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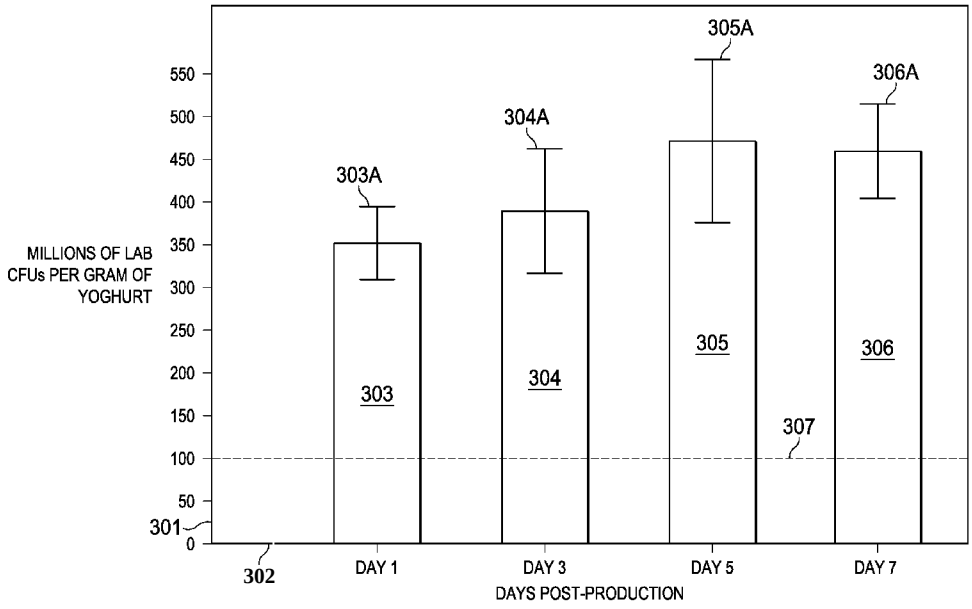
(54) Title: METHOD AND SYSTEM FOR PRODUCING YOGURT-BASED FOOD PRODUCT

FIG. 3



(57) Abstract: The present invention is a method of using a secondary fermentation process on a fermented milk product to produce a double -fermented yogurt product. The present invention is a food product of double -fermented yogurt having reduced lactose content and increased numbers of beneficial live bacteria. Specifically, the present invention is a method of making a double -fermented yogurt product utilizing mesophilic-adapted bacterial cultures in a second fermentation process to produce a double -fermented yogurt having reduced lactose content and increased numbers of beneficial live bacteria. The present method can use unfermented milk, semi-fermented milk and fermented milk as starting materials to produce the double- fermented yogurt product.

[Continued on next page]

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— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(in))*

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METHOD AND SYSTEM FOR PRODUCING YOGURT-BASED FOOD PRODUCT

RELATED APPLICATION DATA

[0001] This application claims the benefit of U.S. Provisional Patent Application Serial No. 62/455,079 filed February 6, 2017.

TECHNICAL FIELD

[0002] The present invention relates to a double-fermentation process that produces a yogurt-based food product and the yogurt product produces therefrom.

BACKGROUND OF THE INVENTION

[0003] The background of the invention will address fermentation of milk products and yogurt production.

Fermentation of Milk Products

[0004] Milk products prepared by lactic acid fermentation (e.g. yogurt) or a combination of this and yeast fermentation (e.g. Kefir) are called fermented or cultured milks. Fermented milk is the collective name for products such as yogurt (yoghurt), ymer, kefir, cultured buttermilk, filmjolk (Scandinavian sour milk), cultured cream and koumiss (a product based on mares' milk).

[0005] Fermented milk is a milk product that has been inoculated with a starter culture which converts part of the lactose to lactic acid. Depending on the type of lactic acid bacteria used, different end products are formed in the conversion process, such as carbon dioxide, acetic acid, diacetyl, acetaldehyde and several other substances; and, these end products give the fermented milk product different characteristic tastes and aromas. The microorganisms used in the production of kefir and koumiss also produce ethyl alcohol.

[0006] Fermented milk originates from the Near East and subsequently became popular in Eastern and Central Europe. The first example of fermented milk was presumably produced accidentally by nomads. This milk turned sour and coagulated under the influence of certain microorganisms, and the bacteria used in this fermentation were of the harmless, acidifying type as opposed to toxin-producing organisms.

[0007] A legend holds that yogurt and kefir were born on the slopes of Mount Elbrus in the Caucasus range by a miracle of nature. According to this legend, microorganisms of various kinds happened to land in a pitcher of milk at the same time and at the right temperature on the southern slope of Mount Elbrus; and, in this environment, they were found to live in symbiosis. Microorganisms, on this southern slope, thrive in relatively high temperatures 40-45 °C, and these microorganisms produced a product that the Turks called "yogurut." Some sources say that this name was introduced in the 8th Century, and that it was changed in the 11th Century to its present form, *yogurt* or *yoghurt.*

[0008] Kefir, the legend goes on to say, was created on the northern slope Mount Elbrus by a mixture of microorganisms that are fond of a cooler climate, which means these microorganisms thrive best at 25 - 28 °C. The name kefir may be derived from Turkish with the first syllable of the name, "kef," meaning pleasurable, which was probably the shepherd's first comment on the flavor. Kefir contains several different types of microorganisms, among which yeast is most famous as it is capable of forming alcohol. The maximum alcohol content of kefir is about 0.8%.

[0009] In the production of fermented milk, the best possible growth conditions must be created for the starter culture. These are achieved by heat treatment of the milk to destroy any competing microorganisms. In addition, the milk must be held at the optimum temperature for the relevant starter culture. When the best possible flavor and aroma have been achieved, the cultured milk must be cooled quickly, to stop the fermentation process. If the fermentation time is too long or too short, the flavor will be impaired and the consistency of the product may suffer.

[0010] Fermentation is technically the metabolic process that converts sugars to acids, gases, or alcohols. Fermentation is commonly used to describe the bulk growth of microorganisms on a medium, usually with the goal of producing a specific chemical end product. Microorganisms, such as yeast and bacteria, can ferment most naturally derived food products, with milk being a commonly fermented food.

[0011] Fermentation is a naturally occurring process for breaking down materials, and indeed most naturally derived food materials will eventually undergo some form of fermentation unless steps are taken to prevent it. The key to making palatable fermented foods, such as yogurt, is to control the fermentation process by selecting the conditions and starting cultures that will yield the desired end product.

[0012] Starter cultures for milk fermentation typically use lactic acid producing bacteria, such as *Lactobacillus spp., Lactococcus spp., Leuconostoc spp.* or some species of *Streptococcus*. Milk fermentation will produce different end products, depending on the type of starter culture used. Thermophilic bacteria, which thrive at relatively high temperatures, are generally used in milk fermentation, and several types can be used as starter cultures for yogurt production.

[0013] The most prevalent types are *Lactobacillus delbrueckii subspecies bulgaricus* and *Streptococcus thermophilus.* The function of the starter cultures is to ferment, or break down, sugar in the milk (lactose) to produce lactic acid. The lactic acid acidifies the milk and acts on the milk proteins causing the acidic taste and thickening associated with yogurt. During lactic acid fermentation, milk undergoes a coagulation process where the solid portion (curd) tend to contract and separate from some of the watery portion (whey).

[0014] Adequate heat treatment and homogenization of the milk, sometimes combined with methods to increase the Milk Solids-Not Fat (MSNF) content, as for milk intended for yogurt, are essential "foundation stones" for the construction of the coagulum during the incubation period. The production techniques for other fermented products have many similarities; the pre-treatment of the milk, for example, is almost the same.

[0015] While it is claimed that yogurt acts as a "preservative" in humans against human aging; in actuality, the conversion of lactose into lactic acid does has a preservative effect on milk. The low pH of cultured milk inhibits the growth of putrefactive bacteria and other detrimental organisms, thereby prolonging the shelf life of the milk product. Acidified milk, on the other end of the pH scale, is a very favorable environment for yeasts and molds. The digestive systems of some people lack the lactase enzyme. As a result, lactose is not broken down in the digestive process into simpler types of sugars. These people can consume only very small volumes of ordinary milk, but these people can consume fermented milk in which the lactose is already partly broken down by the bacterial enzymes.

Yogurt

[0016] Yogurt, or yoghurt, is one of many foods produced by bacterial fermentation of milk. The original function of fermenting milk was to extend the shelf life of milk before storage means, such as refrigeration, were available. With the advent of fermented milk products

came additional advantages, such as an improved taste, increased stability, enhanced bioavailability of vitamins, and greater digestibility of the milk sugars and proteins.

[0017] The bacteria used to make yogurt are known as "yogurt cultures" or "active bacterial cultures." Fermentation of lactose (milk sugar) by these bacterial yogurt cultures produces lactic acid, which gives yogurt the distinctive tart flavor and causes the milk proteins to coagulate giving yogurt the characteristic thickened texture.

[0018] Cow's milk is available worldwide and is the most common type of milk used for yogurt production. Milk from water buffalo, goats, sheep, mares, camels, yaks and zebu, can also be used to produce yogurt, and these may be the preferred type of milk where locally available. There are many types of fermented milk products other than yogurt, including cheeses, buttermilk, sour cream, kefir, laban (leben), labneh, lassi, and kumis.

[0019] Milk used for yogurt may be either raw (i.e. unheated) or pasteurized. Pasteurization is the process of heat-processing a liquid, such as milk, to kill pathogenic bacteria which may exist in the raw milk. Use of pasteurization in milk products has helped reduce the transmission of milk-borne bacterial diseases; and, for this reason, pasteurized milk is most commonly used in the commercial production of yogurt. Yogurt can be pasteurized after fermentation; however this results in inactivation of the beneficial live cultures normally found in yogurt.

