APPENDICES

APPENDIX A

Culture Media and Chemicals

1. MRS Broth

Ingredients	g/L
Pepton	10.0
Meat extract	10.0
Yeast Extract	5.0
D(-) Glucose	20.0
Tween 80	1.0 ml
K_2HPO_4	2.0
Sodium acetate	5.0
Triammonium citrate	2.0
$MgSO_4.7H_2O$	0.2
$MnSO_4.4H_2O$	0.05
Deionized water	1000 ml

All ingredients were dissolved in deionized water and pH was adjusted to 6.2±0.4. Medium was dispensed into Duran bottles and sterilized by autoclaving at 121°C for 15 min.

2. MRS Agar

Ingredients	g/l
Pepton	10.0
Meat extract	10.0
Yeast Extract	5.0
D(-) Glucose	20.0
Tween 80	1.0 ml
K_2HPO_4	2.0
Sodium acetate	5.0
Triammonium citrate	2.0
$MgSO_4.7H_2O$	0.2
$MnSO_4.4H_2O$	0.05
Agar	15.0
Deionized water	1000ml

All ingredients were dissolved in deionized water and pH was adjusted to 6.2±0.4. Medium was sterilized by autoclaving at 121°C for 15 min.

3. Modified-MRS media for testing the sugar utilization

Ingredients	g/l
Pepton	10.0
Meat extract	10.0
Yeast Extract	5.0
Sugar	20.0
Tween 80	1.0 ml
K_2HPO_4	2.0
Sodium acetate	5.0
Triammonium citrate	2.0
$MgSO_4.7H_2O$	0.2
$MnSO_4.4H_2O$	0.05
Deionized water	1000 ml

** For sugar utilization, in this study used Lactose, Raffinose, and FOS compared with glucose as a control

All ingredients were dissolved in deionized water and pH was adjusted to 6.3. Medium was dispensed into test tubes and sterilized by autoclaving at 121°C for 15 min.

APPENDIX B

Questionnaires for Sensory Evaluation

9-Point Hedonic Scale

Name……Date…….Time……...

Product Name **Probiotic soya beverage**

Please rinse your mouth with water before starting observe and taste samples. Observe, tasting and write down the liking scores.

Liking scores

9 = Like extremely
8 = Like very much
7 = Like moderlately
6 = Like slightly
5 = Neither like nor dislike
4 = Dislike slightly
3 = Dislike moderately
2 = Dislike very much
1 = Dislike extemely

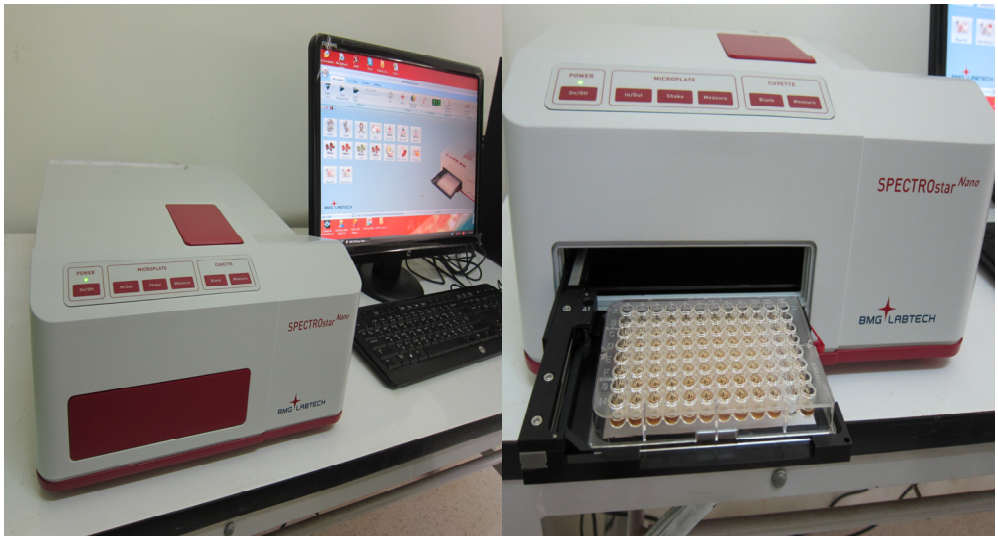
Sample code	Attributes					
	Appearance	color	odor	taste	Mouth-feel	Overall acceptance

