

A METHOD FOR MINIMISING POST-ACIDIFICATION IN CULTURED PRODUCT

FIELD OF THE INVENTION

5 This invention relates to a method for minimising post-acidification in cultured product. More particularly, this invention relates to a method for minimising post-acidification in a cultured product by controlling the amount of protein and lactose present in the cultured product, controlling buffering capacity of the cultured product and maintaining pH of the cultured product within a predetermined range.

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BACKGROUND OF THE INVENTION

Cultured products such as yoghurts and fermented beverages are naturally acidified by lactic acid bacteria which convert lactose and other sugars to lactic acid. Acid
15 production usually continues even after the cultured products are manufactured as at least 1×10^6 cfu/g of live culture is required to be present in the cultured products after the cultured products are manufactured and during the storage of the cultured products. The continuation of acid production is known as post-acidification. This post-acidification is responsible for degradation of flavour and texture of the cultured
20 products during their storage. It also reduces the starter culture survival in the cultured products (Donker *et al.*, 2006, "Effect of acidification on the activity of probiotics in yoghurt during cold storage", International Dairy Journal 16, 1181-1189).

Post-acidification even occurs under refrigerated or chilled conditions and this reduces
25 the shelf-life of the cultured products. The shelf-life of cultured products is usually very short, about 21 days under refrigerated conditions.

Many efforts have been made and methods have been developed to extend the shelf-life of cultured products. However, the methods developed thus far often involve
30 modification of cultures, use of special strain of yoghurt cultures or probiotics or involve expensive processing treatment.

For example, US2010/0021586A1, US7563436B2, US2007/0292561A1, EP1893032B1 and Ongol *et al.*, 2007, "Yoghurt fermented by *Lactobacillus delbrueckii*
35 subsp. *bulgaricus* H⁺-ATPase-defective mutants exhibits enhanced viability of

Bifidobacterium breve during storage”, International Journal of Food Microbiology 116, 358-366 describe methods that require genetic manipulation of cultures. KR2009001111A describes a method that requires addition of bacteriocin, and Kailasapathy, K (2006), “Survival of free and encapsulated probiotic bacteria and their effect on the sensory properties of yoghurt”, LWT 39, 1221-1227 describes a method that requires LAB/probiotics to be encapsulated. The method disclosed in De Ancos *et al.* (2000), “Characteristics of stirred low-fat yoghurt as affected by high pressure”, International Dairy Journal 10, 105-111 requires high pressure treatment of lactic acid bacteria/probiotics.

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Most prior art discussed extending the shelf-life of cultured products by minimising post-acidification of cultured products under refrigerated or chilled conditions. For example, EP1035777B1 describes a method for producing fermented soy milk with cereal hydrolysate and/or almond milk. The method includes controlling post-acidification of the fermented soy milk during storage, under chilled condition of 10°C for 28 days. US2010/0009034A1 however describes a process for preparing a fermented milk beverage and extending the shelf-life of the beverage by keeping high viable cell count at ambient temperature. The milk beverage produced by the process can be stored at ambient temperature for 1 to 6 months. This process however involves the use of a special strain of culture to prepare the beverage.

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Alvarez, F., *et al.* (1998), “Fermentation of concentrated skim-milk. Effects of different protein/lactose ratios obtained by ultrafiltration-diafiltration”, Journal of the Science of Food and Agriculture 76, 10-16 describes a method that uses skim milk with a specific protein/lactose ratio of 3.5 for producing fermented product using ultrafiltration and diafiltration. This method was used to produce fermented product with high protein concentration and low lactose that have good texture and flavour. However, no storage trial of the fermented product was conducted and there was no mention of controlling the post-acidification of the fermented product. The method is also not suitable for industrial application as there is a limit on the amount of lactose that can be reduced through ultrafiltration and diafiltration.

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US2008/0063752A1, WO 2009/065724A1 and WO 2009/065725A1 disclose methods using low lactose or fermentable sugars protein ingredients to produce fermented

products. However, there is no discussion on controlling post-acidification of the fermented products after they are produced.

Therefore, there remains a need to provide a method that seeks to address at least one
5 of the above problems, or at least to provide an alternative.