[0020] Milk used in the production of yogurt may also be homogenized to prevent separation of the milk fats; or, the milk can be left un-homogenized, which produces markedly different results in the final product. Homogenization is a process by which the milk fat is emulsified in the aqueous portion of the milk to prevent separation of the two components. Un-homogenized milk produces yogurt with a layer of higher fat cream on the top of the lower fat yogurt layer. Yogurt can also be homogenized after the fermentation process to combine the two layers of the final product.

[0021] One known process for making yogurt starts with heating the milk to denature the whey proteins and to reduce the number of microorganisms that cause spoilage. In commercial yogurt production for this process, the milk is typically pasteurized at 185°F (85°C) for 30 minutes or at 203°F (95°C) for 10 minutes. The milk is then cooled to around 100-1 15°F (38-46°C) to bring the milk to the correct temperature for growth of the thermophilic bacteria in the starter culture. Starter cultures are mixed with the cooled milk

and the milk is maintained at around 99-1 15°F (37-46°C) for between 4-12 hours to allow for fermentation of the milk sugar to produce lactic acid.

[0022] Completion of this prior fermentation process is usually determined by the pH of the yogurt and is considered complete at approximately pH 4.4-4.6. Once the fermentation period is completed, the yogurt is rapidly cooled to stop the fermentation. After fermentation, yogurt will have the characteristic tart flavor and thickened consistency, with longer fermentation times producing tarter yogurt. Straining the yogurt after fermentation to remove some of the liquid whey content produces a thicker "Greek-style" yogurt with a higher protein content.

[0023] Strained yogurt, also known as "Greek" or "Greek-style" yogurt, is a type of yogurt that is usually thicker, creamier and more acidic than traditional-type yogurt. Although this type of yogurt initially originated from Greece and the Eastern Mediterranean region in general (e.g., Lebanon, Egypt, Armenia, Turkey), it is now being produced worldwide and has taken up a significant part of the dairy products market in USA, Canada, Australia and Europe.

[0024] The production of strained yogurt conventionally starts from the fermentation of milk (fresh or powdered) at 37-46°C with the aid of thermophilic lactic acid bacteria and involves straining away part of the whey, i.e., the aquatic phase of the fermented product. The straining process may be performed by gravity filtration, vacuum filtration, ultrafiltration, centrifugation, or other processes to reduce the whey content in the yogurt. The strained product may be homogenized and pasteurized before packaging. To date, no alternative starting dairy materials other than milk have been used for the production of strained yogurt.

SUMMARY OF THE INVENTION

[0025] The present invention is a method of double-fermentation that utilizes two separate milk fermentation steps to produce a yogurt product that is substantially lower in lactose and has substantially higher numbers of beneficial bacteria in the finished double-fermented yogurt product. The double-fermentation process can use unfermented (fresh), semi-fermented or fermented milk and milk products as a starting material. Full fat, reduced fat and non-fat milk and milk products can be used in the disclosed double-fermentation process. Milk from cows, water buffalo, goats, sheep, mares, camels, yaks, zebu and combinations thereof can be used in the claimed method.

[0026] The method provided herein can utilize semi-fermented milk derivatives instead of fresh milk as a starting dairy material to produce yogurt. Semi-fermented milk products, such as laban (leben), can be advantageously used for double-fermentation. Full-fat, reduced-fat or non-fat semi-fermented dairy materials can be used. Other fermented milk products, such labneh, buttermilk, soured milk, yogurt, lassi, kefir and combinations thereof, can also be advantageously used for double-fermentation.

[0027] The disclosed method for producing a double-fermented yogurt-based food product comprises the steps of: (a) placing a full-fat, reduced fat or non-fat fermented milk product into a fermentation tank at 22-30°C; (b) inoculating the fermented milk product with an active bacterial starter culture adapted for mesophilic fermentation comprising lactic acid producing bacteria; (c) incubating the inoculated fermented milk product at 22-30°C without agitation during a fermentation process; (d) fermenting the inoculated fermented milk product until a pH range of 4.1-4.3 is achieved resulting in a double-fermented yogurt product; (e) stirring the double-fermented yogurt product at low speed (10-30 revolutions per minute) for 2-5 minutes; (f) straining the double-fermented yogurt product to reduce a starting volume by 1/4-3/4; (g) homogenizing the strained double-fermented yogurt product; and (h) cooling the resulting strained double-fermented yogurt product to 4 °C.

[0028] The method provided herein utilizes a mesophilic bacterial starter culture added to a fermented or semi-fermented milk starting material for a second fermentation step. The mesophilic bacterial starter culture can be a culture comprising *Streptococcus thermophilus* and *Lactobacillus delbrueckii subspecies bulgaricus,* a culture comprising *Streptococcus thermophilus, Lactobacillus delbrueckii subspecies bulgaricus, Bifidobacterium sp., Lactobacillus casei* and *Lactococcus lactis,* or an activated culture of cottage cheese (labneh).

[0029] The double-fermented yogurt product is preferably strained to reduce the initial volume by 2/3. Straining is accomplished by gravity filtration, vacuum filtration, centrifugation, ultrafiltration, or combinations thereof. The double-fermented yogurt product has a lactose content of less than 1%.

[0030] Alternatively, the double-fermented yogurt product is produced from an unfermented (fresh) milk product that is fermented in a first fermentation process having the steps of (a) placing a full-fat, reduced fat or non-fat unfermented milk product into a fermentation tank at 30-37°C; (b) inoculating the unfermented milk product with an active culture of thermophilic bacteria comprising *Streptococcus thermophilus* and *Lactobacillus delbrueckii subspecies*

bulgaricus; (c) incubating the inoculated unfermented milk product at 30-37°C during a first fermentation process; (d) fermenting the inoculated unfermented milk product until a pH range of 4.5-4.6 is achieved resulting in a fermented milk product; and (e) cooling the fermented milk product resulting from the first fermentation process to 4°C.

[0031] The product of the first fermentation is then fermented in a second fermentation process having the further steps of (f) placing the fermented milk product resulting from the first fermentation process into a fermentation tank at 22-30°C for a second fermentation process; (g) inoculating the fermented milk product with an active bacterial starter culture adapted for mesophilic fermentation comprising lactic acid producing bacteria; (h) incubating the inoculated fermented milk product at 22-30°C without agitation during the second fermentation process; (i) fermenting the inoculated fermented milk product until a pH range of 4.1-4.3 is achieved resulting in a double-fermented yogurt product; (j) stirring the double-fermented yogurt product at a low speed (10-30 revolutions per minute) for 2-5 minutes; (j) straining the double-fermented yogurt product to reduce a starting volume by 1/4-3/4; (k) homogenizing the strained double-fermented yogurt product; and (1) cooling the resulting strained double-fermented yogurt product to 4 °C.

[0032] Also disclosed herein is a double-fermented yogurt product prepared by a process comprising the steps of: (a) providing a starting fermented milk product; (b) inoculating the starting fermented milk product with an active bacterial starter culture having lactic acid producing bacteria adapted for mesophilic fermentation; (c) incubating the inoculated fermented milk at 22-30 °C without agitation until a pH of 4.1-4.3 is reached resulting in a double-fermented yogurt; (d) stirring the double-fermented yogurt for 2-5 minutes at low speed (10-30 revolutions per minute) to break-up curds; (e) straining the stirred double-fermented yogurt to reduce a starting volume by 2/3; (f) homogenizing the strained double-fermented yogurt; and (g) cooling the homogenized strained double-fermented yogurt to 4°C.

[0033] The mesophilic bacterial starter culture for producing the double-fermented yogurt product can be a culture comprising *Streptococcus thermophilus* and *Lactobacillus delbrueckii subspecies bulgaricus,* a culture comprising *Streptococcus thermophilus, Lactobacillus delbrueckii subspecies bulgaricus, Bifidobacterium sp., Lactobacillus casei* and *Lactococcus lactis,* or an activated culture of cottage cheese (labneh).

[0034] The double-fermented yogurt product is preferably strained to reduce the initial volume by 2/3. Straining is accomplished by gravity filtration, vacuum filtration,

centrifugation, ultrafiltration, or combinations thereof. The double -fermented yogurt product has greater than 200 million Colony Forming Units (CFU) of lactic acid-forming bacteria per gram of yogurt and has a lactose content of less than 1%.

BRIEF DESCRIPTION OF THE DRAWINGS

[003S] The above, and other objects and advantages of the present invention will be understood upon consideration of the following detailed description taken in conjunction with the accompanying drawings, in which like reference characters refer to like parts throughout, and in which:

[0036] FIG 1. is an Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster tree of the bacterial communities of yogurt produced by the double-fermentation method and in other fermented dairy products.

[0037] FIG. 2A is a graph of rarefaction curves of the bacterial communities of yogurt produced by the double-fermentation method and in other kinds of fermented dairy products

[0038] FIG. 2B is a bar graph of taxonomic composition at the Genus level of bacterial communities of the yogurt produced by the double-fermentation method and in other fermented dairy products.

[0039] FIG. 3 is a bar graph showing total lactic acid bacteria (LAB) colony-forming unit (CFU) counts per gram of yogurt over a period of 7 days post-production.

[0040] While the invention is susceptible to various modifications and alternative forms, specific embodiments thereof have been shown by way of example in the drawings and are herein described in detail. It should be understood that the description herein of specific embodiments described herein are not intended to limit the invention to the particular forms disclosed. On the contrary, the claimed invention is meant to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the invention as defined by the appended claims and described herein.

DETAILED DESCRIPTION

[0041] The present invention is a method of for making a double-fermented strained yogurt product that involves two separate fermentation steps instead of a single step as in conventional methods. The present invention is a method of double-fermentation that utilizes two separate milk fermentation steps to produce a yogurt product that is substantially lower

in lactose and has substantially higher numbers of beneficial bacteria in the finished double-fermented yogurt product. The double-fermentation process can utilize fresh (unfermented), semi-fermented, or fermented milk as a starting material to produce a double-fermented strained yogurt. Double-fermented strained yogurt produced by the present method has a substantially reduced percentage of lactose and substantially higher levels of beneficial live bacteria as compared to conventionally prepared (single-fermentation) yogurt.

