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Research Article

Investigation of *Spirulina* supplementation into UHT milk and modified soymilk for yoghurt fermentation

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Abstract

The purpose of this study was to investigate the supplementation of *Spirulina* supplementation into cow's milk and modified soymilk for yoghurt fermentation with a view to products creating new generating diverse sensory and nutritional effects. Through the process of experimentation, using two strains of *Lactobacillus*, the following results were obtained. *Lactobacillus* In MRS broth, *acidophilus* bacteria reached biomass after 15 hours of culturing, *Lactobacillus bulgaricus* while bacteria reached biomass after 18 hours of culturing. *Lactobacillus* In milk, *acidophilus* and *Lactobacillus bulgaricus* both reached bacterial biomass after 15 hours of culturing. The collection time was 6 hours incubation, while the parameters of the fermentation process were; institutional distribution ratio between soymilk and UHT milk is 8:2, duration 3 hours fermentation, brewed at 43^{o}C, inoculate 10% fermented milk at the same rate transplanted between *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* is 6:4, saccharose sugar supplements is 8%, additional algae *Spirulina* concentrations of 6%, 5% gelatine. Storage time for optimal product is 10 days at 4^{o}C.

Keywords: *Lactobacillus acidophilus, Lactobacillus bulgaricus, Spirulina platensis,* dairy product, MRS broth, LAB, Vietnam.

Introduction

Soybeans are considered one of the Earth's oldest crops. Many studies show that soy is derived from China. It is grown on different soil types and climate-wide scope, ranging from the tropical regions of Brazil to Hokkaido Island full of snow in Northern Japan. With the development of science and technology; people have found new roles for soy in many areas, especially in the area of food. So, soybeans are spreading more and more around the world.

Soymilk is one kind of milk made from soy and, according to researchers the persistent use soymilk daily will reduce the blood cholesterol, as well as lowering blood pressure and prevent hardening of the arteries in older people. In addition to a large amount of protein, soymilk also contains up to eight types of amino acids important for human consumption (leucine, lysine, methionine, isleucine, phenylalanine, threonine, tryptophane, and valine). Unsaturated fatty acids in soybeans also act as barrier against cancer such as; Bowman- Bird Inhibitor (BBI), protease inhibitor, phytate, phytosterols, isoplavones and phenolic acid substances.

Cow's milk is also a nutrient rich food, providing almost all requirements of vitamins and minerals to the body. Thanks to the beneficial effects, milk and dairy products are very popular with everyone. The market of milk and dairy products is quite rich in variety, meeting the needs of people of all ages.

Yoghurt, a fermented milk product, provides essential bacteria that enhance can human health. Lactic bacteria introduced into the intestine will compete with bacterial rot and overwhelm the bacteria. According to various studies, yoghurt is very beneficial for the body, as it has anti-aging effects, reduces cholesterol in the blood, limits intestinal disorders, relieves side effects due to the use of antibiotics, increases interferon, reduces stomach ulcers, osteoporosis prevention, etc.

Algae are a group of microorganisms capable of self-supporting photosynthesis. They obtain their energy from sunlight. *Spirulina* has been shown to have many advantages, especially in terms of nutritional value and contains many valuable substances in the prevention and treatment of some diseases. *Spirulina* has been used as a food source or supplement to provide nutrition and health improvements for people because it is rich in nutrients like protein, amino acid, essential fatty acids, vitamins and minerals.

A number of previous studies have been undertaken on soymilk, *Spirulina* and yoghurt. Paul*, et al.* [1] provided a summary of the commercial developments in microalgal biotechnology. A number of important advances have occurred in recent years that are slowly moving the field into new areas. New products are being developed for use in the mass commercial markets as opposed to the "health food" markets. These include algal-derived long-chained polyunsaturated fatty acids, mainly docosahexaenoic acid, for use as supplements in human nutrition and animals. Large-scale production of algal fatty acids is possible through the use of heterotrophic algae and the adaptation of classical fermentation systems providing consistent biomass under highly controlled conditions that result in a very high quality product. New products have also been developed for use in the development of pharmaceutical and research products. These include stable-isotope biochemicals produced by algae in closed-system photobioreactors with extremely bright fluorescent pigments. Cryopreservation has also had a tremendous impact on the ability of strains to be maintained for long periods of time at low cost and maintenance while preserving genetic stability.

