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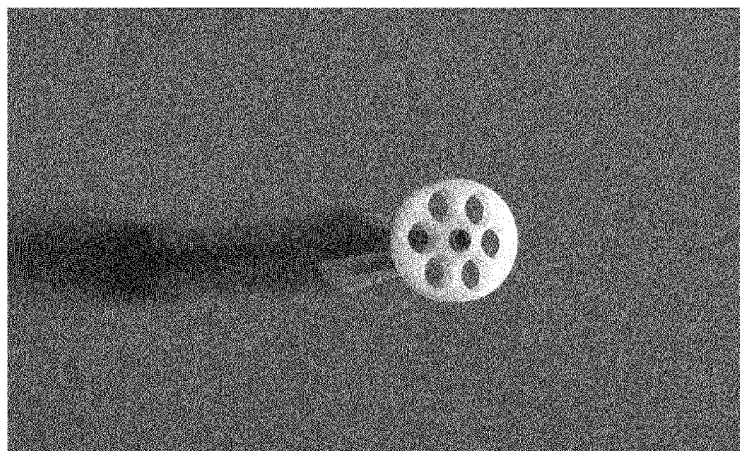
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(54) **Title:** METHODS AND TOOLS FOR MANUFACTURING FERMENTED MILK PRODUCTS IN MICRO CONTAINERS

FIGURE 9



(57) **Abstract:** The present invention relates to an automatic process of preparing one or more dairy product samples independently in one or more micro containers where each container comprises one dairy product sample. The present invention also relates to micro tools such as micro tools for use in the process of the present invention.

TITLE: Methods and tools for manufacturing fermented milk products in micro containers

FIELD OF THE INVENTION

The present invention relates to an automatic process of preparing one or more dairy product samples independently in multiple micro containers where each container comprises one dairy product sample. In particular, the present invention relates to an automatic process of preparing one or more dairy product samples. Moreover, the present invention relates to micro tools such as micro tools for use in the process of the present invention.

BACKGROUND OF THE INVENTION

In the dairy industry, it is usual to measure chemical and physical properties of dairy products in order to ensure a standardized product.

Fermented products from milk, whey or other raw materials should meet specific requirements in terms of acidity, flavor, viscosity and yield. Dairy products, for example yogurt, often have a specific acidity and a specific viscosity. The acidity of the medium is strongly related to the viscosity and the yield of the final product as it is the driving force for protein agglomeration and creation of the protein networks.

Changes in the process parameters have a significant effect on the quality of the final product. Often in fermentations of milk, lactic acid or other (NLAB) bacteria have been added to the milk to provide a desired fermentation. However, different lactic acid bacterial cultures used in the fermentation may result in different lactic acid or other compound production such as EPS, CO₂ or VOC that can cause altered flavor, altered viscosity and poor yield. It is then common to culture the lactic acid bacteria in specific media and then measure the properties in the product. This is often done on model products during development. Many resources are needed to create such model products and measure properties of the products in a range of different applications, as samples might ferment differently. Most of these model products are in a range of volumes of 100 ml to 20 L. The volume of these models combined with the wish for extensive number of designed experiments for product or process optimization, make it difficult to automate the procedures, which in turn makes the screening work very laborious.

It is desired to provide an automated method for making large amounts of fermentation samples in a low volume system under controlled process conditions and measuring of properties as acidity,

viscosity and yield of the fermented products is handled for each sample individually. It is preferred that the method can be subjected to automation and handling of a large number of samples, and that the samples used can be contained in micro plates.

In terms of pH measurements of milk related products and for many samples in micro plates, the use of colorimetric determination is described in the US patent publication No. US 2009/0215027A1.

Standard rheology measurements require relatively large samples of the medium to be tested.

Measurements of viscosity parameters can be performed by different methods where shear rates are applied to the measured product under highly controlled conditions (dimension of measuring systems, speed, temperatures, etc.). The resulting stress parameters are recorded. Although these types of measurement are accurate and reproducible, they are highly demanding in respect of the time required per sample, technical skills and precision.

Yield measurements are unusual and cumbersome in small scale experiments. They are based on mass calculations of products in larger scale where all ingredients can be weighted before and after process. In small scale experiments some ingredients are used in very low proportions making it very difficult to measure. If the number of samples is large then this type of measurement becomes extremely laborious.

The way the sample is fermented and processed during and after fermentation, are critical factors influencing the measuring accuracy. Usage of Liquid handling robots can reassure a uniform treatment of such samples.

Industrial production of fermented products such as stirring yoghurt implies the transfer of the acidified milk curd from fermenters to buffer tanks and coolers through pumps and tubes. Such processes lead to the breakdown and stirring of the product.

For model products the same or similar techniques are used in smaller scale in order to simulate the process stress on the fermented product. Due to the sample size, these techniques can be applied individually to each sample generating extra workload to prepare the samples for measurement.

Until now, no automatic, small scale, easy methods and systems for multiple sample processing, and measurement of fermented products have been provided due to the lack of specific tools for sample treatment.

Among other advantages, the method of the present invention makes it possible to automatically process numerous samples (such as 8-384), suitable for high throughput screening of viscosity of

fermented milk products e.g. of yoghurts and yield experiments for cheese products e.g. cottage cheese, without manual intervention and process variations.

SUMMARY OF THE INVENTION

The present invention provides designed micro tools and a method to process and prepare product samples for determination of acidity, viscosity, flavor and yield and correlates the measurement with specific product or process properties. In particular, the present invention provides a method and a system for determining the acidity, viscosity, flavor and yield of small scale fermented products, such as a stirred yogurt with accuracy and speed and without requiring manual work for sample preparation, treatment and measuring. The present invention allows automatic processing of large numbers of small scale fermented products and an interactive simulation of a process stress or process control by using online measurements from individual samples.

In a first aspect the present invention relates to an automatic process of preparing one or more dairy product samples independently in one or more micro containers where each container comprises one dairy product sample, the process comprising:

- (i) programming a robot for automatic handling of one or more independent processes according to specific individual recipes for each dairy product sample;
- (ii) adding by the robot a milk as a raw material, and optionally further additives according to the specific recipe, to each micro container to obtain a milk sample to be processed;
- (iii) adding by the robot an acidifying agent to each micro container;
- (iv) start the process in each micro container by the robot and on-line monitoring of one or more specific parameter(s) of the process in each container, which parameter(s) are used to determine when the milk sample being processed has reached a specific stage where physical manipulation of the sample is necessary;
- (v) monitoring the parameter(s) of each milk sample being processed to determine when the processed milk sample has reached the specific stage for physical manipulation;
- (vi) physical manipulation of each individual processed milk sample upon reaching the specific stage with a micro tool to be operated by a robot in an automatic process of preparing one or more dairy product samples independently in one or more micro containers, the micro tool being adapted to fit into each micro container and having a size and shape that can physically manipulate each processed milk sample independently without removing the processed milk sample; and
- (vii) obtaining the one or more dairy product samples when at least one milk sample has been physically manipulated.