Suggestion……
……
……

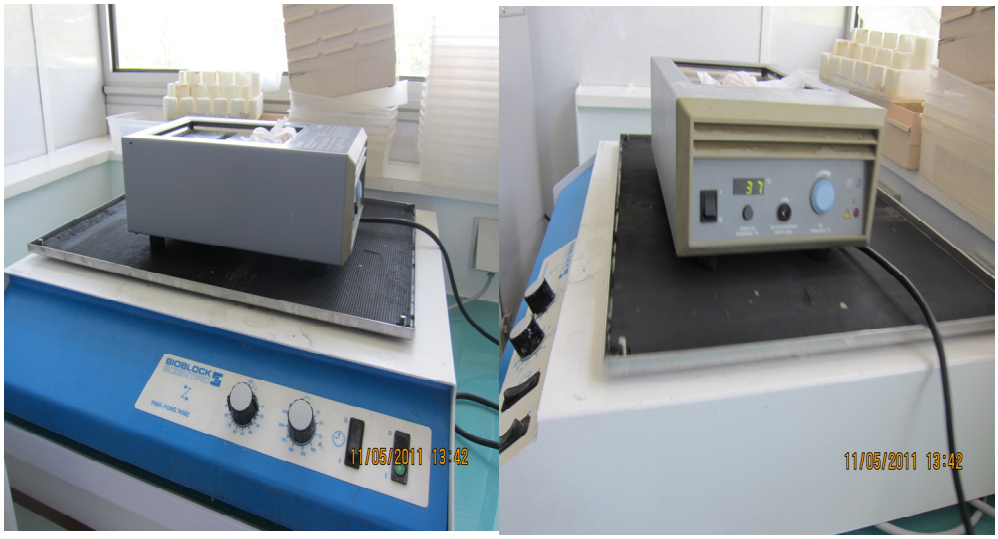
Thank you for your participation

APPENDIX C

Instrument and soya beverage products



UV-Vis spectrophotometer microplate reader (SPECTROstar Nano)



Thermostatically controlled shaker (Bioblock Scientific Ping-Pong 74582)

Densimat (bioMérieux, Marcy l'Etoile, France)