Tuomola, *et al*. [2] posed quality assurance criteria for probiotic bacteria. Acid and bile stability and intestinal mucosal adhesion properties are among the criteria used to select probiotic microbes. The quality control of probiotic cultures in food traditionally has relied solely on tests to ensure that an adequate number of viable bacteria are present in the products throughout their shelf lives. Viability is an important factor, but not the only criterion for quality assurance. To be effective, probiotic strains must retain the functional health characteristics for which they were originally selected. Such characteristics include the ability to survive transit through the stomach and small intestine and to colonize the human gastrointestinal tract. *In vitro* test protocols can be readily adopted to examine the maintenance of a strain's ability to tolerate acidic conditions, survive and grow in the presence of bile and metabolize selective substrates. Molecular techniques are also available to examine strain stability. Adhesion characterization may be an important quality-control method for assessing gut barrier effects. Adhesion has been related to shortening the duration of diarrhea, immunogenic effects, competitive exclusion and other health effects. Adhesion properties should be carefully monitored, including adhesion to intestinal cells (eg, Caco-2) and human intestinal mucus. Tuomola's research outlines the types of *in vitro* testing that can be used to ensure quality control of functional probiotic strains.

Gloria, *et al.* [3] showed the effect of *Spirulina platensis* biomass on the growth of lactic acid bacteria in milk. The stimulatory effect of aqueous suspensions of *Spirulina platensis* dry biomass extracted at pH 6.8 and 5.5 was studied on four lactic acid bacteria (LAB) grown in milk. The addition of dry *S. platensis* to milk (6 mg/ml) stimulated growth of *Lactococcus lactis* by 27%. The growth of other strains was also promoted.

Saito [4] reviewed the selection of useful probiotic lactic acid bacteria from the *Lactobacillus acidophilus* group and their applications to functional food. In the present review, a new mass screening system for selecting probiotic strains from *Lactobacillus acidophilus* group lactic acid bacteria (LAB) with strong adhesion to the human intestinal tract is described. Characteristics of antimicrobial peptides (bacteriocin), lactose-hydrolyzing enzymes and immunostimulative oligo DNA motifs in *L. gasseri* strains are also described. Finally, the use of *L. acidophilus* LAB, selected by this screening method, that have strong adhesion to the human colonic mucosa in functional yogurt products is described. Adhesiveness to the human intestine is one of the most important characteristics of probiotic LAB. A new screening system that involves a combination of three methods is proposed: rat colonic mucin (RCM)-micro plate assay, Carnoy's histochemical staining method and carbohydrate probe binding assay. By using an RCM-coated poly-vinylidene-diflouride membrane that mimics the human colonic mucous layer, a new lectin was isolated and its structure was clarified by gene cloning. Furthermore, the structures and functions of a new cyclic bacteriocin (gassericin A), new lactose-hydrolyzing enzymes and new immunostimulating oligo DNA motifs from *Lactobacillus gasseri* (B1 subgroup) were clarified. A new functional yogurt 'Fit down' is proposed, that is fermented by an adhesive strain of *L. acidophilus* LA67 selected by screening and contains antihypertensive peptides derived from whey proteins by protease digestion. In the future, superior functional food containing more effective probiotic LAB is expected to be developed by the use of the proposed mass screening system.

Otieno, *et al*. [5] demonstrated the stability of β-glucosidase activity produced by *Bifidobacterium* and *Lactobacillus* spp. in fermented soymilk during processing and storage. Fifteen probiotic microorganisms including *Bifidobacterium*, *Lactobacillus acidophilus* and *Lactobacillus casei* were screened for β-glucosidase activity using p-nitrophenyl-β-d-glucopyranoside as a substrate. Six strains were selected on the basis of β-glucosidase activity produced during fermentation of soymilk. The stability of the enzyme activity was assessed during incubation for up to 48 h and storage for 8 week at frozen (-80°C), refrigerated (4°C), room (24.8°C) and incubation (37°C) temperatures. *L. casei* strains showed the highest β-glucosidase activity after 24 h of incubation followed by *L. acidophilus* strains, whereas *Bifidobacterium* strains showed least activity. However, p-glucosidase from *Bifidobacterium animalis* BB12 showed the best stability during the 48 h fermentation. Lower storage temperatures (-80°C and 4°C) showed significantly higher ($P < 0.05$) β-glucosidase activity and better stability than that at higher temperatures (24.8°C and 37°C). The stability of β-glucosidase from these microorganisms should be considered for enzymic biotransformation during storage of isoflavone β-glucosides to bioactive isoflavone aglycone forms with potential health benefits