The present invention may be used in methods for automatic preparation and processing of a large number of small scale fermented milk samples comprising:

- a) A robotic device to prepare and process samples in a micro plate format
- b) A sequence of process handlings for different milk products being fermented
- 5 c) Specifically designed micro tools for the different processes
- d) A method to control specially designed micro tools for different treatment of samples in the micro plate

10 In particular, the present invention makes use of a robotic device to prepare, and process samples in a micro plate comprising:

- i. aspirating and/or dispensing a sample using a device equipped with at least one pipetting tip capable of moving along X (horizontal), Y (vertical) and Z (into plane) axis
- ii. collecting the specific tools with at least one pipetting tip
- iii. coordinating pipetting tips to non-ordered locations within an array sequence of a micro plate
- 15 iv. ejecting the pipetting tips to specific locations within an array sequence of a washing station

In particular, the present invention relates to a sequence of process handlings for different milk products being fermented comprising:

- i. inoculation of the milk base with the appropriate microorganisms in the correct dosage
- 20 ii. fermentation of the milk base at controlled temperature conditions
- iii. application of process stress as heating, cooling, stirring, cutting and pressing at predefined time or predefined pH

25 In particular, the present invention relates to specifically designed micro tools for the different processes comprising:

- i. a micro tool storage station
- ii. homogenizing micro disc with through-holes for stirring of fermented milk gels in micro plate well
- iii. micro network of strings with specific dimensions for cutting fermented milk gels in micro plate well
- 30 iv. micro weights for pressing under whey in micro plate well
- v. a micro tool washing station

In particular, the present invention relates to a method to control specially designed micro tools for different treatment of samples in the micro plate comprising:

- i. an online database of pH driven colorimetric changes of the individual wells of a micro plate
- 5 ii. a personal computer
- iii. a software system for the control of the robotic system
- iv. a predefined sequence of sample arrays

In a further aspect the present invention relates to an automatic process of preparing one or more yoghurt samples independently in one or more micro containers where each container comprises one
10 yoghurt sample, the process comprising:

- (i) programming a robot for automatic handling of one or more independent processes according to specific individual recipes for each yoghurt sample;
- (ii) adding by the robot a milk as a raw material, such as skimmed milk, and optionally
15 further additives according to the specific recipe, to each micro container to obtain a milk sample to be fermented;
- (iii) adding by the robot a bacteria to each micro container;
- (iv) start fermentation in each micro container by the robot and at a temperature of about 43°C, and on-line monitoring of pH of the process in each container, which pH is used to
20 determine when the fermented milk sample has reached pH 4.55 where stirring of the milk sample is necessary;
- (v) monitoring the pH of each fermented milk sample to determine when the milk sample has reached the specific stage of pH 4.55 for stirring;
- (vi) stirring of each individual fermented milk sample upon reaching pH 4.55 with a micro
25 tool to be operated by a robot in an automatic process of preparing one or more yoghurt samples independently in one or more micro containers, the micro tool being adapted to fit into each micro container and having a size and shape that can physically manipulate each fermented milk sample independently without removing the milk sample;
- (vii) obtaining the one or more yoghurt samples when at least one milk sample has been
30 stirred.

In a further aspect the present invention relates to an automatic process of preparing one or more cheese and whey samples independently in one or more micro containers where each container comprises one cheese and whey sample, the process comprising:

- (i) programming a robot for automatic handling of one or more independent processes according to specific individual recipes for each cheese sample;
- (ii) adding by the robot a milk as a raw material, such as skimmed milk, and optionally further additives according to the specific recipe, to each micro container to obtain a milk sample to be fermented;
- (iii) adding by the robot a bacteria to each micro container;
- (iv) start fermentation in each micro container by the robot and at a temperature of about 37°C,
- (v) adding by the robot a coagulating agent, such as rennet, according to the specific recipe, to each micro container to obtain the milk sample to be fermented;
- (vi) on-line monitoring of pH of the process in each container, which pH is used to determine when the fermented milk sample has reached a pH between 4.7-6.6, e.g. 5.2, where cutting of the fermented milk sample is necessary;
- (vii) cutting of each individual fermented milk sample upon reaching pH between 4.7-6.6 with a micro tool to be operated by a robot in an automatic process of preparing one or more cheese samples independently in one or more micro containers, the micro tool being adapted to fit into each micro container and having a size and shape that can physically manipulate each fermented milk sample independently without removing the fermented milk sample; and
- (viii) pressing of each individual cheese sample according to the recipe with a micro tool to be operated by a robot in an automatic process of preparing one or more cheese samples independently in one or more micro containers, the micro tool being adapted to fit into each micro container and having a size and shape that can physically manipulate each cheese sample independently without removing the cheese sample, to obtain the one or more cheese and whey samples when at least one milk sample has been pressed.
- 25 In a further aspect the present invention relates to a micro tool to be operated by a robot in an automatic process of preparing one or more dairy product samples independently in one or more micro containers, the micro tool being adapted to fit into each micro container and having a size and shape that can physically manipulate each processed milk sample independently without removing the milk sample.
- 30 Further objects and advantages of the present invention will appear from the following description, and claims.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows a general diagram for micro scale fermented dairy products, such as yoghurt and cheese, according to the present invention.

Figure 2 shows a specific diagram for low fat stir yoghurt according to the present invention.

Figure 3 shows an embodiment of the cutting net of the present invention with a mesh for cutting for instance a cheese.

Figure 4 shows an embodiment of the disc of the present invention with through holes.

- 5 Figure 5 shows another embodiment of the disc of the present invention with through holes.

Figure 6 shows another embodiment of the cutting net of the present invention with a mesh for cutting for instance a cheese.

Figure 7 shows an embodiment of the perforated weight of the present invention.

Figure 8 shows an embodiment of the prototype disc with through holes design used for SLA models

- 10 Figure 9 shows an embodiment of the prototype disc as shown in figure 8 attached to a tip of a pipette.

Figure 10 shows an embodiment of pressure curves recorded during aspiration of yogurt samples.

Figure 11 shows an embodiment of pressure curves recorded during dispensing of yogurt samples.

DESCRIPTION OF THE INVENTION

- 15 In the automatic process of preparing one or more dairy product samples in principle as few as one sample may be prepared, and typically at least two product samples and in principle as many as the skilled person considers necessary and typically up to 384 samples or more. In one embodiment of the present invention the one or more dairy product samples are at least two, such as from 2 to 10.000, such as 4 to 5000, such as 6 to 2000, such as 8 to 1000, e.g. from 8 to 384.
- 20 The number of product samples usually matches the number of micro containers; however, obviously the number of product samples can be less, such as 8 samples in a 384 micro titer well plate. In an embodiment of the present invention the one or more micro containers are selected from at least two micro containers, such as such as from 2 to 10.000, such as 4 to 5000, such as 6 to 2000, such as 8 to 1000, e.g. from 8 to 384.
- 25 As used herein the term “micro container” is intended to mean any container of a small lab scale size suitable for carrying out experiments to be later analyzed in order to obtain data information that can be used for implementation in a larger scale process, such as industrial scale process. Typically, the micro container is selected from a well of a micro titer plate (MTP), such as MTP with at least 1 well, typically 8 to 384 wells.

As used herein the term “independently” in respect of the process of preparing one or more dairy product samples independently in one or more micro containers is intended to mean that each milk sample in each micro container is processed in accordance with the process steps of the process of the present invention, and that the process in each micro container is carried out without being influenced by any one of the processes going on in the other micro containers. Thus, if for instance a MTP of 96 wells is used, then 96 different processes may be running and they may be finished at different times in accordance with the progression of the process in each micro container.

The programming of a robot as described in step (i) may be done by a person skilled in the art of programming and simply requires that a computer is operably connected to the robot and that relevant data are loaded into the computer in accordance with the recipe of the dairy product to be prepared. The specific recipe for each dairy product to be prepared may be different and the term “individually” as used herein is intended to cover specific recipes that may be the same or different for each product sample, thus, if for instance a MTP of 96 wells is used, then 96 different specific recipes may be used or some or all of the recipes may be the same.

The term “specific recipe” as used herein is intended to mean that the recipe is pre-made for preparing a specific dairy product sample and that the skilled person can enter the recipe data into a computer that may then communicate with the robot to carry out the automatic process.

In a further embodiment the dairy product is selected from a milk product, such as yoghurt, buttermilk, sour cream or a cheese. In an embodiment the dairy product is a yoghurt.

In step (ii) the robot adds a milk as a raw material, and optionally further additives according to the specific recipe, to each micro container to obtain a milk sample to be processed.

As used herein the term “milk sample being processed” or “processed milk sample” is intended to mean that the milk sample is in progress to become the final dairy product sample, but has not reached this stage yet. Thus, a processed milk sample is an intermediate product sample that may change over time and the final product sample is obtained when the final step, such as step (vii), of the process of the present invention is fulfilled.