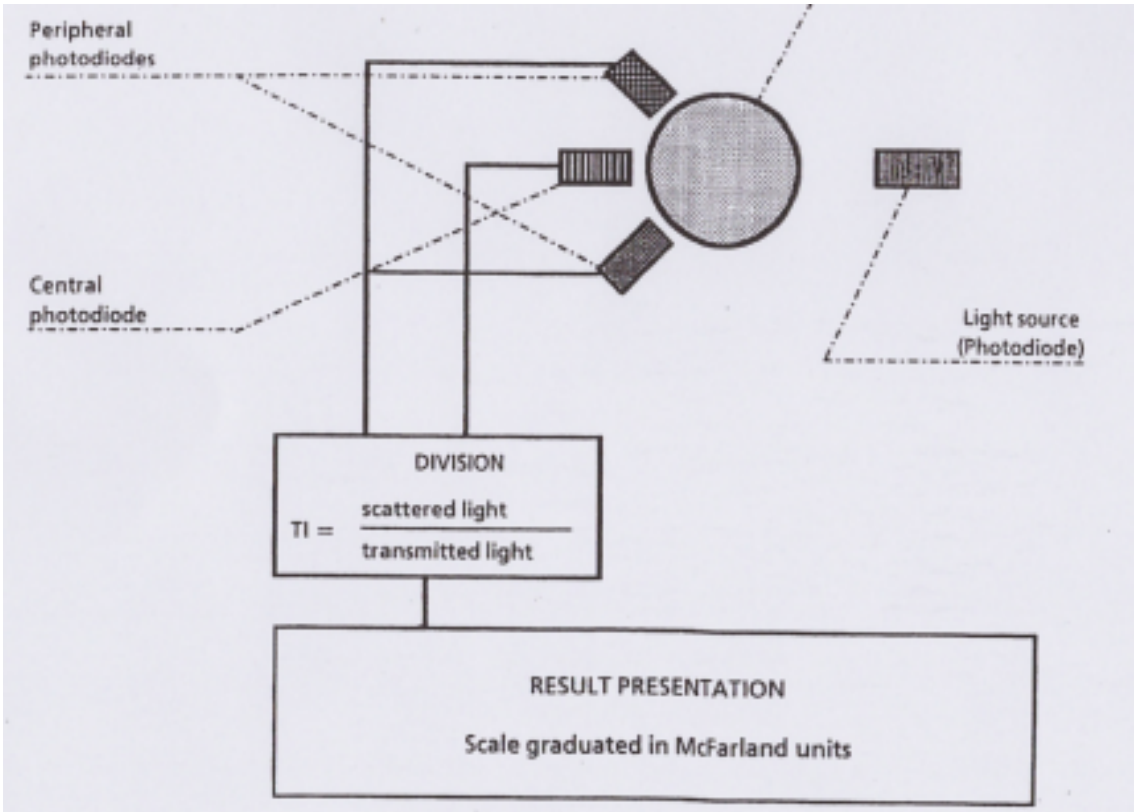


Fig. 1 - Reading Principle of ***DENSIMAT***

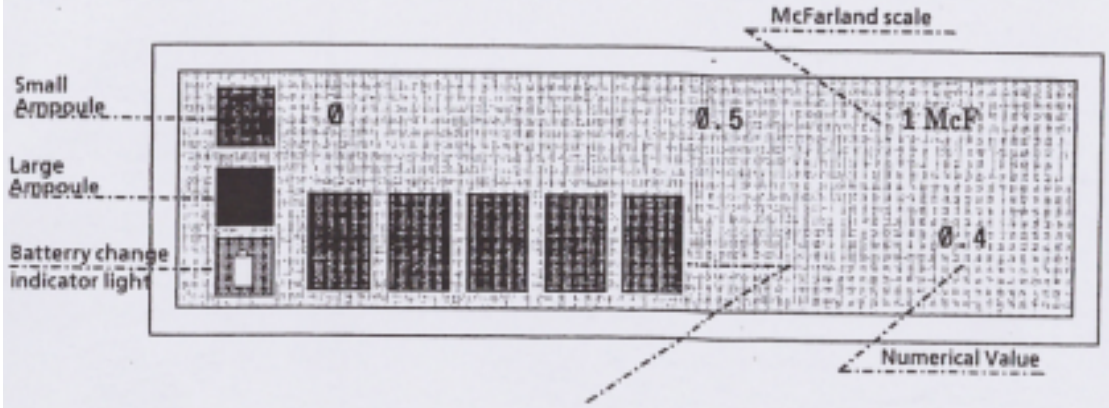


Fig. 2 - ***DENSIMAT*** Display Panel

DENSIMAT enables precise determination of bacteria density by two measurements.
An incident beam of light is passed through the ampoule and two subsequent measurements are obtained:

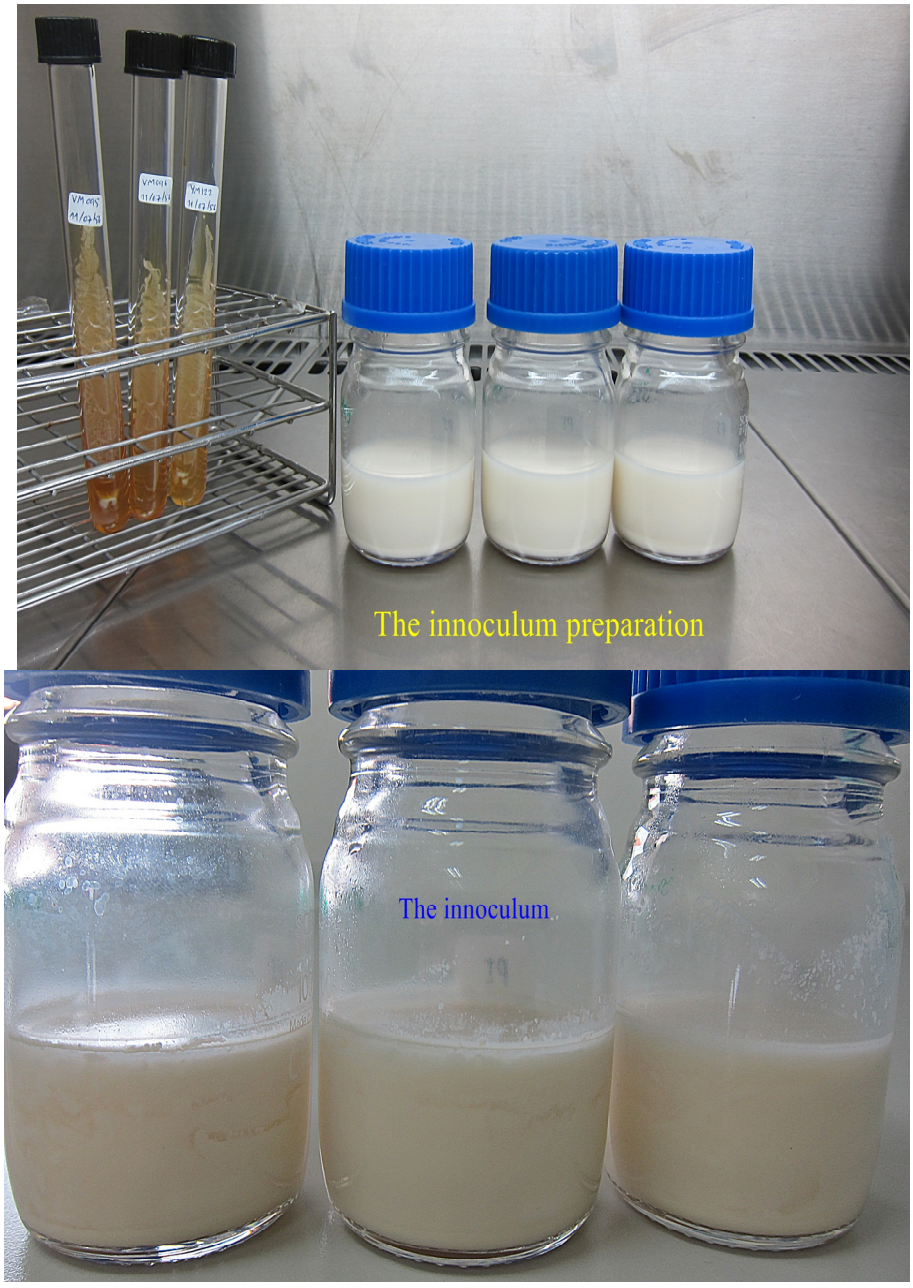
- Scattered light S (two photodiodes).
- Transmitted light T (one photodiode).