Rekha and Vijayalakshmi [6] studied biomolecules and nutritional quality of soymilk fermented with probiotic yeast and bacteria. Soymilk was fermented with five isolates of probiotic lactic acid bacteria and in combination with probiotic yeast *Saccharomyces boulardii*. Nutritional profiles such as fat, protein, ash, pH, acidity, polyphenol and protein hydrolysis were analyzed. Polyphenol content decreased from 265.88 to 119 microg/ml with different cultures. Protein hydrolysis ranged from 2.46 to 2.83 mmol l(-1) with different cultures. The antioxidant activity was assessed using different methods like 1, 1-diphenyl-2-picrylhydrazyl free radical-scavenging assay, inhibition of ascorbate autoxidation and measurement of reducing activity. The activities varied with the starters used but, nevertheless, were significantly higher than those found in unfermented soymilk. Bioconversion of the isoflavone glucosides (daidzin + genistin) into their corresponding bioactive aglycones (daidzein + genistein) was observed during soymilk fermentation. Total glucosides in soymilk were 26.35 mg/100 ml. In contrast, aglycones genistein and daidzein were quantitatively lesser accounting 2.91 mg/100 ml (genistein 1.17 mg/100 ml and daidzein 1.19 mg/100 ml). Soymilk fermented with probiotic cultures resulted in the reduction of glycosides ranging from 0.40 mg to 1.36 mg/100 ml and increase in aglycones ranging from 6.32 mg to 13.66 mg/100 ml.

Following on from the work outlined above, the purpose of this study was to investigatethe supplementation of S*pirulina* into cow's milk and modified soymilk for fermentation of yoghurt in order to create new products generating diverse sensory and nutritional effects.

Materials and Methods

Materials

Soybean was collected in Tra Vinh province, Vietnam. Sterilized milk (non-sugar) was supplied from Vinamilk JSC, Vietnam. *Spirulina* was purchased from Japan. Refines sugar was bought in a super market in Tra Vinh province, Vietnam. Strain *Lactobacillus acidophilus, Lactobacillus bulgaricus* were sources from Pasteur Institute, HCM City, Vietnam.

Growth curve of Lactobacillus acidophilus, Lactobacillus bulgaricus on medium

Lactobacillus acidophilus and *Lactobacillus bulgaricus* bacteria in the form of MRS agar chain transplants were incubated at 37^{o}C at intervals of 0, 5, 10, 15, 20 hours. The number of bacteria was determined by the method of disk dump.

Growth curve of Lactobacillus acidophilus, Lactobacillus bulgaricus on milk (soymilk:UHT milk, 1:1)

Just like bacteria in the environment MRS agar chain transplants through the environment MRS loose, incubated at 37^{o}C at optimum time. Take the bacteria in the environment MRS liquid to the milk sugar-free environment, incubated at 37^{o}C for 4 h. Bacteria from the environment UHT milk was transplanted to raw milk and pasteurized milk mixed with a ratio of 1: 1, incubated at 37^{o}C across time periods and the number of bacteria determined. The number of bacteria is determined by the method of pouring plates.

Stock fermented milk

Bacteria from the environment MRS loose chain implantation through the environment fresh milk UHT and incubated at 43^{o}C for 4 hours. Continue processing plant bacteria from raw milk to the milk fluid environment (soymilk and UHT milk with a 1: 1 ratio), incubated at 43^{o}C during the period of disposition as above. Sensory product surveys over time.

Fermentation

Fermentation time

pH monitoring, product characteristics over time and in combination with the growth curve of bacteria in soymilk, from there select the proper fermentation period such that the product ensures the necessary bacteria so that it acts as probiotic.

Effect of soymilk and sterilized milk non-sugar

Each test performed according to the procedures stated. But the ratio of institutional coordination between the soy milk and UHT milk varies according to the distribution scale parameter: mode between soy milk and UHT milk as follows: 4: 6, 5: 5, 6: 4.

Effect of saccharose supplementation

Preparation of soymilk from results of experiments is executed. In turn the saccharose was supplemented into milk by the various surveys rate 6%, 8%, 10% (w/v). Monitor pH and sensory evaluation by the method of products align comparision. From there select the appropriate sugar added milk.

Effect of bacteria inoculation ratio

Preparation of soy milk results from experiments. To change the ratio of seed to plant *Lactobacillus acidophilus* and *Lactobacillusratio bulgaricus* changed as follows 6:4, 5:4, 5:6 (v/v). Monitor pH and sensory evaluation by the method of products align comparision. From there select the appropriate transplant varieties rate added to the milk to ferment.

Effect of mixture ratio (UHT milk + soymilk) with Spirulina

Each test performed according to the procedures stated. But the ratio of institutional coordination between the room and algae milk (soymilk + milk UHT) changes according to the parameters: the ratio of institutional coordination between the room and *Spirulina* milk (soymilk + UHT milk) with the aspect ratio as follows: 6%, 8%, and 10% (v/v).

Effect of fermented milk preservation

The product is stored at a temperature of 4^{o}C; track pH, the acid and the number of lactic acid bacteria in products by the date preserve for optimum storage time for products (product that has just reached on the value perception has just achieved the nutritional value of probiotic). After the optimal storage time, product sensory assessment is performed by scoring method to determine the extent of tastes to accept consumer products.

Method of analysis

Colony forming unit: by pouring Petri dishes

pH of fermented milk: by pH Hanna 210.