As used herein the term “milk” is intended to mean any raw and/or processed milk material that can be subjected to fermentation according to the method of the invention. Thus, useful milk substrates include, but are not limited to, solutions/suspensions of any milk or milk like products comprising

protein, such as whole or low fat milk, skim milk, buttermilk, reconstituted milk powder, condensed milk, dried milk, whey, whey permeate, lactose, mother liquid from crystallization of lactose, whey protein concentrate, or cream. Obviously, the milk substrate may originate from any mammal (such as a cow, a goat, or a pig), e.g. being substantially pure mammalian milk, or reconstituted milk powder.

- 5 In a further embodiment the raw material is selected from low to high fat milk, such as skimmed milk having a fat content of from 0-2% w/w.

When milk is filled into the micro containers, this typically is taking place at a temperature of about 15-60°C, such as 30-45°C, such as about 43°C. The specific recipe will determine which temperature is desired.

- 10 The robot may have one robotic arm that carries out each independent process in each micro container or may have more robotic arms. In a further embodiment each robotic arm is equipped with a pipette. During the process the robotic arm may exchange such pipettes as many times as is required to avoid contamination of the samples.

- Typically, the robot may be any robot that can be programmed to handle one or more operations; such
15 is an automated pipetting station e.g. a robot of the type Hamilton Robotics MicroLab Star. Such robots that are suitable in the present process are described in for instance a JANUS® Automated Workstation from Perkin Elmer or a Xantus modular robotic pipetting platform from Sias AG.

In step (iii) an acidifying agent is added by the robot (typically, via the robotic arm(s) to each micro container.

- 20 As used herein the term "acidifying agent" is intended to mean any agent in solid, fluid or liquid form, which upon administration to a micro container is able to lower the pH in the container compared to the pH in the container before adding the acidifying agent. In a further embodiment the acidifying agent is selected from a microorganism and acid. In one embodiment the acidifying agent is selected from a microorganism. In another embodiment the acidifying agent is selected from an acid.

- 25 As used herein, the term "microorganism" designates a gram-positive, microaerophilic or anaerobic bacterium, which ferments sugars with the production of acids including lactic acid as the predominantly produced acid, acetic acid and propionic acid. The industrially most useful lactic acid bacteria are found within the order "Lactobacillales" which includes Lactococcus spp., Streptococcus spp., Lactobacillus spp., Leuconostoc spp., Pediococcus spp., Brevibacterium spp., Enterococcus spp.
30 and Propionibacterium spp. Lactic acid bacteria, including bacteria of the species Lactobacillus sp. and Streptococcus thermophilus, are normally supplied to the dairy industry either as frozen or freeze-dried cultures for bulk starter propagation or as so-called "Direct Vat Set" (DVS) cultures, intended

for direct inoculation into a fermentation vessel or vat for the production of a dairy product, such as a fermented milk product. Such cultures are in general referred to as “starter cultures” or “starters”.

In a still further embodiment the acidifying agent is a microorganism selected from bacteria, such as lactic acid bacteria within the order Lactobacillales, such as lactic acid bacteria within the species

5 *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*.

In a further embodiment the acidifying agent is an acid selected from an inorganic or organic acid, such as lactic acid, acetic acid or propionic acid.

In step (iv) the process in each micro container is started by the robot and on-line monitoring of one or more specific parameter(s) of the process in each container, which parameter(s) are used to determine

10 when the dairy product sample has reached a specific stage where physical manipulation of the product sample is necessary. Typically, the specific parameter to be monitored is one or more of pH, O₂, CO₂, viscosity and yield. In one embodiment the specific parameter to be monitored is pH. The timing of the physical manipulation is determined by the specific stages to be reached, and by monitoring the specific parameters, such as one or more of pH, O₂, CO₂, viscosity and yield, it can be
15 determined when the specific stage is reached. The specific stage is reached when the specific parameter as determined before starting the process has reached a pre-determined level, for instance, if the specific stage is determined to be a certain pH, e.g. pH = 4.55, then monitoring when the specific parameter pH reaches the specific stage pH 4.55 in any one of the samples will determine the initiation of the physical manipulation in step (vi) of the process of the present invention.

20 As used herein the term “physical manipulation” is intended to mean that the micro tool is in physical contact with the product sample and manipulates the product sample by pressure and/or movements, for instance by moving up and/or down, causing stirring, homogenizing, whipping, cutting, shredding, milling of the sample, or by resting on the sample surface applying pressure on the sample.

In step (v) monitoring the parameter(s) of each milk sample being processed to determine when the
25 processed milk sample has reached the specific stage for physical manipulation to be carried out. This monitoring may be carried out by on-line monitoring via a computer.

In step (vi) physical manipulation of each individual processed milk sample upon reaching the specific stage with a micro tool to be operated by a robot in an automatic process of preparing one or more dairy product samples independently in one or more micro containers is carried out, wherein the
30 micro tool being adapted to fit into each micro container and having a size and shape that can physically manipulate each processed milk sample independently without removing the processed milk sample.

As used herein the term “micro tool” is intended to mean a tool of small dimensions, the micro tool can have the form of a cutting net with variable mesh distance, a perforated weight of variable weight, a disc with through holes of variable diameter to be operated by a robot in the automatic process of the present invention and independently physically manipulate processed milk samples or dairy product samples in the micro containers. The micro tool being adapted to fit into each micro container and having a size and shape that can physically manipulate each processed milk sample or dairy product sample independently without removing the sample. It is clear to the skilled person that by getting into physical contact the micro tool may remove insignificant amounts of the sample, but the amounts will be without relevance for the process, the monitoring and the data collection.

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In an embodiment the robot may have one or more robotic arms, wherein each robotic arm is equipped with a pipette, and the pipette tip is equipped with the micro tool.

In a further embodiment the micro tool is used to stirring, cutting, pressing or draining the product sample in each micro container.

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In an embodiment the micro tool is selected from a cutting net. Typically, the tool is a cutting net with mesh width of 0.1 to 3 mm, such as 0.5 to 2 mm. Typically, the cutting net has a size with dimension 5-15 mm in length/depth or diameter and 2-20 mm in height.

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In another embodiment the micro tool is selected from a perforated weight. Typically, the perforated weight is from 1g to 50 g, such as 5 to 25 g. Typically, the perforated weight has a size with dimension 5-15 mm in length/depth or diameter and 2-20 mm in height.

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In a further embodiment the micro tool is selected from a disc with through holes. Typically, the disc has a diameter of 3-10 mm and a height of 0.5-5 mm, such as 5-8 mm and a height of 1-3 mm. The through holes typically have a diameter of 0.5-2.5 mm, such as 1-2 mm, e.g. 1.2-1.7 mm.

In a still further embodiment the micro tool, such as the disc, is used to stirring the sample in each micro container from 5 to 75 times, such as 10 to 50 times, e.g. 10 to 30 times.

In a further embodiment the micro tool is applied to the sample in the micro container when pH is about 4.55.

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In step (vii) the one or more dairy product samples are obtained when at least one milk sample has been processed.

In a further embodiment of the invention after step (vi) each product sample is moved after physical manipulation with the micro tool to a different micro container with a different temperature than the process e.g. at low temperature, and optionally adding one or more additives to the product sample according to the specific recipe. A main effect of this process step is to stop or slow down the processing, such as fermentation, of the product sample.

In a still further embodiment the one or more dairy product samples are post treated by physical manipulation of each product sample in the different micro container with the micro tool operated by the robot in an automatic process of preparing one or more dairy product samples independently in one or more micro containers, the micro tool being adapted to fit into each micro container and having a size and shape that can physically manipulate each dairy product sample independently without removing the product sample. A main effect of this process step is to make the sample ready for measuring, such as measuring of viscosity.

In a further embodiment the product sample is moved after treatment with the micro tool to a different micro container which is maintained at a temperature of about 4°C. A main effect of this process step is to stop or slow down the processing, such as fermentation, of the product sample.

In a still further embodiment the product samples are maintained for 1 to 30 days at constant temperature, such as at about 4°C. In further embodiments the product samples are maintained for 2 to 26 days, such as 4 to 24, such as 6 to 20, such as 8 to 16, e.g. 10 to 14 days, at constant temperature. A main effect of this process step is to simulate the shortest time before the dairy product is used.