[0042] The method provided herein can utilize semi-fermented milk derivatives instead of fresh milk as a starting dairy material to produce yogurt. Semi-fermented milk products, such as laban (leben), can be advantageously used for double-fermentation. Full-fat, reduced-fat or non-fat semi-fermented dairy materials can also be used. Other fermented milk products, such labneh, buttermilk, soured milk, yogurt, lassi, kefir and combinations thereof, can also be advantageously used for double-fermentation.

[0043] In one embodiment of the present invention, a fermented or semi-fermented milk product is utilized as the starting dairy product. The fermented or semi-fermented milk is combined with a mesophilic bacterial starter culture and the combination is incubated at 22-30°C (approximately 72-86°F) for a second fermentation period. The method provided herein utilizes a mesophilic bacterial starter culture added to a fermented or semi-fermented milk starting material for a second fermentation step.

[0044] The mesophilic bacterial starter culture can be a culture comprising *Streptococcus thermophilus* and *Lactobacillus delbrueckii subspecies bulgaricus*, a culture comprising *Streptococcus thermophilus, Lactobacillus delbrueckii subspecies bulgaricus, Bifidobacterium sp., Lactobacillus casei* and *Lactococcus lactis,* or an activated culture of cottage cheese (labneh). The mesophilic bacterial starter culture can also be a mixture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subspecies bulgaricus,* a mixture of *Streptococcus thermophilus, Lactobacillus delbrueckii subspecies bulgaricus, Bifidobacterium sp., Lactobacillus casei* and *Lactococcus lactis,* or an or an activated culture of cottage cheese (labneh).

[0045] Activated cultures of cottage cheese (labneh) can be prepared by diluting (1:10 v/v) cottage cheese (labneh) in a fermented milk product, such as soured milk or laban, and incubating the mixture at 25-28°C (approximately 77-82°F) for approximately one hour prior to inoculating the fermented milk starting material. The lactic acid producing bacteria in the mesophilic bacterial starter culture have been pre-adapted to mesophilic (lower temperature)

conditions and are able to ferment lactose remaining in the fermented milk produced in the first fermentation period.

[0046] The second fermentation is allowed to proceed without agitation until the fermented milk reaches a pH of 4.1-4.3, which can take from 4-24 hours. After the appropriate pH has been reached, the double-fermented yogurt is stirred at low-speed for 2-5 minutes to gently break the curd resulting from the fermentation. The low speed is approximately 10-30 revolutions per minute. Preferably, the low speed is 20 revolutions per minute.

[0047] The disclosed method for producing a double-fermented yogurt-based food product comprises the steps of: (a) placing a full-fat, reduced fat or non-fat fermented milk product into a fermentation tank at 22-30°C; (b) inoculating the fermented milk product with an active bacterial starter culture adapted for mesophilic fermentation comprising lactic acid producing bacteria; (c) incubating the inoculated fermented milk product at 22-30°C without agitation during a fermentation process; (d) fermenting the inoculated fermented milk product until a pH range of 4.1-4.3 is achieved resulting in a double-fermented yogurt product; (e) stirring the double-fermented yogurt product at low speed (10-30 revolutions per minute) for 2-5 minutes; (f) straining the double-fermented yogurt product to reduce a starting volume by 1/4-3/4; (g) homogenizing the strained double-fermented yogurt product; and (h) cooling the resulting strained double-fermented yogurt product to 4 °C.

[0048] Alternatively, the double-fermented yogurt product is produced from an unfermented (fresh) milk product that is fermented in a first fermentation process having the steps of (a) placing a full-fat, reduced fat or non-fat unfermented milk product into a fermentation tank at 30-37°C; (b) inoculating the unfermented milk product with an active culture of thermophilic bacteria comprising *Streptococcus thermophilus* and *Lactobacillus delbrueckii subspecies bulgaricus;* (c) incubating the inoculated unfermented milk product at 30-37°C during a first fermentation process; (d) fermenting the inoculated unfermented milk product until a pH range of 4.5-4.6 is achieved resulting in a fermented milk product; and (e) cooling the fermented milk product resulting from the first fermentation process to 4°C.

[0049] The product of the first fermentation is then fermented in a second fermentation process having the further steps of (f) placing the fermented milk product resulting from the first fermentation process into a fermentation tank at 22-30°C for a second fermentation process; (g) inoculating the fermented milk product with an active bacterial starter culture adapted for mesophilic fermentation comprising lactic acid producing bacteria; (h) incubating

the inoculated fermented milk product at 22-30°C without agitation during the second fermentation process; (i) fermenting the inoculated fermented milk product until a pH range of 4.1-4.3 is achieved resulting in a double-fermented yogurt product; (j) stirring the double-fermented yogurt product at a low speed (10-30 revolutions per minute) for 2-5 minutes; (j) straining the double-fermented yogurt product to reduce a starting volume by 1/4-3/4; (k) homogenizing the strained double-fermented yogurt product; and (1) cooling the resulting strained double-fermented yogurt product to 4 °C.

[0050] Also disclosed herein is a double-fermented yogurt product prepared by a process comprising the steps of: (a) providing a starting fermented milk product; (b) inoculating the starting fermented milk product with an active bacterial starter culture having lactic acid producing bacteria adapted for mesophilic fermentation; (c) incubating the inoculated fermented milk at 22-30°C without agitation until a pH of 4.1-4.3 is reached resulting in a double-fermented yogurt; (d) stirring the double-fermented yogurt for 2-5 minutes at low speed (10-30 revolutions per minute) to break-up curds; (e) straining the stirred double-fermented yogurt to reduce a starting volume by 2/3; (f) homogenizing the strained double-fermented yogurt; and (g) cooling the homogenized strained double-fermented yogurt to 4°C.

[0051] The mesophilic bacterial starter culture for producing the double-fermented yogurt product can be a culture comprising *Streptococcus thermophilus* and *Lactobacillus delbrueckii subspecies bulgaricus,* a culture comprising *Streptococcus thermophilus, Lactobacillus delbrueckii subspecies bulgaricus, Bifidobacterium sp., Lactobacillus casei* and *Lactococcus lactis,* or an activated culture of cottage cheese (labneh).

[0052] The double-fermented yogurt product is preferably strained to reduce the initial volume by 2/3. Straining is accomplished by gravity filtration, vacuum filtration, centrifugation, ultrafiltration, or combinations thereof. The double-fermented yogurt product has greater than 200 million Colony Forming Units (CFU) of lactic acid-forming bacteria per gram of yogurt and has a lactose content of less than 1%.

[0053] The stirred double-fermented yogurt product is strained to reduce whey content or optionally, left unstrained after the second fermentation. Preferably the double-fermented yogurt product is strained. The straining process may be optionally achieved by gravity filtration, vacuum filtration, ultrafiltration, centrifugation, and combinations thereof. Preferably, the straining is performed by ultrafiltration or centrifugation. The straining

process optionally removes about 1/4-3/4 of the initial volume of the fermented milk starting material. Preferably, the straining removes about 2/3 of the initial volume.

[0054] The stirred double-fermented yogurt product is also optionally smoothed or homogenized after the second fermentation to evenly distribute the yogurt solids (curd). Optionally, the stirred double-fermented yogurt product is not homogenized or smoothed following after the second fermentation. Preferably, the double-fermented yogurt product is smoothed or homogenized. The smoothing or homogenization process is achieved by mixing at 30°C to provide a smooth consistency or by high pressure homogenization (100-150 bars). Preferably, the double-fermented yogurt product is homogenized.

[0055] Following the second fermentation, the double-fermented yogurt product is cooled to approximately 4°C (approximately 40°F) to stop the fermentation. If the optional steps of straining and/or homogenization are performed, the double-fermented yogurt product is cooled to approximately 4°C following the straining or homogenization. The double-fermented yogurt product is maintained at this temperature until used.

[0056] The starting fermented or semi-fermented dairy product can be acidophilus milk, acidofilne milk, acidofilne mlieko, acidofilni mleko, a-fil, airag, aludttej, amasi, arag, ariani, ayran, bland, boruga, buttermilk, byaslag, calpis, chaas,, chal/shubat, chalap, clabber, dadiah, dahi, doogh, ergo, filmjolk, ghara, huruud, jocoque, ksernemselk, karnemelk, kashk, kefir, kefiri, kefirs, kefyras, kimiz, kiselo mleko, kiselo mlijeko, kiselo mlyako, kishk, kislo mleko, kivuguto, kule naoto, kulturmelk, kumis, laban, labneh, lacto, laezh-ribod, langfil, lapte acru, lapte batut, lassi, leben, leche agria, mabisi, matsoni, mattha, matzoon, maziwa lala, mishti doi, mursik, piima, prostokvasha, qatiq, qatyq, quark, qurt, rob, riigpienis, ruguspiens, ryazhenka, sana, sauermilch, shrikhand, skabputra, skyr, soured milk, surmelk, surmjolk, suzma, tvorog, twaróg, tykmselk, urubu, varenets, viili, yogurt, ymer, xynogalo, or combinations thereof. Preferably, the fermented or semi-fermented dairy product is laban or labneh. Laban is a fermented milk derivative that is widespread in the Middle East and equivalent products are known in other locales by alternative names, some of which are listed above. Labneh is a fermented cottage cheese-type product.

[0057] The starting fermented or semi-fermented dairy product can be pasteurized, unpasteurized or a combination of pasteurized and unpasteurized fermented or semi-fermented dairy products. Optionally, the double-fermented yogurt product can be pasteurized following the second fermentation. Preferably, the starting fermented or semi-

fermented dairy product is pasteurized. Preferably, the double-fermented yogurt product is not pasteurized following the second fermentation in order to preserve the beneficial active bacteria in the final yogurt product.