SUMMARY OF THE INVENTION

The above and other problems are solved and an advance in the art is made by a
10 method for minimising post-acidification in cultured products in accordance with this invention. A first advantage of the method in accordance with this invention is that the method allows end pH of the cultured products to be determined and this allows a more accurate prediction of post-acidification of the cultured products. A second advantage
15 of the method in accordance with this invention is that the method provides a solution for extending the shelf-life of chilled and non-chilled cultured products and improving the quality of the cultured products, without having to change any production process and/or extending fermentation time of the cultured products during production process. A third advantage of the method in accordance with this invention is that the method is
20 simple and inexpensive to implement as it can be used without having to genetically manipulate any cultures or involve any costly processing treatments or pre-treatments of any ingredients used in producing the cultured products.

In accordance with an embodiment of this invention, a method for minimising post-acidification in a cultured product is provided. The method comprises determining a
25 first value that defines a ratio of protein to lactose in a cultured product, determining a second value that defines buffering capacity of the cultured product, determining a third value that defines pH of the cultured product using the first value and the second value obtained, controlling the amount of protein to lactose in the cultured product within a predetermined ratio, controlling the buffering capacity of the cultured product within a
30 predetermined value, and maintaining the pH of the cultured product within a predetermined pH.

In accordance with an embodiment of this invention, the predetermined ratio of protein to lactose is in the range of 2.5:1 to 6:1 when the cultured product does not contain any
35 probiotics. In accordance with another embodiment of this invention, the predetermined

ratio of protein to lactose is in the range of 8:1 to 9:1 when the cultured product contains probiotics.

5 In accordance with an embodiment of this invention, the predetermined pH of the cultured product is in the range of 4.0 to 4.5. The pH of the cultured product is maintained in the range of 4.0 to 4.5 by buffering action of the protein and buffer salts in the cultured product.

10 In accordance with an embodiment of this invention, the predetermined value of the buffering capacity of the cultured product is in the range of 0.0042 to 0.0117. The buffering capacity of the cultured product is controlled by adding buffer salts of known buffering capacity to the cultured product.

15 In accordance with some embodiments of this invention, the cultured product has a shelf-life of at least about 28 days under non-refrigerated or refrigerated condition.

20 In accordance with another embodiment of this invention, a cultured product without probiotics comprising protein and lactose in a ratio of 2.5:1 to 6:1 and having a pH in the range of 4.0 to 4.5 obtainable by the method in accordance with this invention is provided.

25 In accordance with yet another embodiment of this invention, a cultured product with probiotics comprising protein and lactose in a ratio of 8:1 to 9:1 and having a pH in the range of 4.0 to 4.5 obtainable by the method in accordance with this invention is provided.

BRIEF DESCRIPTION OF THE DRAWINGS

30 The above and other features and advantages of the present invention will more clearly understood from the following detailed description taken in conjunction with the accompanying drawings:

35 Figure 1 is a graph showing the pH profile of yoghurts (3.5% protein) made from various protein/lactose ratios and stored at 30°C for 10 days. Varying levels of lactose were added to 3.5% protein blend from pea and milk protein ingredients. The yoghurt

culture used was *Streptococcus (S.) thermophilus* and *Lactobacillus(L.) delbrueckii ssp bulgaricus* (0.024%).

Figure 2 is a graph showing the effect of buffering salt, sodium citrate, on the pH profile of yoghurts made from milk protein concentrate (MPC, 3.5% protein). The yoghurts were inoculated with 0.04% of yoghurt culture and fermented at 43°C until the pH values reached about 4.3. The yoghurts were then stored at 30°C for 9 days. Control yoghurt was prepared from MPC only and other yoghurts had varying levels of sodium citrate added to MPC.

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Figure 3 is a graph showing the pH profile of yoghurt made from yoghurt cultures supplied by three commercial companies. The yoghurts were prepared from pea and milk protein ingredients containing 3.5% protein and 0.6% lactose stored at 30°C for 10 days.