In a further embodiment the product samples after maintenance at constant temperature for 1-7 days are stirred at 13°C, and then aspirated and dispensed in an amount sufficient for data recording. When such aspiration and dispensing is performed this is done with an automated pipetting station e.g. a robot of the type Hamilton Robotics MicroLab Star.

A particular aspect of the present invention is preparation of one or more yoghurt samples independently in one or more micro containers where each container comprises one yoghurt sample.

All embodiments in respect of the first aspect also apply to the preparation of one or more yoghurt samples.

The legal definition of yoghurt in many countries requires *Streptococcus thermophilus* alongside *Lactobacillus delbrueckii* subsp. *bulgaricus*.

Thus, in a preferred embodiment, the acidifying agent of step (iii) is at least one bacterium, such as a *Streptococcus thermophilus* bacterium and a *Lactobacillus delbrueckii* subsp. *bulgaricus* bacterium.

In particular, after step (vi) each yoghurt sample is moved after stirring with the micro tool to a different micro container, where temperature is kept constant at about 4°C, and optionally add one or more additives to the yoghurt sample according to the specific recipe.

Furthermore, the one or more yoghurt samples may be post treated by stirring of each yoghurt sample in the different micro container with the micro tool operated by the robot in an automatic process of preparing one or more dairy product samples independently in one or more micro containers, the micro tool being adapted to fit into each micro container and having a size and shape that can physically manipulate each dairy product sample independently without removing the product sample. In a further embodiment the post treatment is initiated after 1-7 days after the last yoghurt sample is moved to the different micro container. Hereafter the one or more fermented yoghurt samples may be stirred at about 13°C and then aspirated and dispensed in an amount sufficient for data recording.

In an embodiment of the particular aspect the robot may have one or more robotic arms, wherein each robotic arm is equipped with a pipette, and the pipette tip is equipped with the micro tool.

In a further embodiment of the particular aspect the micro tool is used to stirring, cutting, pressing or draining the product sample in each micro container.

In an embodiment of the particular aspect the micro tool is selected from a cutting net. Typically, the tool is a cutting net with mesh width of 0.1 to 3 mm, such as 0.5 to 2 mm. Typically, the cutting net has a size with dimension 5-15 mm in length/depth or diameter and 2-20 mm in height.

In another embodiment of the particular aspect the micro tool is selected from a perforated weight. Typically, the perforated weight is from 1g to 50 g, such as 5 to 25 g. Typically, the perforated weight has a size with dimension 5-15 mm in length/depth or diameter and 2-20 mm in height.

In a further embodiment of the particular aspect the micro tool is selected from a disc with through holes. Typically, the disc has a diameter of 3-10 mm and a height of 0.5-5 mm, such as 5-8 mm and a height of 1-3 mm. The through holes typically have a diameter of 0.5-2.5 mm, such as 1-2 mm, e.g. 1.2-1.7 mm.

In a still further embodiment of the particular aspect the micro tool, such as the disc, is used to stirring the sample in each micro container from 5 to 75 times, such as 10 to 50 times, e.g. 10 to 30 times.

In a further aspect the present invention relates to an automatic process of preparing one or more cheese and whey samples independently in one or more micro containers where each container comprises one cheese and whey sample.

- 5 All embodiments in respect of the first aspect also apply to the preparation of one or more cheese samples.

In particular in step (vii) the pH should reach 4.7 to 6.6, such as 4.9 to 6.2, such as 5.0 to 6.0, e.g. about 5.2.

- 10 In an embodiment after step (viii) each whey sample is removed after pressing with the micro tool to a different micro container, where temperature is kept constant at about 4°C.

- In a further embodiment the one or more cheese samples are post treated by pressing of each sample with the micro tool operated by the robot in an automatic process of preparing one or more cheese and whey product samples independently in one or more micro containers, the micro tool being adapted to fit into each micro container and having a size and shape that can physically manipulate each cheese and whey product sample independently without removing the product sample.
- 15

In a still further embodiment the one or more whey samples are aspirated and dispensed in an amount sufficient for data recording.

In an embodiment of the cheese aspect the robot may have one or more robotic arms, wherein each robotic arm is equipped with a pipette, and the pipette tip is equipped with the micro tool.

- 20 In a further embodiment of the cheese aspect the micro tool is used to cutting, pressing or cutting and pressing the product sample in each micro container.

- In an embodiment of the cheese aspect the micro tool is selected from a cutting net. Typically, the tool is a cutting net with mesh width of 0.1 to 3 mm, such as 0.5 to 2 mm. Typically, the cutting net has a size with dimension 5-15 mm in length/depth or diameter and 2-20 mm in height.
- 25

In another embodiment of the cheese aspect the micro tool is selected from a perforated weight. Typically, the perforated weight is from 1g to 50 g, such as 5 to 25 g. Typically, the perforated weight has a size with dimension 5-15mm in length/depth or diameter and 2-20mm in height.

- In a further aspect the present invention relates to a micro tool as defined above, that is a micro tool to be operated by a robot in an automatic process of preparing one or more dairy product samples independently in one or more micro containers, the micro tool being adapted to fit into each micro container and having a size and shape that can physically manipulate each dairy product sample or
- 30

each milk sample being processed independently without removing the sample. In an embodiment the micro tool is attached to a pipetting tip mounted on a robotic arm, or is an integrated part of pipetting tip mounted on a robotic arm, or the micro tool is mounted on a robotic arm. In a further embodiment the micro tool is used to stirring, cutting, pressing or draining the product sample in each micro container. In an embodiment the micro tool is selected from a cutting net. Typically, the tool is a cutting net with mesh width of 0.1 to 3 mm, such as 0.5 to 2 mm. Typically, the cutting net has a size with dimension 5-15mm in length/depth or diameter and 2-20mm in height. In another embodiment the micro tool is selected from a perforated weight. Typically, the perforated weight is from 1g to 50 g, such as 5 to 25 g. Typically, the perforated weight has a size with dimension 5-15mm in length/depth or diameter and 2-20mm in height. In a further embodiment the micro tool is selected from a disc with through holes. Typically, the disc has a diameter of 3-10 mm and a height of 0.5-5 mm, such as 5-8 mm and a height of 1-3 mm. The through holes typically have a diameter of 0.5-2.5 mm, such as 1-2 mm, e.g. 1.2-1.7 mm. In a still further embodiment the micro tool, such as the disc, is used to stirring the sample in each micro container from 5 to 75 times, such as 10 to 50 times, e.g. 10 to 30 times.

All references, including publications, patent applications and patents, cited herein are hereby incorporated by reference to the same extent as if each reference was individually and specifically indicated to be incorporated by reference and was set forth in its entirety herein.

All headings and sub-headings are used herein for convenience only and should not be construed as limiting the invention in any way.

Any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

Recitation of ranges of values herein are merely intended to serve as a short method of referring individually to each separate value falling within the range, unless other-wise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein.

Unless otherwise stated, all exact values provided herein are representative of corresponding approximate values (*e.g.*, all exact exemplary values provided with respect to a particular factor or measurement can be considered to also provide a corresponding approximate measurement, modified by "about", where appropriate).

All methods described herein can be performed in any suitable order unless other-wise indicated herein or otherwise clearly contradicted by context.

The terms “a” and “an” and “the” and similar referents as used in the context of de-scribing the invention are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. Thus, “a” and “an” and “the” may mean at least one, or one or more.

- 5 The use of any and all examples, or exemplary language (*e.g.*, “such as”) provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise indicated. No language in the specification should be construed as indicating any element is essential to the practice of the invention unless as much is explicitly stated.

- 10 The citation and incorporation of patent documents herein is done for convenience only and does not reflect any view of the validity, patentability and/or enforceability of such patent documents.

- The description herein of any aspect or embodiment of the invention using terms such as “comprising”, “having”, “including” or “containing” with reference to an element or elements is intended to provide support for a similar aspect or embodiment of the invention that “consists of”, “consists essentially of”, or “substantially comprises” that particular element or elements, unless
15 otherwise stated or clearly contradicted by context (*e.g.*, a composition described herein as comprising a particular element should be understood as also describing a composition consisting of that element, unless otherwise stated or clearly contradicted by context).

