3 mg MY087 (from TEXEL) as yoghurt forming organisms;

the strain 4 samples contain 6 mg of MA016 (from TEXEL) as yoghurt forming organisms;

TABLE I

pH change over 24 hours

Milk	Condition	Sample	0	1	2	3	4	5	6	7
5	IFM Fridge	Blank		6.68	6.68	6.73	6.85	6.55	6.67	6.89
		Strain 1		6.68	6.67	6.69	6.74	6.44	6.53	6.77
		Strain 2		6.50	6.50	6.57	6.65	6.43	6.54	6.69
		Strain 3		6.50	6.49	6.54	6.46	6.32	6.64	6.80
10	IFM Ambient	Strain 4	6.46	6.44	6.40	6.34	6.26	6.24	6.14	6.03
		Blank		6.65	6.64	6.62	6.44	6.40	6.47	6.54
		Strain 1		6.64	6.60	6.57	6.40	6.27	6.09	5.95
		Strain 2		6.65	6.64	6.68	6.40	6.60	6.62	6.72
15	IFM Tropical	Strain 3		6.63	6.60	6.60	6.35	6.22	6.16	6.10
		Strain 4	6.50	6.39	6.19	6.01	5.73	5.38	5.12	4.76
		Blank		6.47	6.44	6.49	6.49	6.46	6.43	6.30
		Strain 1		6.37	6.05	6.63	4.71	4.26	4.26	4.20
20	IFM Fridge	Strain 2		6.44	6.39	6.35	6.21	5.36	4.78	4.40
		Strain 3		6.42	6.23	5.93	5.53	4.50	4.36	4.30
		Strain 4	6.55	6.15	5.64	5.16	4.77	4.41	4.30	4.23
		Blank		6.53	6.49	6.55	6.66	6.39	6.54	6.73
25	IFM Ambient	Strain 1		6.68	6.47	6.53	6.61	6.33	6.42	6.58
		Strain 2		6.50	6.50	6.57	6.65	6.43	6.54	6.69
		Strain 3		6.50	6.49	6.54	6.46	6.57	6.64	6.80
		Strain 4	6.51	6.54	6.51	6.44	6.44	6.47	6.51	6.55
30	IFM Tropical	Blank		6.48	6.46	6.51	6.53	6.56	6.64	6.75
		Strain 1		6.47	6.45	6.46	6.30	6.25	6.21	6.17
		Strain 2		6.45	6.46	6.51	6.35	6.30	6.43	6.53
		Strain 3		6.47	6.44	6.46	6.20	6.18	6.13	5.98
30	IFM Tropical	Strain 4	6.44	6.40	6.36	6.30	6.25	6.21	6.17	5.91
		Blank		6.26	6.21	6.24	6.15	6.10	5.90	5.40
		Strain 1		6.19	6.04	5.84	5.08	4.85	4.64	4.52
		Strain 2		6.26	6.19	6.14	5.55	4.89	4.63	4.43
30	IFM Tropical	Strain 3		6.23	6.11	5.96	5.49	4.88	4.64	4.48
		Strain 4	6.47	6.22	6.18	6.09	5.97	5.84	5.68	5.27

TABLE I (continuation - 1)

Milk	condition	sample	8	9	10	11	12	13	14	15	
IFM	Fridge	Blank	6.89								
		Strain 1	6.75								
		Strain 2	6.89								
		Strain 3	6.66								
	Ambient	Strain 4	5.78	5.80	5.72	5.40	5.49	5.38	5.27	5.13	
		Blank	6.59	6.59	6.58	6.58					
		Strain 1	5.63	5.30	4.94	4.61					
		Strain 2	6.76	6.63	6.50	6.33					
		Strain 3	5.86	5.55	5.24	4.93					
		Strain 4	4.47	4.30	4.32	4.24	4.24	4.22	4.21	4.18	
		Tropical	Blank	5.83	5.32	4.81	4.30				
			Strain 1	4.15	4.12	4.08	4.03				
Strain 2	4.25		4.18	4.11	3.99						
Strain 3	4.22		4.17	4.12	4.06						
TM	Fridge	Strain 4	4.10	4.08	4.07	4.04	4.04	4.05	4.02	4.02	
		Blank	6.99								
		Strain 1	6.75								
		Strain 2	6.99								
	Ambient	Strain 3	6.66								
		Strain 4	6.46	6.70	6.62	6.55	6.54	6.57	6.65	6.42	
		Blank	6.76	6.70	6.63	6.56					
		Strain 1	5.98	5.68	5.36	5.07					
		Strain 2	6.56	6.43	6.30	6.18					
		Strain 3	5.66	5.30	4.94	4.57					
		Strain 4	5.81	5.66	5.48	5.24	5.05	4.89	4.77	4.66	
		Tropical	Blank	4.88	4.65	4.42	4.19				
Strain 1	4.42		4.34	4.26	4.17						
Strain 2	4.28		4.20	4.12	4.02						
Strain 3	4.35		4.27	4.19	4.11						
30	Strain 4	5.08	4.86	4.73	4.60	4.51	4.49	4.46	4.43		

TABLE I (continuation - 2)