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Figure 4 is a graph showing the pH profile of fermented milks prepared from pea protein and pea protein/milk at 0.4% and 0.6% lactose. The protein solutions (3.5% protein) were inoculated with 0.04% of *Lactobacillus(L.) rhamnosus* overnight at 37°C and stored at 30°C for 10 days. The protein/lactose ratios for 0.4% and 0.6% lactose formulations were 8.75 and 5.83 respectively.

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Figure 5 is a graph showing the pH profile of yoghurt prepared from pea protein and pea protein/milk at 0.4% and 0.6% lactose. The protein solutions (3.5% protein) were inoculated with 0.02% of yoghurt culture and 0.02% of *L.rhamnosus* overnight at 37°C and stored at 30°C for 10 days. The protein/lactose ratios for 0.4% and 0.6% lactose formulations were 8.75 and 5.83 respectively.

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Figure 6 is a graph showing the pH profile of yoghurts (3.5% protein) prepared from different protein ingredients: skim milk powder (control) (5.41% lactose) and other protein ingredients that contain 0.4% lactose. They were inoculated with yoghurt cultures and probiotics (*S. thermophilus*, *L. delbrueckii ssp bulgaricus*, *Lactobacillus(L.) acidophilus*, *Bifidobacterium (B.) lactis* and *Lactobacillus(L.) casei*) and stored at 30°C.

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Figure 7 is a graph showing the pH profile of yoghurts prepared from different protein ingredients containing 0.4% lactose stored at 30°C for 4 weeks. Yoghurt cultures and

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probiotics (*S. thermophilus*, *L. delbrueckii ssp bulgaricus*, *L.rhamnosus*, and *L. casei*) were used for the fermentation.

5 Figure 8 is a graph showing the survival of lactic acid bacteria and probiotics in yoghurt prepared from different protein ingredients containing 0.4% lactose stored at 30°C for 4 weeks. Yoghurt cultures and probiotics were used for the fermentation (*S. thermophilus*, *L. delbrueckii ssp bulgaricus*, *L.rhamnosus* and *L. casei*).

10 Figure 9 is a graph showing the predicted pH value versus the actual measured final pH value of both pea protein and soy protein cultured products containing a range of protein/lactose ratios. They were stored at 30°C for 10 days. Yoghurt cultures and probiotics were used for the fermentation (*S. thermophilus*, *L. delbrueckii ssp bulgaricus*, and *L. acidophilus*).

15 DETAILED DESCRIPTION OF THE INVENTION

Definitions

20 The following words and terms have the meanings given below, unless indicated otherwise. These are intended as general definitions and should in no way limit the scope of the present invention to those terms alone, but are put forth for better understanding of the following description.

25 The term “cultured product” as used herein refers to all products obtained by culture, that is, not only the liquid culture itself but also the cells contained therein. For example, cultured product includes, but is not limited to, yoghurt and fermented products containing live lactic acid bacteria and/or probiotics.

30 The term “buffering capacity” as used herein refers to the ability of protein, or protein and buffer salts in cultured product and/or cultured product formulation to resist pH change. Typically, buffering capacity is expressed in terms of the amount of strong acid or base required to change the pH of a composition in a given amount.

35 The present invention relates to a method for minimising post-acidification in a cultured product. In an embodiment of the invention, the method comprises the steps of

determining a first value that defines a ratio of protein to lactose in a cultured product and determining a second value that defines buffering capacity of the protein ingredient in the cultured product. This is followed by determining a third value that defines pH of the cultured product using the first value and the second value obtained thereto. The method also comprises controlling the amounts of protein and lactose present in the cultured product within the predetermined ratio, controlling the buffering capacity of the cultured product within the predetermined value, and maintaining the pH of the cultured product within the predetermined pH.

- 10 In the method of the invention, the first value that defines the ratio of protein to lactose in the cultured product is determined by dividing the percentage of protein content by the percentage of lactose content in the formulation.

15 In one embodiment of the invention, the protein to lactose ratio is in the range of 2.5:1 to 6:1, depending on the type of cultured product used in the method. In a case where the cultured product contains probiotics, the protein to lactose ratio is preferably in the range of 8:1 to 9:1, more preferably 8.75.