This invention includes all modifications and equivalents of the subject matter re-cited in the aspects or claims presented herein to the maximum extent permitted by applicable law.

- 20 The features disclosed in the foregoing description may, both separately and in any combination thereof, be material for realizing the invention in diverse forms thereof.

- The invention described and claimed herein is not to be limited in scope by the specific aspects herein disclosed, since these aspects are intended as illustrations of several aspects of the invention. Any equivalent aspects are intended to be within the scope of this invention. Indeed, various modifications
25 of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims. In the case of conflict, the present disclosure including definitions will control.

- 30 **EXAMPLE**

Equipment used for pipetting

A liquid handling station, Hamilton Robotics MicroLab Star, equipped with pressure sensor inside the air displacement barrel of the individual pipettes was used in the following experiments.

The Hamilton Robotics MicroLab Star has 8 pipetting channels, built upon air-displacement

- 5 technology, which is analogous to a hand held electronic pipette. Each channel can aspirate up to a volume of 1 ml. To aspirate within different volume ranges the channels can accommodate a range of tips with volume from 10, 50, 300 to 1000 μ l.

- Prototype micro tools for homogenizing were made with stereo lithography technique (SLA) with a 0.05 mm thickness of each layer (see figure 8). The dimensions of the homogenizing micro disc with through-holes were designed to fit to any 1-2 ml deep well plate. The outer diameter of the disc is 6.2 mm. The disc is 2 mm high. The disc has 6 through holes of a diameter of 1.7 mm. In the center, the disc has a hole with diameter of 1.55 mm on the one face (top) of the disc and of 1.25 mm on the other (bottom).

- The micro tools were attached with glue to a 1000 μ l disposable tip for a Hamilton MicroLab Star with the bottom face pointing away from the tip (see figure 9).

NUNC Deep well plates and flat bottom low well plates were used for the experiment.

Commercial yoghurt culture used for this test was from Chr. Hansen dairy cultures portfolio.

Fermentation of milk

- Reconstituted milk with a dry matter content of 9.5 %, which had been heat treated to 99°C for 15 minutes in a batch process was used for the experiment.

- A blue color indicator (consisting of bromocresol green and bromocresol purple) was added at a level of 5%. Then the robot has prepared 16 positions of a 2 ml deep well MTP (master plate) with 1500 μ l of the milk prior to inoculation. The wells were then inoculated by the robot with 0.02 % frozen concentrates of Culture 1. 350 μ l of the inoculated milk from each well were then transferred by the robot in 16 wells of a transparent low well flat bottomed MTP (pH plate). Incubation took place for both master and pH plate at 43°C. Sample pH was monitored by means of colorimetric determination with a sampling rate of one scan per 4 min.

Robot processing

- 30 The collected data from the colorimetric determination were imported in a temporary file with an update every 4 min. The robot software was programmed to read the temporary file in 10 min intervals. A work list for the robot was updated and if the pH in designated targets (wells) had reached 4.55, the pipetting arm would pick up the number of tips necessary and would stir the samples individually by moving up and down 10 times in each well. Then the micro tools would be ejected by the robot. Subsequently, 1000 μ l of the samples were aspirated from the master plate with a 1000 μ l

tip to a second deep well plate (target plate) stored at 4°C to cool and stop fermentation. Upon end of process the target plate was stored with a lid seal into a 4°C refrigerator until viscosity measurement. Samples were temperate at 13°C before viscosity measurements. A treatment of the samples with micro tools (stir the samples individually by moving up and down 15 times in each well) was made immediately before the actual viscosity measure.

Analysis of viscosity

Fermented milk samples prepared with a specific culture from CH portfolio, were prepared in deep well MTP plates and were stirred independently and uniformly by an automatic method to obtain homogeneity before transferring sample to target plate. For the viscosity measurement, only one aspiration and dispensing per well of the target plate was done, as the structure of the yoghurt will change when the tip enters. A volume of 500 µl was aspirated (25µl/sec) and dispensed (75µl/sec) and the pressure change was recorded in real time by the robot software.

Samples prepared with the same culture with an ordinary manual process in 3 L scale were measured and used for comparison reasons.

Results and conclusions

Fermented milks incubated with concentrate of yoghurt culture that are currently used in the dairy industry could be produced in small scale (deep well plate) by an automated process. Results from these micro yoghurt productions were comparable results from larger scale products with the same culture by the aspiration and/or dispensing pressure monitored under pipetting (see Figures 10 and 11). As the start volume in the master plate was 1150 µl and taken into account some losses during stirring, the volume moved to target plate was inconsistent. Additionally, some bubble creation during stirring in target plate created pressure drops during aspiration and dispensing. This is deflected on the curves of micro yoghurts in figures 10 and 11. This issue will be solved with method fine-tuning during stirring and volume adjustments.

Claims

1. An automatic process of preparing one or more dairy product samples independently in one or more micro containers where each container comprises one dairy product sample, the process comprising:
 - 5 (i) programming a robot for automatic handling of one or more independent processes according to specific individual recipes for each dairy product sample;
 - (ii) adding by the robot a milk as a raw material, and optionally further additives according to the specific recipe, to each micro container to obtain a milk sample to be processed;
 - (iii) adding by the robot an acidifying agent to each micro container;
 - 10 (iv) start the process in each micro container by the robot and on-line monitoring of one or more specific parameter(s) of the process in each container, which parameter(s) are used to determine when the milk sample being processed has reached a specific stage where physical manipulation of the sample is necessary;
 - (v) monitoring the parameter(s) of each milk sample being processed to determine when the
15 processed milk sample has reached the specific stage for physical manipulation;
 - (vi) physical manipulation of each individual processed milk sample upon reaching the specific stage with a micro tool to be operated by a robot in an automatic process of preparing one or more dairy product samples independently in one or more micro
20 containers, the micro tool being adapted to fit into each micro container and having a size and shape that can physically manipulate each processed milk sample independently without removing the processed milk sample; and
 - (vii) obtaining the one or more dairy product samples when at least one milk sample has been physically manipulated.
- 25 2. The process of claim 1 wherein after step (vi) each product sample is moved after physical manipulation with the micro tool to a micro container at a different temperature, and optionally add one or more additives to the product sample according to the specific recipe.
- 30 3. The process of claim 1 or 2 wherein the one or more dairy product samples are post treated by physical manipulation of each product sample in the different micro container with the micro tool operated by the robot in an automatic process of preparing one or more dairy product samples independently in one or more micro containers, the micro tool being adapted to fit into each micro container and having a size and shape that can physically manipulate each dairy product sample independently without removing the product sample.

4. The process of any one of the preceding claims wherein the micro containers are selected from micro titer plates (MTP), such as MTP with at least 1 well, such as at least two, typically 8 to 384 wells.
5. The process of any one of the preceding claims wherein the robot has one or more robotic arms.
6. The process of any one of the preceding claims wherein each robotic arm is equipped with a pipette.
7. The process of any one of the preceding claims wherein the pipette tip is equipped with a micro tool to be operated by a robot in an automatic process of preparing one or more dairy product samples independently in one or more micro containers, the micro tool being adapted to fit into each micro container and having a size and shape that can physically manipulate each dairy product sample or each milk sample being processed independently without removing the sample.
8. The process of any one of the preceding claims wherein the robot may be any robot that can be programmed to handle one or more operations, such as an automated pipetting station e.g. a robot of the type Hamilton Robotics MicroLab Star.
9. The process of any one of the preceding claims wherein the acidifying agent is selected from a microorganism and acid.
10. The process of any one of the preceding claims wherein the dairy product is selected from a milk product, such as yoghurt, buttermilk, sour cream or a cheese.
11. The process of any one of the preceding claims wherein the dairy product is a yoghurt.
12. The process of any one of claims 9-11 wherein the acidifying agent is selected from bacteria, such as lactic acid bacteria within the order Lactobacillales.
13. The process of any one of the preceding claims wherein the milk as the raw material is selected from low to high fat milk, such as skimmed milk having a fat content of from 0-2% w/w.
14. The process of any one of the preceding claims wherein the milk is filled into the micro containers at a temperature of about 15-60°C, such as 30-45°C, such as about 43°C.