[0058] The starting fermented or semi-fermented dairy product can be homogenized, un-homogenized, or a combination of homogenized and un-homogenized, fermented or semi-fermented dairy products. Optionally the double-fermented yogurt product can be homogenized following the second fermentation. Preferably the double-fermented yogurt product is homogenized following the second fermentation.

[0059] The starting fermented or semi-fermented dairy product can be a full fat (3-4% fat), reduced-fat (0.2. 1-2.9% fat), or non-fat (0-0.2% fat) dairy product. The starting fermented or semi-fermented dairy product can be from the milk of cows, water buffalo, goats, sheep, mares, camels, yaks, zebu and combinations thereof. Preferably, the milk is from cows or goats.

[0060] At the end of the second fermentation period, the double-fermented yogurt product typically has a fat content of 10- 11.5% w/w (if using full-fat milk as the starting material), a protein content of 9- 12.5% w/w, and pH between 4.1 and 4.3. Fat content in the final yogurt product will vary depending on the fat content in the starting dairy material.

[0061] The double-fermented yogurt product is almost completely devoid of lactose (i.e., <1% w/w as measured with the Lane-Eynon volumetric method) due to the double-fermentation process. Yogurt prepared by standard methods has around 4-5% lactose. Reduced lactose in the double-fermented yogurt product is a desirable attribute because the resulting yogurt product can be consumed by lactose-intolerant individuals without the gastrointestinal problems usually experienced following consumption of dairy products.

[0062] The double-fermented yogurt product has increased numbers of beneficial active bacteria over that found in yogurt prepared by traditional meals. The Colony Forming Units (CFU) of beneficial active bacteria in double-fermented yogurt product is significantly higher than the 100 million CFU threshold (307) set by the U.S. National Yogurt Association for a commercial yogurt to receive the "Live & Active Cultures Seal." The double fermented yogurt product has greater than 150 million CFU of lactic acid-forming bacteria per gram of yogurt and may have over 450 million CFU per gram of yogurt. Preferably, the double-

fermented yogurt product has greater than 200 million Colony Forming Units (CFU) of lactic acid-forming bacteria per gram of yogurt.

[0063] In another embodiment of the present method, fresh (unfermented) milk is combined with a thermophilic bacterial mixture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subspecies bulgaricus*, and the combination is incubated at 30-43°C (approximately 86-109°F) for a first fermentation period. Preferably, the incubation temperature range is 30-37°C (approximately 86-99°F) for the first fermentation period. The first fermentation is allowed to proceed until the milk reaches a pH of 4.5-4.6, which takes from 4-24 hours. Preferably, the fermentation period lasts 4-8 hours. When the appropriate pH is reached, the fermented milk is cooled to around 4°C (approximately 40°F).

[0064] After the fermented milk has been adequately cooled, the fermented milk is combined with a mesophilic bacterial starter culture and the combination is incubated at 22-30°C (approximately 72-86°F) for a second fermentation period. Optionally, the mesophilic bacterial starter culture is a mixture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subspecies bulgaricus*, a mixture of *Streptococcus thermophilus*, *Lactobacillus delbrueckii subspecies bulgaricus*, *Bifidobacterium sp.*, *Lactobacillus casei* and *Lactococcus lactis*, or an or an activated culture of cottage cheese (labneh). Activated cultures of cottage cheese (labneh) can be prepared by diluting (1:10 v/v) cottage cheese (labneh) in a fermented milk product, such as soured milk or laban, and incubating the mixture at 25-28°C (approximately 77-82°F) for approximately one hour prior to inoculating the fermented milk starting material. The lactic acid producing bacteria in the mesophilic bacterial starter culture have been pre-adapted to mesophilic (lower temperature) conditions and are able to ferment lactose remaining in the fermented milk produced in the first fermentation period.

[0065] The second fermentation is allowed to proceed without agitation until the fermented milk reaches a pH of 4.1-4.3, which can take from 4-24 hours. After the appropriate pH has been reached, the double-fermented yogurt is stirred at low-speed for 2-5 minutes to gently break the curd resulting from the fermentation. The low speed is approximately 10-30 revolutions per minute. Preferably, the low speed is 20 revolutions per minute.

[0066] The stirred double-fermented yogurt product is strained to reduce whey content, or optionally, left unstrained after the second fermentation. Preferably the double-fermented yogurt product is strained. The straining process may be optionally performed by gravity filtration, vacuum filtration, ultrafiltration, centrifugation, and combinations thereof.

Preferably, the straining is achieved by ultrafiltration or centrifugation. The straining process optionally removes about 1/4-3/4 of the initial volume of the fermented milk starting material. Preferably, the straining removes about 2/3 of the initial volume.

[0067] The stirred double-fermented yogurt product is also optionally smoothed or homogenized after the second fermentation to evenly distribute the yogurt solids (curd). Optionally, the stirred double-fermented yogurt product is not homogenized or smoothed following after the second fermentation. Preferably, the double-fermented yogurt product is smoothed or homogenized. The smoothing or homogenization process is achieved by mixing at 30°C to provide a smooth consistency or by high pressure homogenization (100-150 bars). Preferably, the double-fermented yogurt product is homogenized.

[0068] Following the second fermentation, the double-fermented yogurt product is cooled to approximately 4°C (approximately 40°F) to stop the fermentation. If the optional steps of straining and/or homogenization are performed, the double-fermented yogurt product is cooled to approximately 4°C following the straining or homogenization. The double-fermented yogurt product is maintained at this temperature until used.

[0069] The starting fresh (unfermented) milk product can be pasteurized milk, unpasteurized milk or combinations thereof. Preferably, the starting fresh milk is pasteurized prior to the first fermentation. Optionally, the double-fermented yogurt product can be pasteurized following the second fermentation. Preferably, the double-fermented yogurt product is not pasteurized following the second fermentation in order to preserve the beneficial active bacteria in the final yogurt product.

[0070] The starting fresh milk product can be homogenized milk, un-homogenized milk or combinations thereof. Optionally, the double-fermented yogurt product can be left un-homogenized, homogenized prior to the second fermentation, or homogenized following the second fermentation. Preferably the double-fermented yogurt product is homogenized following the second fermentation. The starting fresh milk product can be full fat, reduced-fat, or, or non-fat milk. The starting fresh milk product can be milk from cows, water buffalo, goats, sheep, mares, camels, yaks, zebu and combinations thereof. Preferably, the milk is from cows or goats.

[0071] At the end of the second fermentation period, the double-fermented yogurt product typically has a fat content of 10-11.5% w/w (if using full-fat milk as the starting material), a

protein content of 9-12.5% w/w, and pH between 4.1 and 4.3. Fat content in the final yogurt product will vary depending on the fat content in the starting dairy material.

[0072] The double-fermented yogurt product is almost completely devoid of lactose (i.e., <1% w/w as measured with the Lane-Eynon volumetric method) due to the double-fermentation process. Yogurt prepared by standard methods has around 4-5% lactose. Reduced lactose in the double-fermented yogurt product is a desirable attribute because the resulting yogurt product can be consumed by lactose-intolerant individuals without the gastrointestinal problems usually experienced following consumption of dairy products.

[0073] The double-fermented yogurt product has increased numbers of beneficial active bacteria over that found in yogurt prepared by traditional meals. The Colony Forming Units (CFU) of beneficial active bacteria in double-fermented yogurt product is significantly higher than the 100 million CFU threshold (307) set by the U.S. National Yogurt Association for a commercial yogurt to receive the "Live & Active Cultures Seal." The double fermented yogurt product has greater than 150 million CFU of lactic acid-forming bacteria per gram of yogurt and may have over 450 million CFU per gram of yogurt. Preferably, the double-fermented yogurt product has greater than 200 million Colony Forming Units (CFU) of lactic acid-forming bacteria per gram of yogurt.

[0074] Microbiological analyses using next generation sequencing (16S rRNA sequencing of the whole community at an Illumina platform) have shown that the bacterial community of the double-fermented strained yogurt product is significantly different from the communities of other conventional yogurts and from the communities of cottage-style cheese (labneh) and the semi-fermented starting material (laban) (FIG. 1). The bacterial community of double-fermented strained yogurt is more diverse than the communities of the other types of products tested (FIG. 2A), containing bacterial taxa that exist both in the laban and in the labneh (FIG. 2B). Moreover, double-fermented strained yogurt contains 350-500 million colony-forming units (CFU) of lactic acid bacteria per gram (FIG. 3), which is 3-5 times greater than the 100 million CFU threshold set by the U.S. National Yogurt Association for a commercial yogurt to receive the "Live & Active Cultures Seal."

[0075] FIG. 1 is an Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster tree of the bacterial communities of the yogurt produced by the method described here for Double-Fermented Strained Yogurt (DFSY) and of other kinds of fermented dairy products tested, including two different types of laban, (LB 1 and LB2), two different types of labneh,

(LN1 and LN2), and one type of commercial yogurt, (YG1). The letters A, B and C indicate different biological replicates. The broken lines in the nodes (108) indicates a bootstrap support of more than 75%, which demonstrates that the topology of the UPGMA tree is preserved in at least 75% of the random permutation tests.

[0076] The three replicates for DFSY (103A, 103B, 103C) cluster together indicating the expected correlation in the bacterial communities for the replicates. Bacterial communities for the Labneh 2 (105A-C) and Yogurt 1 (104A-C) have close similarities to the bacterial communities in the DSFY. The Laban 1 (101B, 101C) and Laban 2 (102A, 102C) bacterial communities are interrelated and also have some commonality to the bacterial communities in the DSFY. The Labneh 1 replicates (106B, 106C) indicate some commonality to the DSFY bacterial communities, but more diversity than seen in the other fermented dairy products tested. The distance scale (107) represents the degree of differences between sequences on the UPGMA cluster tree. Shorter branches represent greater commonality between the pairs.