20 The amounts of protein and lactose present in the cultured product is controlled to be within a predetermined range by increasing or reducing the amount of protein present in the cultured product, or increasing or reducing the amount of lactose present in the cultured product. For example, if the protein/lactose ratio falls below 2.5:1, more protein can be added to the cultured product to bring the ratio to be within 2.5:1 to 6:1. If the protein/lactose ratio is greater than 6:1, more lactose can be added to the culture product to bring the ratio to be within 2.5:1 to 6:1.

25 A method has been designed to measure the buffering capacity of the protein ingredients in the cultured product and/or the cultured product formulation. In the method of the invention, the second value that defines the buffering capacity of the cultured product is determined according to the formula provided by Françoise Salaün *et al.* (2005), "Buffering capacity of dairy products", International Dairy Journal 15, 95-109:

$$\frac{dB}{dpH} = \frac{(\text{weight of 0.2M HCl or 2M lactic acid added}) \times \text{Molarity of the acid}}{(\text{weight of sample}) \times (\text{pH change produced})}$$

In one embodiment of the invention, the buffering capacity of the protein ingredients in the cultured product and/or the cultured product formulation is preferably in the range of 0.0042 to 0.0117. The buffering effect of the cultured product can affect not only the pH, but also the taste of the cultured product. Two cultured products, for example, two yoghurt products may have a similar pH value but the yoghurt that has a higher buffering capacity may be sourer than the yoghurt that has a lower buffering capacity.

The buffering capacity of the cultured product formulation is controlled to be within a predetermined range by using ingredients of known buffering capacity and measuring the buffering capacity of the formulation to confirm the value.

The pH of the cultured product is determined by using the protein/lactose ratio and the buffering capacity of the protein ingredients and/or cultured product formulation obtained thereto to calculate a value that defines a preferred pH of the cultured product. In one embodiment of the invention, the pH of the cultured product is preferably in the range of 4.0 to 4.5.

The pH of the cultured product is strongly influenced by the buffering action of the protein, or the buffering action of the protein and buffer salts present in the cultured product and/or the cultured product formulation. In one embodiment of the invention, the pH is maintained within a predetermined range by controlling the lactose content and the buffering action of the protein and other ingredients in the cultured product formulation. In this embodiment, the cultured product is buffered by the protein ingredients itself, that do not require additional buffer salts to maintain the pH within the predetermined range. The protein ingredients are substantially the only buffering agent.

In another embodiment, to maintain pH, one or more buffer salts may be added. A variety of buffer salts are available for such use. For example, the buffer salts that may be suitable for such use in the method of the invention include, but are not limited to, citrate, phosphate, lactate, carbonate and acetate, etc. The buffer salts used in the method must provide sufficient buffering capacity to maintain the pH within the predetermined range for as long as necessary. Maintaining the correct pH of the cultured product is important as the pH will affect the stability and shelf-life of the cultured product.

The method in accordance with the invention is able to produce a cultured product that has a shelf-life of at least about 28 days, stored under non-refrigerated condition (about 11 to 30°C) or refrigerated condition (about 0 to 10°C), with minimal change in pH and good culture/probiotics survival, with cell count of about 1×10^6 cfu/ml or more.

The implementation of the method in accordance with the invention makes it possible to minimise the post-acidification in the cultured products during their longer storage time even at non-refrigerated conditions. The degree of post-acidification can vary depending on the composition of the culture and the amount of protein/lactose used. However, the post-acidification is always clearly lower than that observed in the case of cultured products, for example, yoghurts obtained with conventional methods. Weak post-acidification is accompanied by good culture/probiotics survival.

The method of the invention can be used in conjunction with any conventional methods used for producing cultured products. The method is suitable for the preparation of all types of cultured products, including those containing protein and lactose. Examples of the cultured products include, but are not limited to, dairy products, non-dairy products or blends. Examples of dairy products include, but are not limited to, yoghurt, no-fat or skim milk, low-fat milk, full-fat or whole milk, milk solids, cream, and the like. Non-dairy products are generally products not made by milk. Examples of non-dairy product include non-dairy yoghurt, but are not limited to, soy, pea, wheat, rice, and the like. Examples of blends include, but are not limited to, soy/milk, pea/milk, soy/pea, and the like.