15. The process of any one of the preceding claims wherein the specific parameter to be monitored is selected from pH, O₂, CO₂, viscosity, and yield.
16. The process of any one of the preceding claims wherein the specific parameter to be monitored is pH.
17. The process of any one of the preceding claims wherein the specific stage is when pH reaches 4.55 in any one of the samples.
18. The process of any one of the preceding claims wherein the micro tool is applied to the sample in the micro container when pH is about 4.55.
19. The process of any one of the preceding claims wherein the micro tool is used to stirring, cutting, pressing or draining the product sample in each micro container.
20. The process of any one of the preceding claims wherein the product sample is moved after treatment with the micro tool to a different micro container which is maintained at a temperature of about 4°C.
21. The process of any one of the preceding claims 2-19 wherein the product samples are maintained for 1 to 7 days at constant temperature, such as of about 4°C.
22. A micro tool to be operated by a robot in an automatic process of preparing one or more dairy product samples independently in one or more micro containers, the micro tool being adapted to fit into each micro container and having a size and shape that can physically manipulate each dairy product sample or each milk sample being processed independently without removing the sample.
23. The micro tool of claim 22 wherein the micro tool is attached to a pipetting tip mounted on a robotic arm, or is an integrated part of pipetting tip mounted on a robotic arm, or the micro tool is mounted on a robotic arm.
24. The micro tool of any one of claims 22-23 wherein the tool is selected from a cutting net, a perforated weight, a disc with through holes.