[0077] FIG. 2A represents rarefaction curves of the bacterial communities of the yogurt produced by the disclosed method (DFSY for Doubly Fermented Strained Yogurt) and of other kinds of fermented dairy products tested. The y-axis (201) is the Rarefaction measurement based on the Chao 1 diversity index (an extrapolation metric of species richness) and the X-axis (202) is in sequences per sample. The mean of three biological replicates is shown, with vertical lines representing one standard deviation.

[0078] The Double Fermented Strained Yogurt (DFSY) prepared by the disclosed method was compared to two different types of laban (Laban A and B), two different types of labneh (Labneh A and B) and one type of commercial yogurt (Yogurt A) for the number of different species in the bacterial communities for the fermented dairy products. The curve for DFSY (203) indicates a greater number of different species in the DSFY sample than in the other dairy products tested. The curves for Labneh A (205A) and Laban A (204A) also have high degrees of species richness, but significantly lower than DSFY. The curves for Laban B (204B) and Labneh B (205B) indicate less species richness, and Yogurt A (206) has the least diversity among the samples tested.

[0079] FIG. 2B is a bar graph showing taxonomic composition at the Genus level of the bacterial communities for the Double-Fermented Strained Yogurt (DFSY) produced by the described method and for the other fermented dairy products seen in FIG. 2A. The bars (223-225) indicate the taxonomic composition for the DSFY yogurt (223), two types of laban,

Laban A (224A) and Laban B (224B), two types of labneh, Labneh A (225A) and Labneh B (225B), and one type of commercial yogurt, Yogurt A (226). Each bar represents the average proportions out of three biological replicates. The bacterial communities in each of the fermented dairy products are primarily composed of Genus Streptococcus (211) and Genus Lactobacillus (213). Genus Streptococcus (211) is the dominant variety in all of the products except for Labneh B (204B) which has Genus Lactobacillus (213) as the dominant genus.

[0080] As seen in the DSFY bar (223), there is significant diversity of taxa seen in the DSFY Yogurt made by the disclosed method. DFSY Yogurt (223) has Genus Streptococcus (211) as the primary genus and Genus Lactobacillus (213) as the secondary genus. DSFY Yogurt also has taxa such as Genus Bifidobacterium (210) that is seen in laban (224A & 224B) and Labneh A (225A), and Genus Lactococcus (212) that is seen in labneh (225A & 225B). In agreement with the FIG. 2A graph, the least diversity was seen in Yogurt A (226).

[0081] Table 1, below, indicates the taxonomy percentages of the bacterial communities found in the different types of dairy products represented in the FIG. 2B bar graph. Bacterial types with less than 0.20% representation in the dairy products are not symbolized in the FIG. 2B bars. The most prevalent Taxa observed in the dairy products are Bifidobacterium (210), Streptococcus (211), Lactococcus (212), and Lactobacillus (213). In most of the tested dairy products, Genus Thermus (214) occurred in very low relative abundance (< 0.20%), with a marginally higher percentage seen in Labneh A (225A). Genera that were Unidentified (215) were also in low relative abundance in all of the dairy products tested.

Table 1: Percentages of Taxa in Fermented Dairy Products

Ref. #	Genus (%)	Laban A -224A	DFSY Yoghurt -223	Laban B -224B	Labneh B -225B	Yoghurt A - 226	Labneh A -225A
210	Bifidobacterium	2.82	1.70	1.17	0.05	0.05	1.37
211	Streptococcus	82.50	89.53	79.96	29.42	95.88	90.75
212	Lactococcus	0.75	2.52	0.00	12.16	0.04	0.47
213	Lactobacillus	13.62	3.76	17.01	56.21	2.24	5.32
214	Thermus	0.14	0.02	0.16	0.04	0.09	0.43
215	Unidentified	0.18	2.46	1.70	2.12	1.70	1.65

[0082] FIG 3 is a bar graph showing total lactic acid bacteria (LAB) colony-forming unit (CFU) counts of the Double-Fermented Strained Yogurt over 7 days post production. The lactic acid bacteria CFU per gram of yogurt (301) were measured over a period of 7 days

post-production (302). Measurements were taken at the first day post-production and every two days thereafter until day 7. The mean of three biological replicates is shown in each bar, with vertical lines representing one standard deviation.

[0083] On Day 1, around 350 million CFU (303) were measured for Double-Fermented Strained Yogurt. On Day 3, the CFU measurement had increased to around 390 million (304) and on Day 5, the CFU had again increased to around 470 million (305). On Day 7, the CFU decreased slightly from the Day 5 counts to around 460 million. The standard deviation (303A, 304A,305A, 306A) for the CFU measurements of three replicates over the days tested indicate that all the measurements over all days were significantly higher than the 100 million CFU threshold (307) set by the U.S. National Yogurt Association for a commercial yogurt to receive the "Live & Active Cultures Seal."

EXAMPLES

Example 1: Second fermentation process with a fermented starting material:

[0084] A fermented dairy derivative, such as fermented milk or laban, is placed into a sanitized or sterilized fermentation vessel at 22-30°C and then inoculated with an active culture of mesophilic-adapted bacteria, including: *Streptococcus thermophilus, Lactobacillus delbrueckii subspecies bulgaricus, Bifidobacterium sp., Lactobacillus casei* and *Lactococcus lactis.* The fermented dairy derivative starting material can be full-fat or reduced-fat, pasteurized or unpasteurized, homogenized or un-homogenized. Alternatively, an activated culture of cottage cheese (labneh) can be used.

[0085] The inoculated fermented dairy derivative is fermented at 22-30°C without agitation until a pH of 4.1-4.3 is reached. After the appropriate pH is reached, the double-fermented yogurt product is stirred at low speed (approximately 20 revolutions per minute) to break-up the curd resulting from the fermentation.

[0086] The stirred double-fermented yogurt is strained to reduce whey content by a centrifugation or ultrafiltration process. Straining is considered complete when 2/3 of the initial volume has been removed. The double-fermented strained yogurt product is homogenized by means suitable for a dairy product and then stored at 4°C.

Example 2: First and second fermentation processes with a fresh starting material:

[0087] Fresh (unfermented) milk is placed into a sanitized or sterilized fermentation vessel at 30-37°C and then inoculated with an active culture of thermophilic bacteria, including:

Streptococcus thermophilus and *Lactobacillus delbrueckii subspecies bulgaricus.* The fresh milk starting material can be full-fat or reduced-fat, pasteurized or unpasteurized, homogenized or un-homogenized.

[0088] The fresh milk is fermented at 30-37°C until a pH of 4.5-4.6 is reached. When the appropriate pH is reached, the fermented milk is cooled to around 4°C (approximately 40°F).

[0089] The cooled milk from the first fermentation is then placed into a sanitized or sterilized fermentation vessel at 22-30°C and inoculated with an active culture of mesophilic-adapted bacteria, including: *Streptococcus thermophilus, Lactobacillus delbrueckii subspecies bulgaricus, Bifidobacterium sp., Lactobacillus casei* and *Lactococcus lactis.* Alternatively, an activated culture of cottage cheese (labneh) can be used.

[0090] After the appropriate pH is reached, the double-fermented yogurt product is strained to reduce whey content by a combination of processes selected from gravity filtration, vacuum filtration, centrifugation, and ultrafiltration. Straining is considered complete when 1/4-3/4 of the initial volume has been removed.

[0091] The double-fermented strained yogurt product is homogenized by high pressure (100-150 bars) and then stored at 4°C.

Example 3: Preparation and use of an activated cottage cheese (labneh) culture for a second fermentation process:

[0092] An activated cottage cheese (labneh) culture is prepared by diluting labneh or cottage cheese in sour milk or laban at a ratio of approximately 1:10 v/v and gently mixing the components. The mixture is then incubated at 25-28°C for approximately one hour to produce an activated mesophilic bacterial starter culture suitable for a mesophilic fermentation process.

[0093] The activated cottage cheese (labneh) culture is used as the mesophilic bacterial starter culture to inoculate a fermented or semi-fermented milk product prior to incubation in a second fermentation process to make a double-fermented yogurt product according to the disclosed method. The method for making a double fermented yogurt product is not limited to the specific steps described in the above examples, but instead the examples are intended as non-limiting representatives of possible steps that may be employed according to the disclosed method.

[0094] Those in the art will understand that a number of variations may be made in the disclosed embodiments, all without departing from the scope of the invention, which is defined solely by the appended claims. Disclosed are materials, compositions, and components that can be used for, can be used in conjunction with, can be used in preparation for, or are products of the disclosed methods and compositions. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed that while specific reference of each various individual and collective combinations and permutations of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein.

[0095] For example, if a method is disclosed and discussed and a number of modifications that can be made to the method are discussed, each and every combination and permutation of the method, and the modifications that are possible are specifically contemplated unless specifically indicated to the contrary. Likewise, any subset or combination of these is also specifically contemplated and disclosed. This concept applies to all aspects of this disclosure including, but not limited to, steps in methods using the disclosed process. Thus, if there are a variety of additional steps that can be performed, it is understood that each of these additional steps can be performed with any specific method steps or combination of method steps of the disclosed methods, and that each such combination or subset of combinations is specifically contemplated and should be considered disclosed.