The method of the present invention uses a combination of the lactose content and the buffering capacity of the protein and/or cultured product formulation to predict the end pH of the cultured product according to the formula below.

$$\text{pH} = 118.58 \cdot \text{BI} + a \cdot L^{-2.0382} + 3.171$$

wherein

BI= buffering capacity at pH 4.2 of protein ingredients and product formulation

$a = 3.85 \cdot \text{EXP}(-7.156)$

L = lactose content in the product formulation

The method allows final pH of the cultured product (with probiotics) to be predicted more accurately if the lactose content is in the range of 0.05% to 0.6%, the buffering capacity is in the range of 0.0042 to 0.0117 at pH 4.2, and the protein content is in the range of 1% to 3%. This is advantageous to manufacturers of cultured products as it allows the manufacturers to target a specific pH with minimal post-acidification, to obtain a cultured product containing live culture of about 10^6 cfu/g during storage temperature of 30°C or less, without having to extend the production fermentation time. The method of the invention provides a solution for extending the shelf-life of chilled and non-chilled cultured products as well as improving the quality of the cultured products. The method also offers manufacturers of cultured products a much wider range of selection of protein ingredients (dairy or non-dairy), protein concentrations, starter cultures/probiotics and temperature of storage and distribution of the cultured products than other conventional methods used in the production of cultured products. In addition, the cultured products produced using the method of the invention have very low levels of lactose and simple sugars. This method is therefore suitable for manufacturing products for people suffering from lactose intolerance, obesity and diabetes.

The method of the invention is simple and inexpensive to implement as it can be used without having to genetically manipulate any cultures or involve any expensive processing treatment. The method also does not require any pre-treatment of the ingredients used in producing the cultured products and the method can be employed using existing, commercially available ingredients. The method further allows the pH of the cultured products to be maintained within the preferred range after the products are produced and during storage of the products, without having to change any production process and/or extending the fermentation time during the production process. This in turns reduces manufacturing and distribution costs.

In accordance with another embodiment of the invention, a cultured product obtainable by the method of the present invention is provided. The cultured product in accordance with the invention has a pH in the range of about 4.0 to 4.5. It has been found that the cultured product of the invention contains less acidity than conventional cultured product. Generally, the cultured product has a shelf life of about at least 28 days when packaged in a sealed container and stored under non-refrigerated condition (about 11

to 30°C) or refrigerated condition (about 0 to 10°C). Moreover, the cultured product of the invention has a protein to lactose ratio in the range of 2.5:1 to 6:1. In a case where the cultured product contains probiotics, the protein to lactose ratio is in the range of 8:1 to 9:1, preferably 8.75.

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The following examples are provided to further illustrate and describe particular specific embodiments of the present invention, and are in no way to be construed to limit the invention to the specific procedures, conditions or compositions described therein.

10 **EXAMPLES**

Many commercial yoghurt cultures and probiotics from different supplier were tested and all were shown to be suitable, even for the strong acidifying cultures.

15 Example 1

Figure 1 is a graph showing the pH profile of yoghurts (3.5% protein) made from the following protein/lactose ratios (Table 1) and stored at 30°C for 10 days. Varying levels of lactose were added to 3.5% protein blend from pea and milk protein ingredients.

20 The yoghurt culture used was *S. thermophilus* and *L. delbrueckii ssp bulgaricus* (0.024%). Yoghurt culture survival was shown to have at least 1×10^6 cfu/g at 10 days of storage.

Ratios of protein/lactose were obtained from the compositional values specified by the certificate of analysis of each ingredients present in the cultured product formulation.

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The pH values of the cultured product were obtained by using a pH meter. Culture survival was monitored using MRS agar.

Table 1

Ratio of protein/lactose	pH of yoghurts
4.38 : 1	4.26 to 4.48
5.83 : 1	4.32 to 4.38
7.00 : 1	4.51 to 4.70
8.75 :1	4.93 to 4.98

11.67 :1	4.80 to 5.22
17.50 : 1	5.22 to 5.61

The results show that when the ratio of protein/lactose is in the range of 2.5:1 to 6:1, the pH of the yoghurts is in the range of 4.0 to 4.5. When the ratio of protein/lactose is not in the range of 2.5:1 to 6:1, the pH of the yoghurts is in the range of 4.51 to 5.70.