FIGURE 1

General diagram for micro scale fermented dairy products

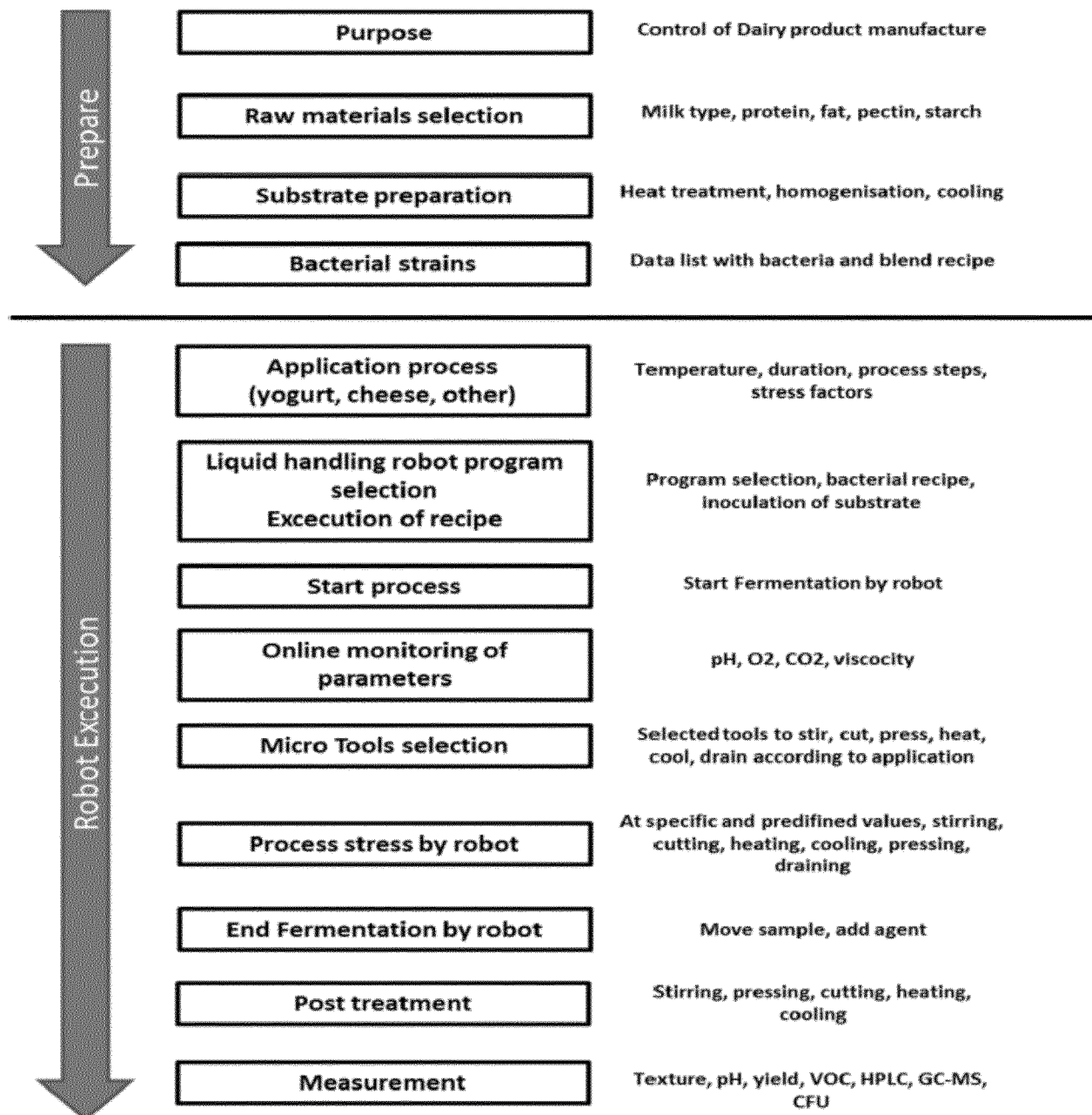


FIGURE 2

Specific diagram for low fat stir yogurt

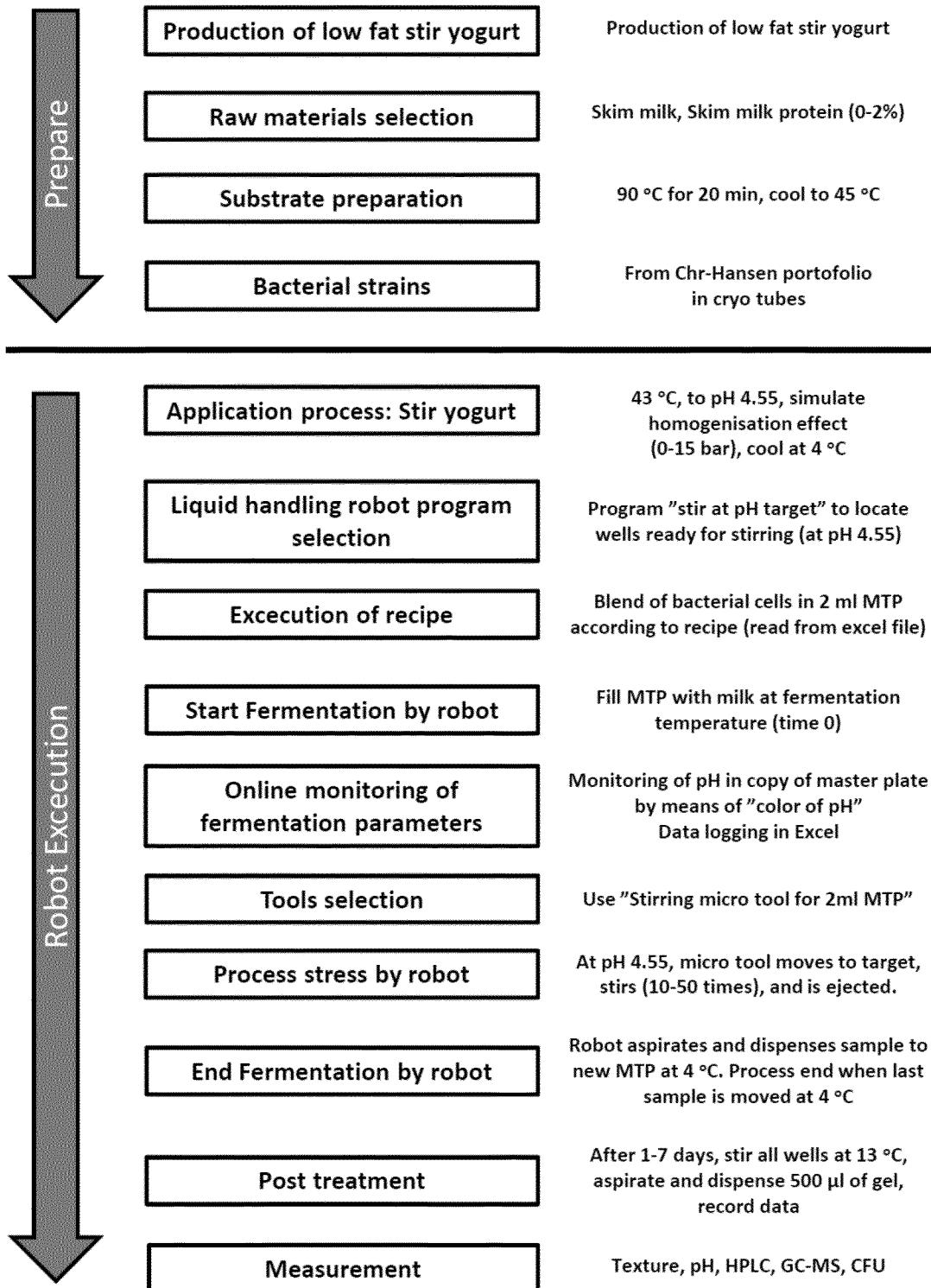


FIGURE 3

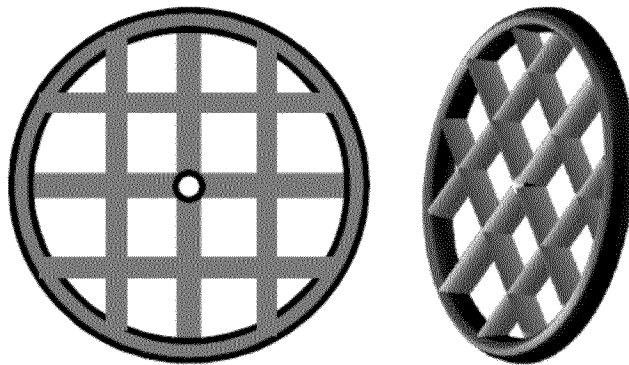


FIGURE 4

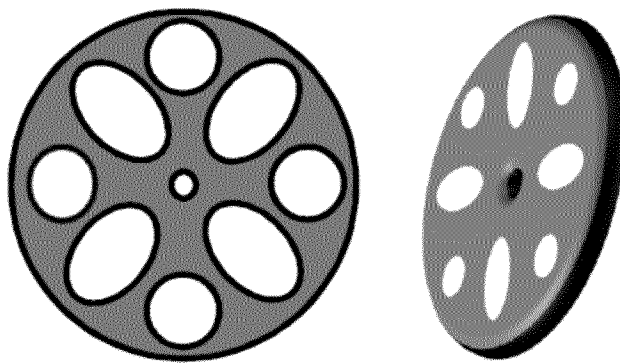


FIGURE 5

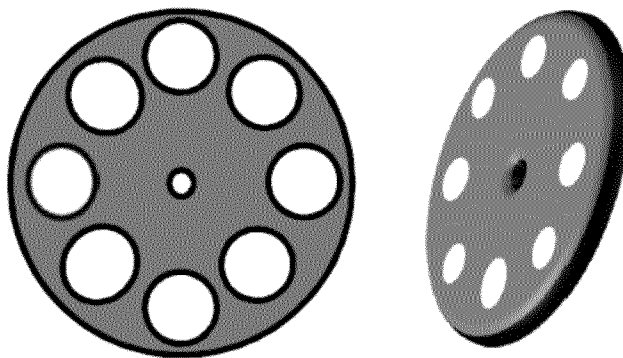


FIGURE 6

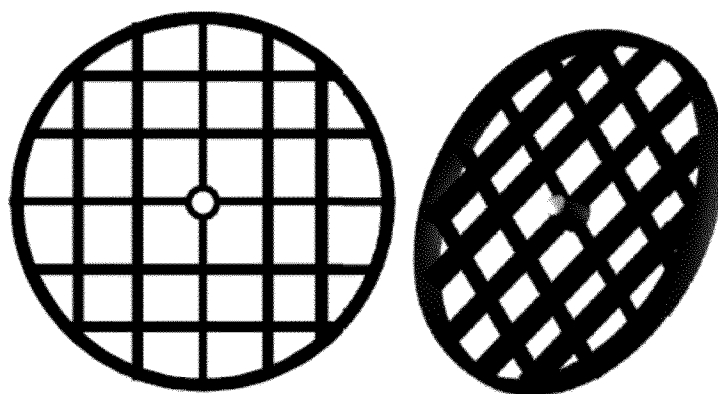


FIGURE 7

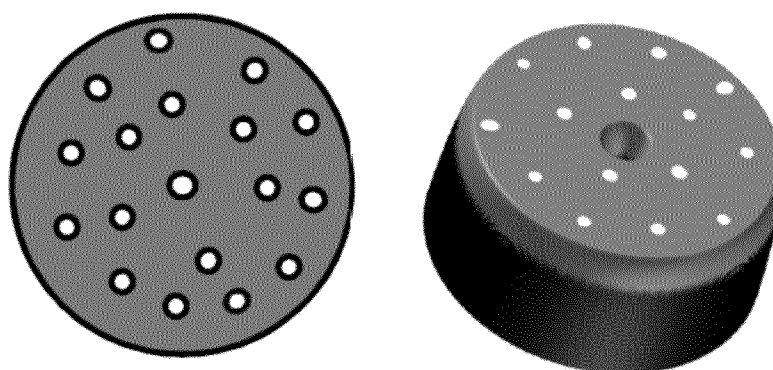


FIGURE 8

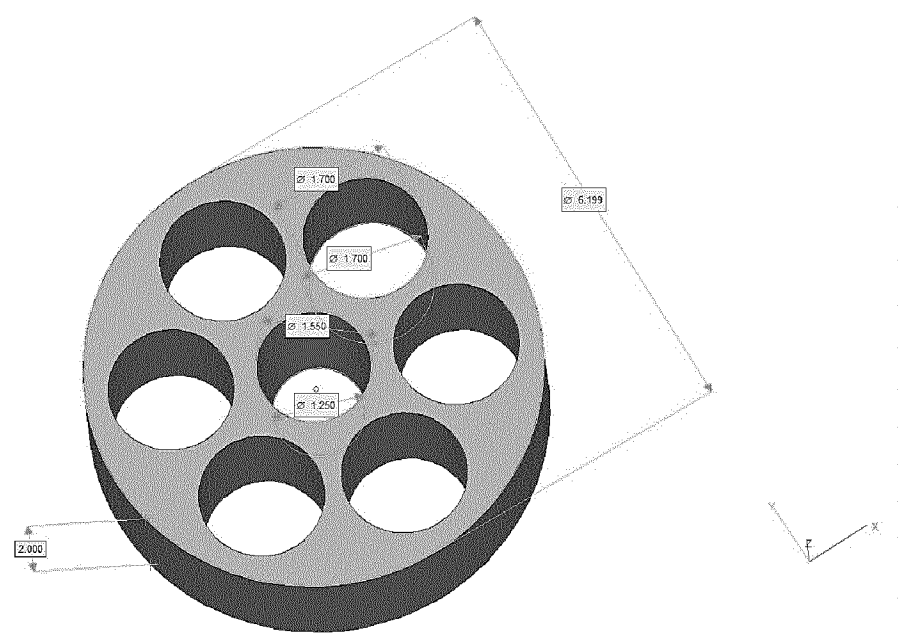


FIGURE 9

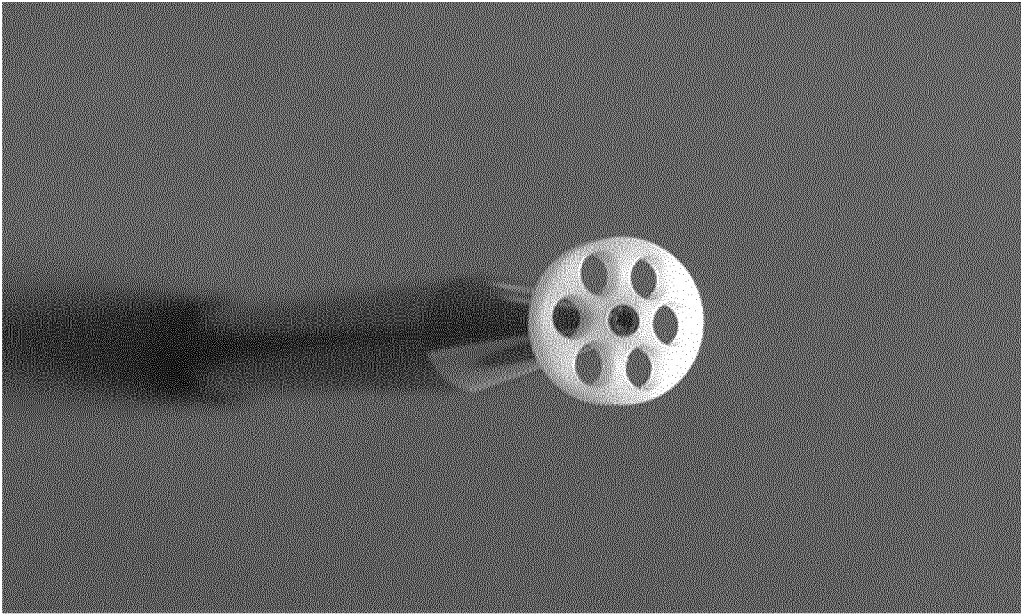


FIGURE 10

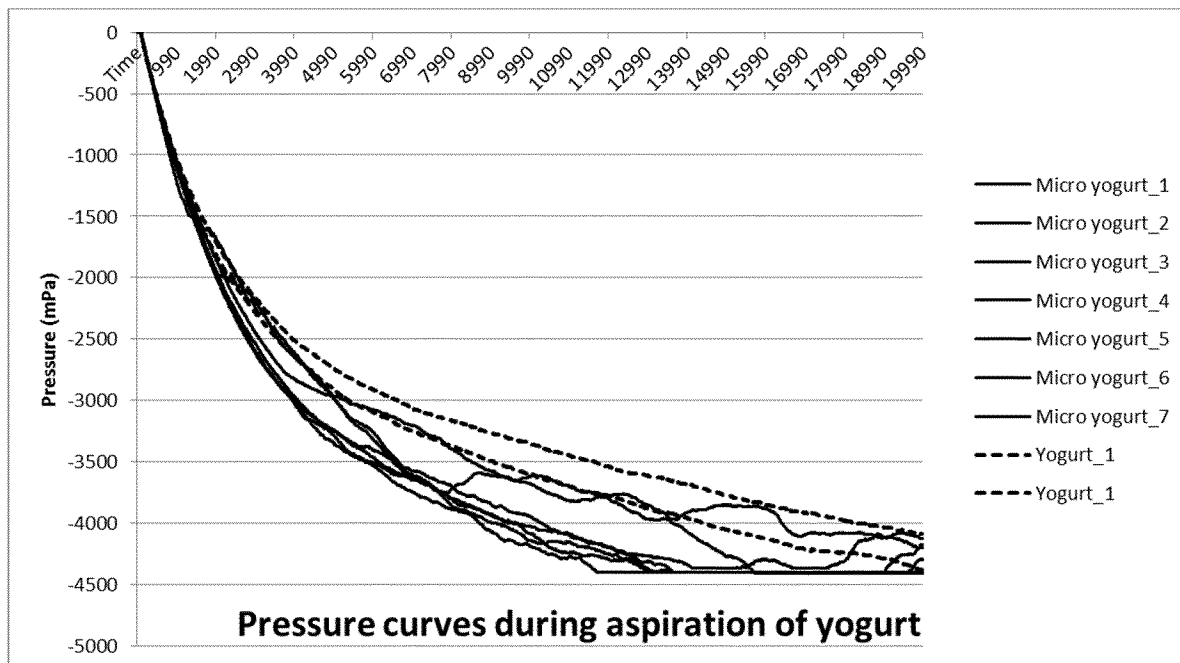
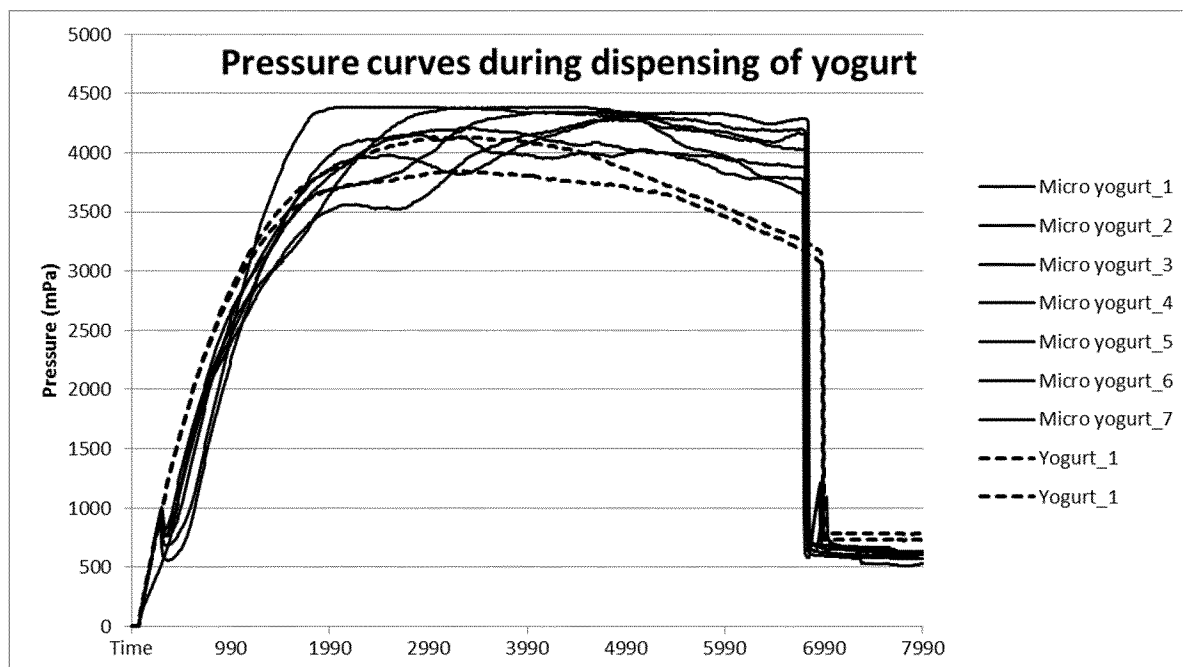


FIGURE 11



INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2014/058542

A. CLASSIFICATION OF SUBJECT MATTER
 INV. G01N33/04 A23C9/12
 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)

G01N B01J B81B 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, WPI Data, FSTA, COMPENDEX, INSPEC, IBM-TDB

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2009/215027 A1 (HOULBERG ULF [DK] ET AL) 27 August 2009 (2009-08-27) cited in the application	22-24
A	the whole document	1-21
A	US 5 172 193 A (PAYNE FREDERICK A [US] ET AL) 15 December 1992 (1992-12-15) abstract; figure 1	1-24
A	FR 2 629 612 A1 (INST NAT SANTE RECH MED [FR]) 6 October 1989 (1989-10-06) page 5, line 13 - page 11, line 7; figure 1	1-24
A	EP 0 106 681 A2 (COMW OF AUSTRALIA [AU]) 25 April 1984 (1984-04-25) page 3, line 14 - page 4, line 5; figure 1	1-24



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"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

21 July 2014

Date of mailing of the international search report

31/07/2014

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Authorized officer

Gilow, Christoph

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2014/058542

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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