The ratio S/T is directly proportional to the density of the bacteria suspension.
The results are displayed on a mobile graduated scale on a liquid crystal display. The number of squares displayed is proportional to the McFarland value. The corresponding numerical value is shown on the right hand side of the display.

Correspondence McFarland scale/Bacterial concentration/Optical density:

Standard McFarland Scale	Bacterial concentration (1) $\times 10^8$ / mL	Theoretical Optical Density (2) at 550 nm
0.5	1.5	0.125
1	3	0.25
2	6	0.50
3	9	0.75
4	12	1.00
5	15	1.25
6	18	1.50
7	21	1.75

(1) The bacterial concentration varies according to the size of the microorganisms. The figures which are reported represent a mean, valid for the bacteria. For the yeasts, due to their bigger size, these figures have to be divided by 30.
(2) The values correspond to the optical densities of bacterial suspension. The Barium Sulphate (BaSO4) solutions do not present the same optical density since the size and the form of the particles are different from those of bacteria. The light is also diffracted differently.



Innoculum preparation

Fermented Soya Milk

Probiotic Soya Beverage

APPENDIX D

Linear equation and Standard Curves

1. Linear equation of standard carbohydrates

Order	Carbohydrates	Retention time (min)	Linear equation	R^2
1	FOS	7.543	Y=192,413x-195,907	1.000
2	Maltotetraose	7.990	Y=31,815x+4,205	0.999
3	Starchyose	8.238	Y=255,096x+6,308	1.000
4	Raffinose	9.120	Y=207,604x-12,767	1.000
5	D(-)Maltose	9.272	Y=349,107x+22,798	1.000
6	Treharose	9.285	Y=316,795x+8,251	1.000
7	Lactose	9.465	Y=329,458x-20,649	1.000
8	Sucrose	trace	-	-
9	D(+)Glucose	11.248	Y=345,487x-30,292	1.000
10	Myo-innositol	11.746	Y=337,576x+19,339	1.000
11	Mannose	11.905	Y=335,045x-22,677	1.000
12	D(+) Galactose	11.931	Y=312,622x+6,497	1.000
13	Xylose	12.042	Y=326,602x+3,026	1.000
14	D(-)Fructose	12.104	Y=341,073x-9,360	1.000
15	Mannitol	12.510	Y=315,119x-1,438	1.000
16	Sorbitol	12.688	Y=274,728x+1,715	1.000
17	L(-)Rhamnose	12.742	Y=277,311x-1,713	1.000
18	L(+)Arabinose	13.031	Y=298,992x+3,359	1.000
19	D(-)Arabinose	13.032	Y=290,168x-12,189	1.000

2. Linear equation of standard Lactic acid and SCFAs

Order	SCFA	Retention time (min)	Linear equation	R^2
1	Acetic acid	3.378	y = 4E+07x - 16049	0.900
2	Lactic acid	3.612	y = 6E+07x - 73861	0.999
3	Propionic acid	8.608	y = 5E+07x - 13881	0.900
4	n- Valeric acid	15.610	y = 3E+06x + 23708	0.900
5	Bytaric acid	21.940	y = 6E+07x - 25967	0.900
6	Iso- bytaric acid	22.906	y = 8E+07x - 35802	0.900

3. Standard curve for the responses (peak areas) to Zearalenone doses (µg).