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Example 2

Figure 2 is a graph showing the effect of buffering salt, sodium citrate, on the pH profile of yoghurts made from milk protein concentrate (MPC, 3.5% protein). The yoghurts were inoculated with 0.04% of yoghurt culture and fermented at 43°C until the pH values reached about 4.3. The yoghurts were then stored at 30°C for 9 days. Control yoghurt was prepared from MPC only and other yoghurts had varying levels of sodium citrate added to MPC. Yoghurt culture survival was shown to have at least 1×10^3 cfu/g at 9 days of storage on MRS agar.

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Citrate contents were obtained from the compositional values specified by the certificate of analysis of each ingredients present in the cultured product formulation. The pH values were obtained by using a pH meter.

20 Table 2

Sodium citrate addition (%)	pH of yoghurts	pH difference
0.00 (Control)	3.76 to 4.39	0.64
0.05	3.82 to 4.31	0.49
0.10	3.83 to 4.39	0.56
0.20	3.89 to 4.38	0.49

The results show that adding a buffer salt such as sodium citrate, the difference in the pH drops after fermentation is less and the end pH of the yoghurt is higher than that of the control. This shows the buffering effect of adding buffer salt, sodium citrate.

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Example 3

Figure 3 is a graph showing the pH profile of yoghurt made from yoghurt cultures supplied by three commercial companies. The yoghurts were prepared from pea and milk protein ingredients containing 3.5% protein and 0.6% lactose stored at 30°C for 10 days. Yoghurt culture survival was shown to have at least 1×10^3 cfu/g at 10 days of storage on MRS agar.

Table 3

Yoghurt culture	pH of yoghurt
YC-350	4.32 to 4.43
Yo-Mix 300	4.31 to 4.36
CY 222	4.36 to 4.42

The results in Figure 3 show that the pH of the yoghurt remains within the range of 4.0 to 4.5 when different commercial yoghurt cultures are tested and when the amount of protein and lactose used in the yoghurt is in the range of 2.5:1 to 6:1

Example 4

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Figure 4 is a graph showing the pH profile of fermented milks prepared from pea protein and pea protein/milk at 0.4% and 0.6% lactose. The protein solutions (3.5% protein) were inoculated with 0.04% of *L.rhamnosus* overnight at 37°C and stored at 30°C for 10 days. The protein/lactose ratios for 0.4% and 0.6% lactose formulations were 8.75 and 5.83 respectively. Culture survival was shown to have at least 1×10^3 cfu/g on MRS agar.

Ratios of protein to lactose contents were obtained from the compositional values specified by the certificate of analysis of each ingredients present in the cultured product formulation. The pH values were obtained by using a pH meter.

Table 4

Formulation	Protein/lactose ratio	End pH of the yoghurt
Pea protein 0.4% lactose	8.75	4.18 to 4.12
Pea protein 0.6% lactose	5.83	3.86 to 3.89

Pea and milk protein 0.4% lactose	8.75	4.21 to 4.25
Pea and milk protein 0.6% lactose	5.83	3.89 to 3.93

The results in Figure 4 show that when different types of protein are used, the pH of the fermented milks can still be maintained within the range of 4.0 to 4.5 when the amount of protein and lactose used in the fermented milks is in the ratios of 8:1 and 9:1 (when the fermented milk contains probiotics).

Example 5

Figure 5 is a graph showing the pH profile of yoghurt prepared from pea protein and pea protein/milk at 0.4% and 0.6% lactose. The protein solutions (3.5% protein) were inoculated with 0.02% of yoghurt culture and 0.02% of *L.rhamnosus* overnight at 37°C and stored at 30°C for 10 days. The protein/lactose ratios for 0.4% and 0.6% lactose formulations were 8.75 and 5.83 respectively. Culture survival was shown to have at least 1×10^3 cfu/g on MRS agar.