WHAT IS CLAIMED IS:

1. A method for producing a double-fermented yogurt product, comprising the steps of:

(a) placing a full-fat, reduced fat or non-fat fermented milk product into a fermentation tank at 22-30°C;

(b) inoculating the fermented milk product with an active bacterial starter culture adapted for mesophilic fermentation comprising lactic acid producing bacteria to produce an inoculated fermented milk product;

(c) incubating the inoculated fermented milk product at 22-30°C without agitation during a fermentation process;

(d) fermenting the inoculated fermented milk product until a pH range of 4.1-4.3 is achieved producing a double-fermented yogurt product;

(e) stirring the double-fermented yogurt product at low speed (10-30 revolutions per minute) for 2-5 minutes;

(f) straining the double-fermented yogurt product to reduce a starting volume by 1/4-3/4, which results in a strained double-fermented yogurt product;

(g) homogenizing the strained double-fermented yogurt product; and

(h) cooling the strained double-fermented yogurt product to 4 °C after the homogenizing step.

2. The method of Claim 1, wherein the fermented milk product is selected from at least one of laban, labneh, buttermilk, soured milk, yogurt, lassi, kefir and combinations thereof.

3. The method of any one of Claims 1-2, wherein the active culture of bacteria adapted for mesophilic fermentation is a mixture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subspecies bulgaricus*, a mixture of *Streptococcus thermophilus*, *Lactobacillus delbrueckii subspecies bulgaricus*, *Bifidobacterium sp.*, *Lactobacillus casei* and *Lactococcus lactis*, or an activated cottage cheese (labneh) starter culture.

4. The method of any one of Claims 1-4, wherein straining the double-fermented yogurt product is accomplished by gravity filtration, vacuum filtration, centrifugation, ultrafiltration, or combinations thereof.

5. The method of any one of Claims 1-4, wherein straining the double-fermented yogurt product reduces the starting volume by 2/3.

6. The method of any one of Claims 1-5, wherein the fermentation results in a double-fermented yogurt product having a lactose content of less than 1% and has greater than 200 million Colony Forming Units (CFU) of lactic acid-forming bacteria per gram of yogurt.

7. The double-fermented yogurt product made by the method of any of Claims 1-6.

8. A method for producing a double-fermented yogurt product, comprising the steps of:

(a) placing a full-fat, reduced fat or non-fat unfermented milk product into a fermentation tank at 30-37°C;

(b) inoculating the unfermented milk product with an active culture of thermophilic bacteria comprising *Streptococcus thermophilus* and *Lactobacillus delbrueckii subspecies bulgaricus* to produce an inoculated unfermented milk product;

(c) incubating the inoculated unfermented milk product at 30-37°C during a first fermentation process;

(d) fermenting the inoculated unfermented milk product until a pH range of 4.5-4.6 is achieved producing a fermented milk product;

(e) cooling the fermented milk product produced from the first fermentation process to 4°C;

(f) placing the fermented milk product produced from the first fermentation process into a fermentation tank at 22-30°C for a second fermentation process;

(g) inoculating the fermented milk product with an active bacterial starter culture adapted for mesophilic fermentation comprising lactic acid producing bacteria to produce an inoculated fermented milk product;

(h) incubating the inoculated fermented milk product at 22-30°C without agitation during the second fermentation process;

(i) fermenting the inoculated fermented milk product until a pH range of 4.1-4.3 is achieved, which produces a double-fermented yogurt product;

(j) stirring the double-fermented yogurt product at a low speed (10-30 revolutions per minute) for 2-5 minutes;

(j) straining the double-fermented yogurt product to reduce a starting volume by 1/4-3/4 to produce a strained double-fermented yogurt product;

(k) homogenizing the strained double-fermented yogurt product; and

(l) cooling the strained double-fermented yogurt product to 4 °C after the homogenizing step.

9. The method of Claim 8, wherein the non-fermented milk product is milk from at least one of cows, water buffalo, goats, sheep, mares, camels, yaks, zebu and combinations thereof.

10. The method of Claim 9, wherein the non-fermented milk product is selected from cow milk, goat milk and combinations of cow milk and goat milk.

11. The method of any one of Claims 8-10, wherein the active culture of bacteria adapted for mesophilic fermentation is a mixture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subspecies bulgaricus*, a mixture of *Streptococcus thermophilus*, *Lactobacillus delbrueckii subspecies bulgaricus*, *Bifidobacterium sp.*, *Lactobacillus casei* and *Lactococcus lactis*, or an activated cottage cheese (labneh) starter culture.

12. The method of any one of Claims 8-11, wherein straining the double-fermented yogurt product is accomplished by gravity filtration, vacuum filtration, centrifugation, ultrafiltration, or combinations thereof.

13. The method of any one of Claims 8-12, wherein straining the double-fermented yogurt product reduces the starting volume by 2/3.

14. The method of any one of Claims 8-13, wherein the second fermentation process results in a double-fermented yogurt product having a lactose content of less than 1% and having greater than 200 million Colony Forming Units (CFU) of lactic acid-forming bacteria per gram of yogurt.

15. The double-fermented yogurt product made by the method of any of Claims 8-14.

16. The method for producing a double-fermented yogurt product, comprising the steps of:

(a) placing a full-fat, reduced fat or non-fat fermented milk product into a fermentation tank at 22-30°C;

(b) inoculating the fermented milk product with an active bacterial starter culture adapted for mesophilic fermentation comprising lactic acid producing bacteria to produce an inoculated fermented milk product;

(c) incubating the inoculated fermented milk product at 22-30°C without agitation during a second fermentation process;

(d) fermenting the inoculated fermented milk product until a pH range of 4.1-4.3 is achieved producing a double-fermented yogurt product;

(e) stirring the double-fermented yogurt product at low speed (10-30 revolutions per minute) for 2-5 minutes;

(f) straining the double-fermented yogurt product to reduce a starting volume by 1/4-3/4 to produce a strained double-fermented yogurt product;

(g) homogenizing the strained double-fermented yogurt product; and

(h) cooling the strained double-fermented yogurt product to 4 °C after the homogenizing step.

17. The method of Claim 16, wherein the fermented milk product is selected from at least one of laban, labneh, buttermilk, soured milk, yogurt, lassi, kefir and combinations thereof.

18. The method of Claim 16, wherein the active culture of bacteria adapted for mesophilic fermentation is a mixture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subspecies bulgaricus*, a mixture of *Streptococcus thermophilus, Lactobacillus delbrueckii subspecies bulgaricus*, *Bifidobacterium sp.*, *Lactobacillus casei* and *Lactococcus lactis*, or an activated cottage cheese (labneh) starter culture.

19. The method of Claim 16, wherein straining the double-fermented yogurt product is accomplished by gravity filtration, vacuum filtration, centrifugation, ultrafiltration, or combinations thereof.

20. The method of Claim 16, wherein straining the double-fermented yogurt product reduces the starting volume by 2/3.

21. The method of Claims 16, wherein the second fermentation results in a double-fermented yogurt product having a lactose content of less than 1% and has greater than 200 million Colony Forming Units (CFU) of lactic acid-forming bacteria per gram of yogurt.

22. A method for producing a double-fermented yogurt product, comprising the steps of:

(a) placing a full-fat, reduced fat or non-fat unfermented milk product into a fermentation tank at 30-37°C;

(b) inoculating the unfermented milk product with an active culture of thermophilic bacteria comprising *Streptococcus thermophilus* and *Lactobacillus delbrueckii subspecies bulgaricus* to produce an inoculated unfermented milk product;

(c) incubating the inoculated unfermented milk product at 30-37°C during a first fermentation process;

(d) fermenting the inoculated unfermented milk product until a pH range of 4.5-4.6 is achieved to produce a fermented milk product;

(e) cooling the fermented milk product produced from the first fermentation process to 4°C;

(f) placing the fermented milk product produced from the first fermentation process into a fermentation tank at 22-30°C for a second fermentation process;

(g) inoculating the fermented milk product with an active bacterial starter culture adapted for mesophilic fermentation comprising lactic acid producing bacteria to produce an inoculated fermented milk product;

(h) incubating the inoculated fermented milk product at 22-30°C stirring at a low speed during the second fermentation process;

(i) fermenting the inoculated fermented milk product until a pH range of 4.1-4.3 is achieved to produce a double-fermented yogurt product;

(j) straining the double-fermented yogurt product to remove whey reducing a starting volume by 1/4-3/4 and to produce a strained double-fermented yogurt product;

(k) homogenizing the strained double-fermented yogurt product; and

(1) cooling the strained double-fermented yogurt product to 4°C after the homogenizing step.

23. The method of Claim 22, wherein the non-fermented milk product is milk from at least one of cows, water buffalo, goats, sheep, mares, camels, yaks, zebu and combinations thereof.

24. The method of Claim 23, wherein the non-fermented milk product is selected from cow milk, goat milk and combinations of cow milk and goat milk.

25. The method of Claim 22, wherein the active culture of bacteria adapted for mesophilic fermentation is a mixture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subspecies bulgaricus,* a mixture of *Streptococcus thermophilus, Lactobacillus delbrueckii subspecies bulgaricus, Bifidobacterium sp., Lactobacillus casei* and *Lactococcus lactis,* or an activated cottage cheese (labneh) starter culture.

26. The method of Claim 22, wherein straining the double-fermented yogurt product is accomplished by gravity filtration, vacuum filtration, centrifugation, ultrafiltration, or combinations thereof.

27. The method of Claim 22, wherein straining the double-fermented yogurt product reduces the starting volume by 2/3.

28. The method of Claim 22, wherein the second fermentation process results in a double-fermented yogurt product having a lactose content of less than 1% and has greater than 200 million Colony Forming Units (CFU) of lactic acid-forming bacteria per gram of yogurt.

29. A double-fermented yogurt product produced by a process comprising the steps of:

(a) providing a starting fermented milk product;

(b) inoculating the starting fermented milk product with an active bacterial starter culture having lactic acid producing bacteria adapted for mesophilic fermentation to produce an inoculated fermented milk;

(c) incubating the inoculated fermented milk at 22-30°C without agitation until a pH of 4.1-4.3 is reached to produce a double-fermented yogurt;

(d) stirring the double-fermented yogurt for 2-5 minutes at low speed (10-30 revolutions per minute) to break-up curds to produce a stirred double-fermented yogurt;

(e) straining the stirred double-fermented yogurt to reduce a starting volume by 2/3 to produce a strained double-fermented yogurt;

(f) homogenizing the strained double-fermented yogurt; and

(g) cooling the strained double-fermented yogurt to 4°C after the homogenizing step.