Milk	condition	sample	16	17	18	19	20	23	24		
5	IFM	Fridge	Blank						6.72		
			Strain 1						5.19		
			Strain 2						6.82		
			Strain 3						6.15		
				5.02	4.94	4.81		4.73	4.69		
	10	Ambient	Blank							4.35	
			Strain 1							4.24	
			Strain 2							4.24	
			Strain 3							4.23	
					4.19	4.19	4.21		4.21	4.19	
		Tropical	Blank								3.80
			Strain 1								3.79
Strain 2										3.64	
Strain 3									3.64		
15	IFM	Fridge	Strain 4	4.02	3.98	3.99		4.00	3.99		
			Blank							6.62	
			Strain 1							5.95	
			Strain 2							6.63	
	20	Ambient	Strain 3							6.24	
			Strain 4	6.40	6.50		6.55		6.45		
			Blank							4.17	
			Strain 1							4.24	
		Tropical	Strain 2							4.33	
			Strain 3							4.13	
			Strain 4	4.60	4.62		4.58		4.19		
			Blank							3.82	
25	IFM	Fridge	Strain 1						4.07		
			Strain 2						3.68		
			Strain 3						3.78		
			Strain 4	4.38	4.38		4.36		4.21		
	Tropical	Blank							3.82		
		Strain 1							4.07		
		Strain 2							3.68		
		Strain 3							3.78		
30	IFM	Fridge	Strain 4	4.38	4.38		4.36		4.21		
			Blank						3.82		
			Strain 1						4.07		
			Strain 2						3.68		
	Tropical	Strain 3							3.78		
		Strain 4	4.38	4.38		4.36		4.21			
		Blank							3.82		
		Strain 1							4.07		

35 From TABLE I, it appears that both infant formula and therapeutic milks show a similar trend under cool conditions that is there is no drop in pH for any sample. Further a general trend is observed in infant formula milk and therapeutic milk in that only the samples containing RA024 show any pH drop at ambient temperature. That drop is initially noted after 8 hours storage and drops to a pH 4.5 in both cases after 11 hours.

At tropical conditions:

Infant formula: The pH shows an initial drop after 3 hours in all samples except the blank.
After 5 hours both samples containing RA024 have reached a pH of 4.
After 7 hours the sample containing MY087 only has reached a pH of 4.
After 11 hours the blank itself has drop to a pH of 4.5.

5

Therapeutic milk: All samples containing probiotic show a drop in pH after 2 hours.
All samples appear to act similarly. A after 5 hours reaching a pH of 4.5,
except the blank which reaches a pH of 4.5 after 11 hours.

10 In this TABLE, tropical conditions would appears to be the optimum conditions to induce pH reduction i.e. culture growth, producing yoghurt.

The best culture is probably either the MA016 or the RA024. Both induce a quick drop in pH in both tested milks.

CLAIMS

1. Self-rehydrating container comprising a semi-permeable membrane which allows the preparation of substantially sterile water solutions using osmotically driven filtration, containing a carbohydrate, a fermented milk product forming starter culture, milk powder and eventually other edible solutes.
5
2. Self-rehydrating container according to claim 1, characterized in that the carbohydrate is selected from the group consisting of lactose, sucrose and dextrose and preferably is lactose.
3. Self-rehydrating container according to claim 1 or 2, characterized in that the container is divided into two compartments separated by a non permanent seal, a watertight compartment containing a fermented milk product forming starter culture, milk powder and eventually other edible materials, and a second osmotically active compartment containing a carbohydrate and eventually other osmotically active solutes.
10
4. Self-rehydrating container according to claim 3 characterized in that the watertight compartment is also substantially oxygen-tight.
15
5. Yoghurt production process characterized in that it comprises the steps of
 - a) immersing in water a self rehydrating container comprising a semi-permeable membrane and containing a carbohydrate, a yoghurt forming starter culture, milk powder and eventually other solutes;
 - b) leaving the container immersed until the filtered water has reached a sufficient level; and
 - c) optionally, keeping the bag at a temperature above 25°C for a sufficient further period of time allowing the fermented milk product to be formed.
20
6. Yoghurt production process using a self-rehydrating container according to any one of claims 3 or 4 comprising
 - a) immersing the container in water,
 - b) leaving the container immersed until the filtered water has reached a sufficient level,
 - c) breaking the non-permanent seal separating the two compartment,
 - d) allowing the watertight compartment to be filled with the sterile water of the other compartment,
 - e) allowing the starter culture and milk powder to dissolve,
 - f) keeping the bag at a temperature above 25°C and allowing for the fermented milk product to be formed.
25
30
35
7. Fermented milk product obtained using a process according to any one of claims 5 or 6.

INTERNATIONAL SEARCH REPORT

Int. onal Application No
PCT/BE 98/00036

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A23C9/123 A23C9/15 A23C9/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A23C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4 195 561 A (G. CASTANIS) 1 April 1980 see column 3, line 1 - line 12; claims 1-9 ---	1-7
A	US 4 920 105 A (A. ZELLMAN) 24 April 1990 see column 7, line 21 - line 31; claims 1-23; figures 1,4 ---	1-4
A	US 3 696 931 A (W. HOUGH) 10 October 1972 see column 5, line 34 - column 6, line 2; claims 1-15; figures 1-3 ---	1-4
A	EP 0 360 612 A (HAMPSHIRE ADVISORY AND TECHNICAL SERVICES) 28 March 1990 see page 4, line 1 - line 47; claims 1-17; figures 1-4 ---	1-4
A	FR 781 005 A (P. DIGEAN) 9 May 1935 see claims 1-3; figure 2 -----	1-7

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

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06/07/1998

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