Table 5

Formulation	Protein/lactose ratio	End pH of the yoghurt
Pea protein 0.4% lactose	8.75	4.10 to 4.15
Pea protein 0.6% lactose	5.83	3.84 to 4.01
Pea and milk protein 0.4% lactose	8.75	4.23 to 4.29
Pea and milk protein 0.6% lactose	5.83	3.90 to 3.93

The results in Figure 5 show that when using a combination of yoghurt culture and probiotic, the pH of the yoghurts can still be maintained within the range of 4.0 to 4.5 when the amount of protein and lactose used in the fermented milks is in the ratios of 8:1 and 9:1.

Example 6

Figure 6 is a graph showing the pH profile of yoghurts (3.5% protein) prepared from different protein ingredients: skim milk powder (control) (5.41% lactose) and other protein ingredients that contain 0.4% lactose. They were inoculated with yoghurt cultures and probiotics (*S. thermophilus*, *L. delbrueckii ssp bulgaricus*, *L. acidophilus*, *B. lactis* and *L. casei*) and stored at 30°C. Except for the control protein/lactose ratio of 0.65, all other formulations had their protein/lactose ratios of 8.75. Culture survival was shown to have at least 1×10^3 cfu/g on MRS agar.

Table 6

Formulation	Protein/lactose ratio	pH of the yoghurt
Control	0.65	3.28 to 3.51
Pea protein	8.75	4.20 to 4.25
Pea and milk protein	8.75	4.20 to 4.31
Soy	8.75	4.17 to 4.20
Soy and milk protein	8.75	4.17 to 4.20
Milk protein	8.75	4.40 to 4.44

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The results in Figure 6 show that when different types of cultures and different protein ingredients are used, the pH of the yoghurts can still be maintained within the range of 4.0 to 4.5 when the amount of protein and lactose used in the fermented milks is in the ratios of 8:1 and 9:1 (when the yoghurt contains different types of probiotics). In contrast, much lower yoghurt pH is observed for normal yoghurt ingredient and skim milk powder which has a much lower protein/lactose ratio.

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Example 7

Figure 7 is a graph showing the pH profile of yoghurts prepared from different protein ingredients containing 3.5% protein and 0.4% lactose stored at 30°C for 4 weeks. Yoghurt cultures and probiotics (*S. thermophilus*, *L. delbrueckii ssp bulgaricus*, *L. rhamnosus*, and *L. casei*) were used for the fermentation. Culture survival was shown to have at least 1×10^3 cfu/g on MRS agar.

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Table 7

Formulation	pH of the yoghurt
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Pea protein	4.21 to 4.37
Pea and milk protein	4.26 to 4.35
Soy protein	4.15 to 4.30
Soy and milk protein	4.30 to 4.31

The results in Figure 7 show that the shelf-life of cultured products prepared from different protein ingredients containing cultures and probiotics can be extended to about 4 weeks (or 28 days) under non-refrigerated condition at 30°C, with minimal change in pH, maintained within the range of 4.0 to 4.5.

Example 8

Figure 8 is a graph showing the survival of lactic acid bacteria and probiotics in yoghurt prepared from different protein ingredients containing 3.5% protein and 0.4% lactose stored at 30°C for 4 weeks. Yoghurt cultures and probiotics were used for the fermentation (*S. thermophilus*, *L. delbrueckii ssp bulgaricus*, *L.rhamnosus*, and *L. casei*). Culture survival was monitored weekly using MRS agar plating.

Table 8

Formulation	Lactic acid bacteria and probiotic cell counts at 4 weeks of storage (30°C), cfu/ml of yoghurt
Pea protein	9.25E+05
Pea and milk protein	1.76E+06
Soy protein	2.27E+06
Soy and milk protein	1.07E+06

The results in Figure 8 show that the shelf-life of cultured products prepared from different protein ingredients containing cultures and probiotics can be extended to about 4 weeks (or 28 days) under non-refrigerated condition at 30°C, with good survival of lactic acid bacteria and probiotics, with cell counts of about 1×10^6 cfu/ml.