30. The double-fermented yogurt product of Claim 29, wherein the active bacterial started culture having lactic acid producing bacteria adapted for mesophilic fermentation is a mixture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subspecies bulgaricus,* a mixture of *Streptococcus thermophilus, Lactobacillus delbrueckii subspecies bulgaricus, Bifidobacterium sp., Lactobacillus casei* and *Lactococcus lactis,* or an activated cottage cheese (labneh) starter culture.

31. The double-fermented yogurt product of Claim 29, wherein the starting fermented milk product is at least one of laban, labneh, buttermilk, soured milk, yogurt, lassi, kefir and combinations thereof.

32. The double-fermented yogurt product of Claim 29, wherein the starting fermented milk product has at least one of fermented cow milk, fermented water buffalo milk, fermented goat milk, fermented sheep milk, fermented mare milk, fermented camel milk, fermented yak milk, and fermented zebu milk.

33. The double-fermented yogurt product of Claim 29, wherein the double-fermented yogurt product has greater than 200 million Colony Forming Units (CFU) of lactic acid-forming bacteria per gram of yogurt.

34. The double-fermented yogurt product of Claim 29, wherein the double-fermented yogurt product has a lactose concentration of less than 1%.

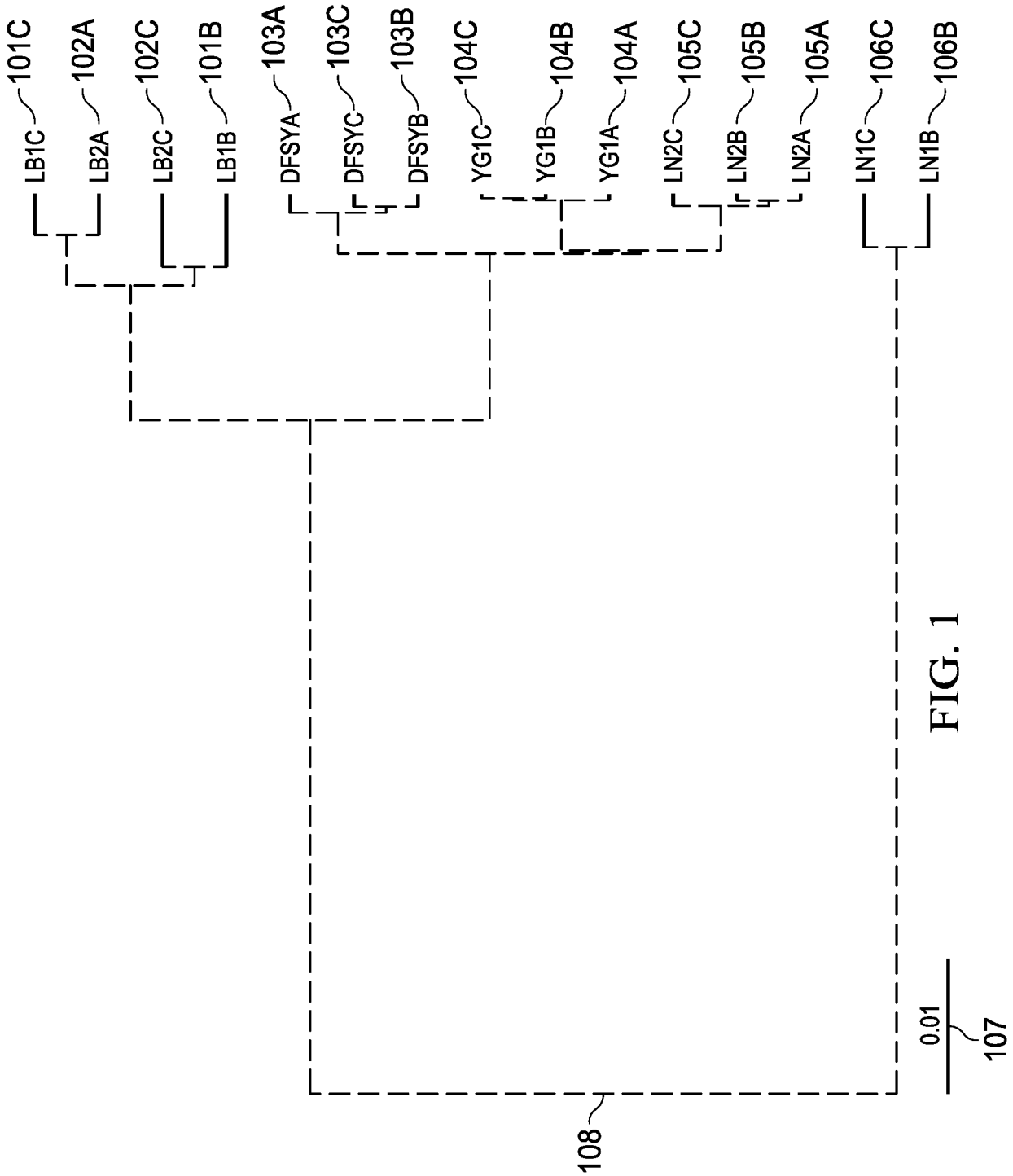

LB1C
101C
LB2A
102A
LB2C
102C
LB1B
101B
DFSYA
103A
DFSYC
103C
DFSYB
103B
YG1C
104C
YG1B
104B
YG1A
104A
LN2C
105C
LN2B
105B
LN2A
105A
LN1C
106C
LN1B
106B
0.01
107
108

FIG. 1

FIG. 2A

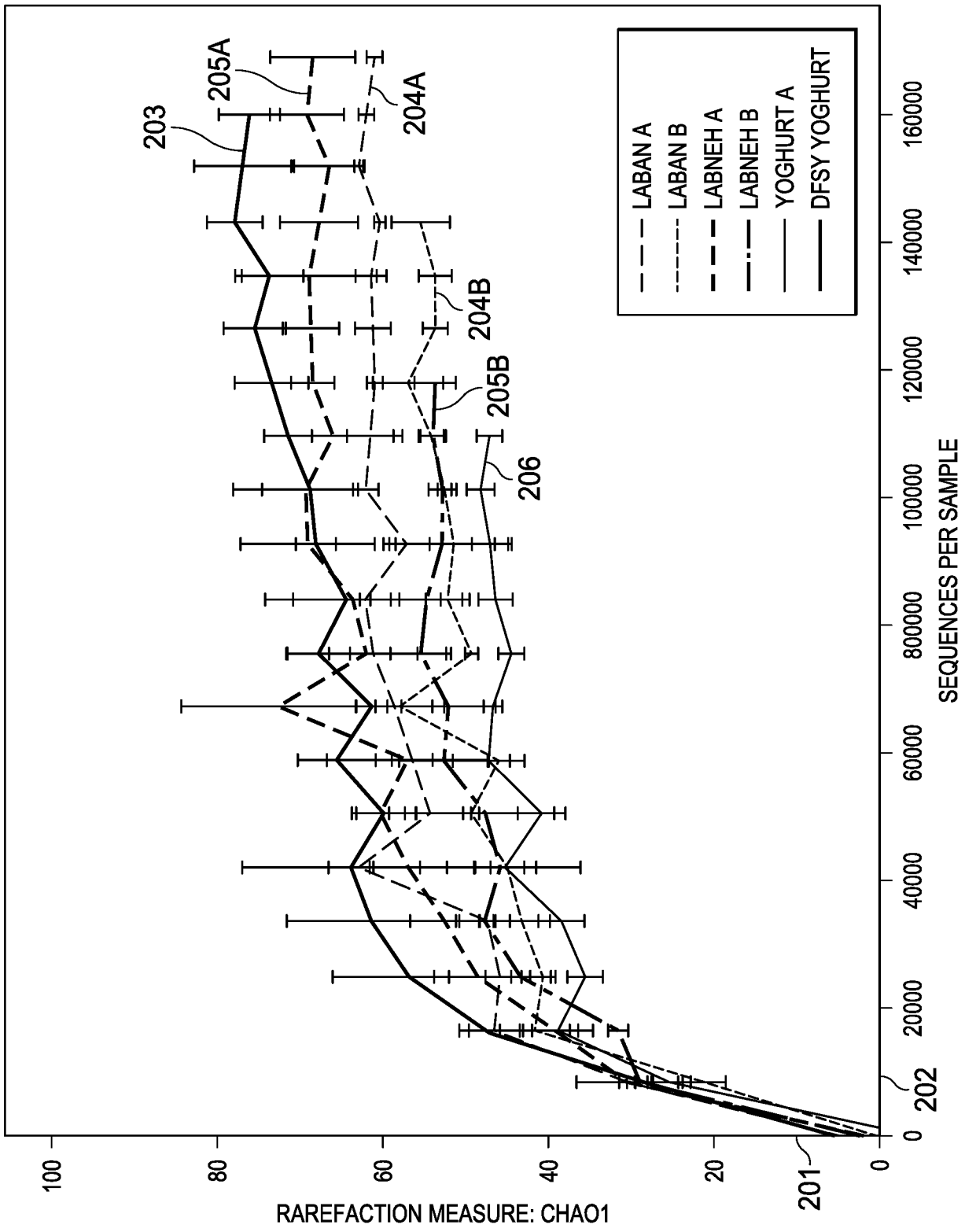

RAREFACTION MEASURE: CHAO1
SEQUENCES PER SAMPLE
LABAN A
LABAN B
LABNEH A
LABNEH B
YOGHURT A
DFSY YOGHURT
203
205A
204A
204B
205B
206
201
202
0
20
40
60
80
100
0
20000
40000
60000
80000
100000
120000
140000
160000