Example 9

Figure 9 is a graph showing the predicted pH values versus the actual measured pH values of two types of yoghurts, pea yoghurts and soy yoghurts of a range of protein content (1 to 3%) and lactose content (0.05 to 0.6%) and buffering capacities at pH 4.2 (0.0042 to 0.0117). They were inoculated with yoghurt cultures and probiotics (*S. thermophilus*, *L. delbrueckii ssp bulgaricus*, and *L. acidophilus*) and stored at 30°C. Culture survival was shown to have at least 1×10^3 cfu/g on MRS agar.

Table 9

Protein content (%)	Lactose content (%)	Buffering capacity at pH 4.2	Protein/lactose ratio	Predicted pH	Actual pH
1.00	0.60	0.0035	1.67	3.59	3.66
1.00	0.40	0.0035	2.50	3.60	3.85
1.00	0.20	0.0035	5.00	3.66	3.73
1.50	0.20	0.0052	7.50	3.87	3.90
1.65	0.20	0.0058	8.25	3.93	4.00
1.00	0.10	0.0035	10.00	3.91	4.23
2.01	0.20	0.0070	10.05	4.08	4.20
2.50	0.20	0.0087	12.50	4.29	4.40
3.00	0.20	0.0105	15.00	4.49	4.53
1.00	0.05	0.0035	20.00	4.93	4.82

The results in Figure 9 show that actual pH of the yoghurt can be accurately predicted according to the following formula if protein is 1 to 3% and lactose is 0.05 to 0.6% and buffering capacity is 0.0042 to 0.0117 at pH 4.2.

$$\text{pH} = 118.58 \cdot \text{BI} + a \cdot L^{-2.0382} + 3.171$$

15

where:

BI = buffering capacity at pH 4.2 of protein ingredients and product formulation

$a = 3.85 \cdot \text{EXP}(-7.156)$

L = lactose content in the product formulation

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The above is a description of the subject matter the inventor regards as the invention. It is foreseeable that those skilled in the art can and will design alternative methods and compounds that include this invention based on the above disclosure.

CLAIMS:

1. A method for minimising post-acidification in a cultured product, the method comprising:
 - determining a first value that defines a ratio of protein to lactose in a cultured product;
 - determining a second value that defines buffering capacity of the cultured product;
 - determining a third value that defines pH of the cultured product using the first value and the second value obtained;
 - controlling the amount of protein to lactose in the cultured product within a predetermined ratio;
 - controlling the buffering capacity of the cultured product within a predetermined value; and
 - maintaining the pH of the cultured product within a predetermined pH.
2. The method according to claim 1, wherein the predetermined ratio of protein to lactose is in the range of 2.5:1 to 6:1 when the cultured product does not contain any probiotics.
3. The method according to claim 1, wherein the predetermined ratio of protein to lactose is in the range of 8:1 to 9:1 when the cultured product contains probiotics.
4. The method according to claim 1, wherein the predetermined value of the buffering capacity of the cultured product is in the range of 0.0042 to 0.0117.
5. The method according to claim 1, wherein the predetermined pH of the cultured product is in the range of 4.0 to 4.5.
6. The method according to claim 1, wherein the pH of the cultured product is maintained within the predetermined pH by buffering action of protein ingredients and buffer salts in the cultured product.

7. The method according to claim 1, wherein the buffering capacity of the cultured product is controlled by adding buffer salts of known buffering capacity to the cultured product.
- 5 8. The method according to claim 1, wherein the cultured product has a shelf-life of at least about 28 days under non-refrigerated condition.
9. The method according to claim 1, wherein the cultured product has a shelf-life of at least about 28 days under refrigerated condition.
- 10 10. The method according to claim 1, wherein the cultured product is a product selected from the group consisting of yoghurt, fermented product, dairy product, non-dairy product and blends.
- 15 11. A cultured product without probiotics comprising protein and lactose in a ratio of 2.5:1 to 6:1 and having a pH in a range of 4.0 to 4.5 obtainable by the method according to claim 1.
12. A cultured product with probiotics comprising protein and lactose in a ratio of 8:1
20 to 9:1 and having a pH in a range of 4.0 to 4.5 obtainable by the method according to claim 1.
13. The cultured product according to claim 11 or 12, wherein the cultured product is a product selected from the group consisting of yoghurt, fermented product, dairy
25 product, non-dairy product and blends.