FIG. 2B

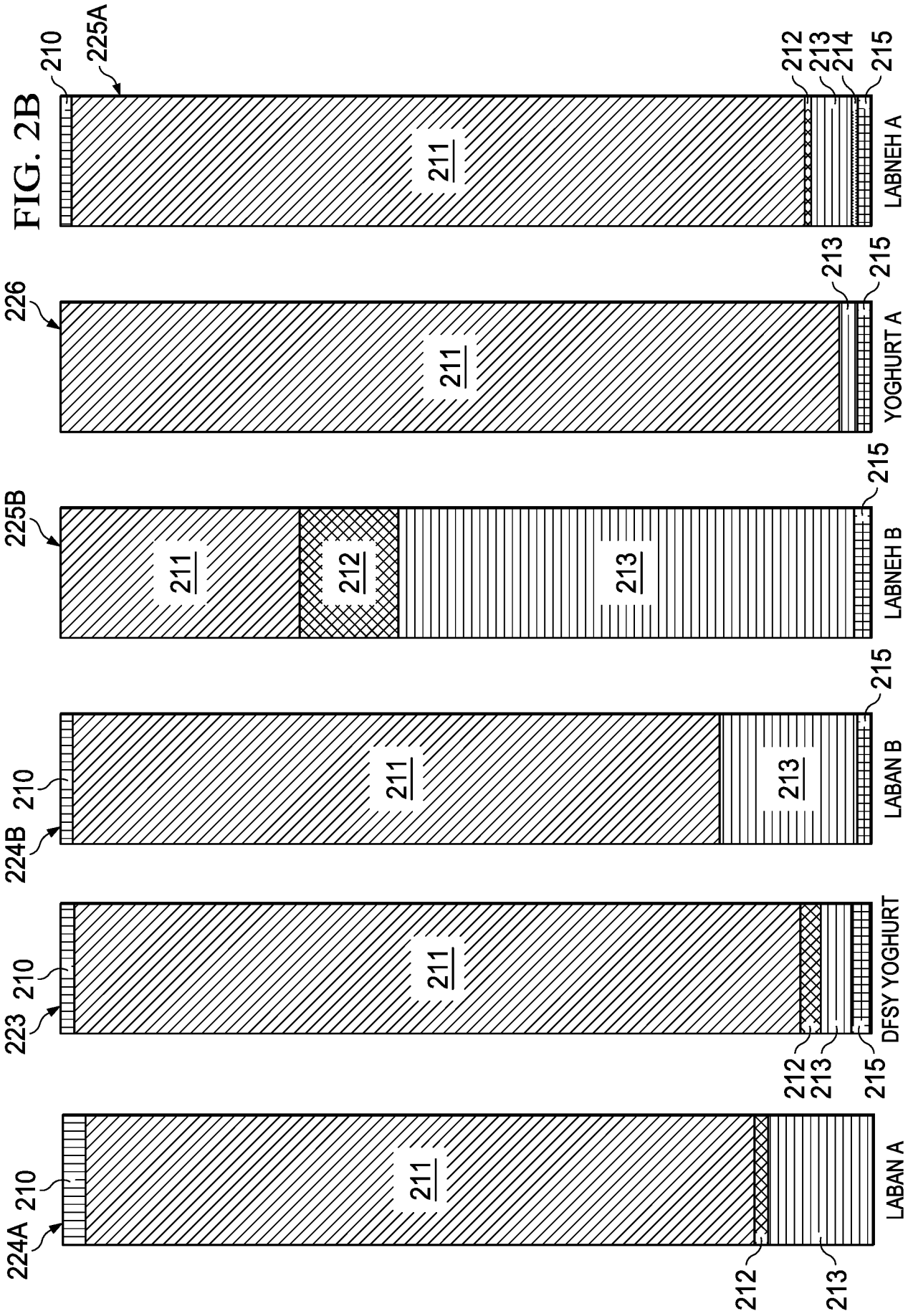
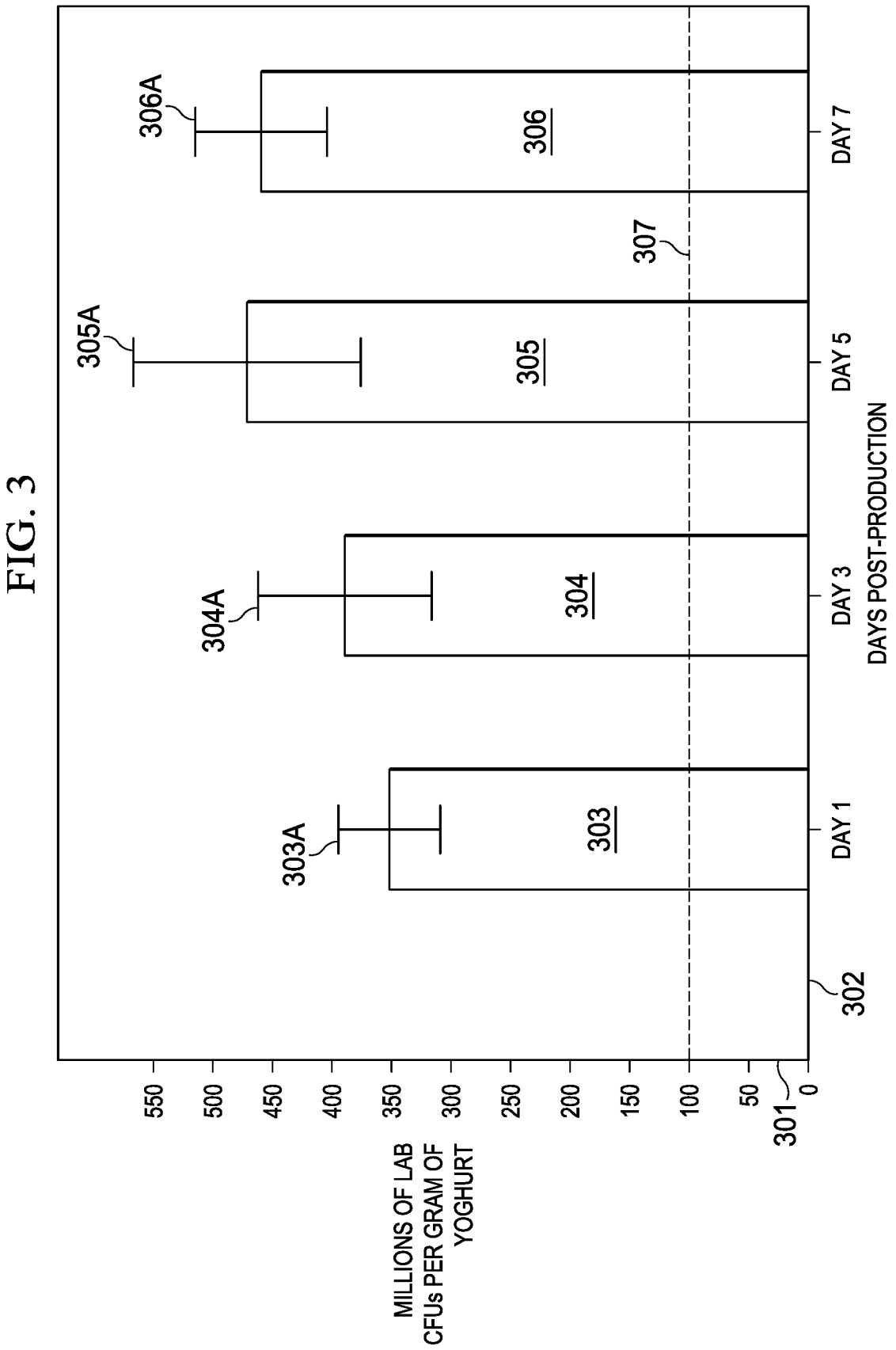

210
225A
211
212
213
214
215
LABNEH A
226
211
213
215
YOGHURT A
225B
211
212
213
215
LABNEH B
224B
210
211
213
215
LABAN B
223
210
211
212
213
215
DFSY YOGHURT
224A
210
211
212
213
LABAN A

FIG. 3


MILLIONS OF LAB CFUs PER GRAM OF YOGHURT
550
500
450
400
350
300
250
200
150
100
50
0
301
302
303
303A
304
304A
305
305A
306
306A
307
DAY 1
DAY 3
DAY 5
DAY 7
DAYS POST-PRODUCTION

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2017/053095

A. CLASSIFICATION OF SUBJECT MATTER
INV. A23C9/12 A23C9/123 A23C9/127
ADD.

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)
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 practicable, search terms used)
EPO-Internal , BIOSIS, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 122 104 A2 (ROBERTS JAMES GORDEN) 17 October 1984 (1984-10-17) page 8, line 7 - page 12, line 6; claims 1-8 page 20, line 22 - line 28 page 22, line 30 - page 25, line 28	1-34
X	US 4 034 115 A (ROBERTS JAMES GORDON) 5 July 1977 (1977-07-05) column 3, line 22 - column 4, line 51; claims 1-7	1-34
X	WO 03/090546 Al (MAHDAVI J) 6 November 2003 (2003-11-06) page 4, line 10 - page 7, line 15; examples 1,6	1-34

-/- .

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 July 2017	25/08/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Munteanu, I

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2017/053095

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CN 104 904 850 A (NANJING DADI REFRIGERATION FOOD CO LTD) 16 September 2015 (2015-09-16) claim 4; examples 1-4; table 1	1-34
A	W0 2016/043776 Al (GEN MILLS INC [US]) 24 March 2016 (2016-03-24) paragraph [0028] - paragraph [0031]	1-34

INTERNATIONAL SEARCH REPORT

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International application No
PCT/IB2017/053095

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		EP 3193624 A1	26-07-2017
		WO 2016043776 A1	24-03-2016