Acidity titration: Four 10 ml milk into 100 ml triangle in the jar, then put in about 3 drops of phenoltalein 1%. Using NaOH 0, 1N titration (the solution appears pink powder in 10 seconds). % acid= n. 0.09. Whereas n: ml NaOH 0.1N.

Product sensory evaluation: by align comparision, score evaluation.

*Laboratory layout method and handle the result***:** by 1 factor arrangement. The result is processed by software Statgraphic 7.0.

Results and Discussion

Growth of Lactobacillus acidophilus, Lactobacillus bulgaricus on MRS broth

Colony morphology on MRS agar

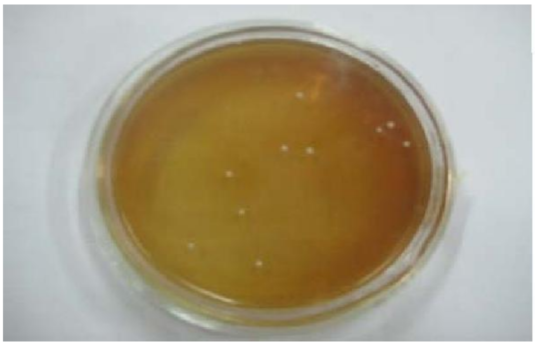

Figure 1. Morphology of *L. acidophilus*.

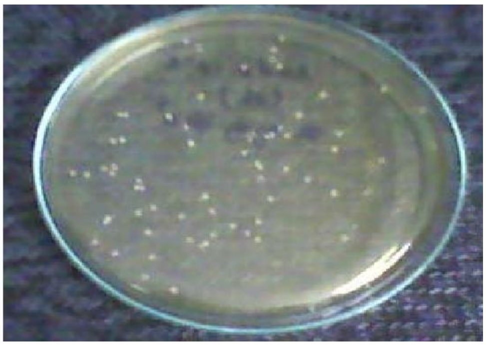

Figure 2. Morphology of *L. bulgaricus*.

Stray bacteria *L. acidophilus* on the environment MRS agar has a rounded size 2-5 mm, convex, white opaque and no colour. Stray bacteria *L. bulgaricus* on the environment MRS agar flat with yellowish, diameter 2-3 mm.

Growth curve of Lactobacillus acidophilus, Lactobacillus bulgaricus on MRS broth

The experiment then proceeded to erect the growth curve of bacteria *Lactobacillus acidophilus, Lactobacillus bulgaricus* on the environment MRS broth, aimed at qualitative research microorganisms over time and to determine the time of maximum cell density from which continue to proliferate in the soymilk. The bacteria are cultured in MRS 37^{o}C temperature, pH = 5.7. Just be transplanted directly from the tube holding the jelly-like tilt.

Table 1. Growth of *L. acidophilus* by culturing time in MRS.

Time (hours)	Density of *L. acidophilus*, Log (CFU/ml)	Density of *L. bulgaricus*, Log (CFU/ml)
0	1.89	5.00
3	2.33	5.30
6	4.81	6.00
9	6.08	6.95
12	7.30	7.08
15	8.33	7.15
18	8.37	8.20
21	8.34	8.08
24	8.30	8.18
27	8.28	8.00
30	8.22	7.70
33	7.80	6.96
36	7.31	6.00

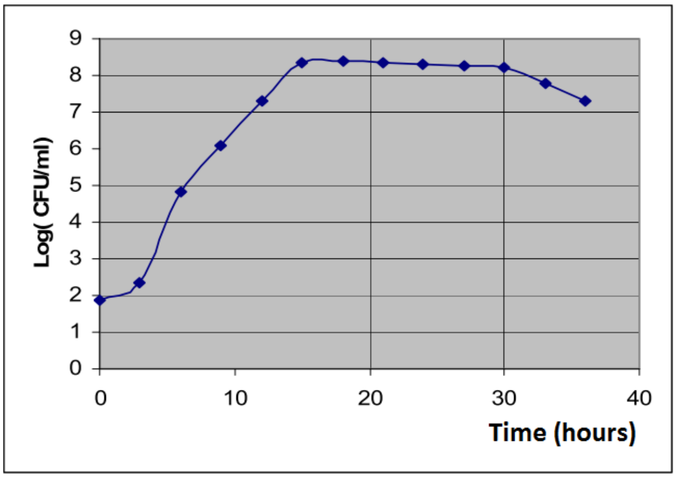

Figure 3. Growth curve of *L. acidophilus* in MRS broth.

From the growth curve of bacteria *L. acidophilus* it was noticed that a potential play lasts around 2-3 hours early due to the bacterial cell number increases not negligible. The length of a relatively short development lead demonstrates the bacterial cells were young, quickly adapting to the environment MRS broth. From 3 to 15 hours, the bacteria grow and develop under exponentiation, bacteria reproduce, biomass growth, here are a number of bacteria. The number of bacterial cells reached in 15 minutes, is the beginning of a balance. Equilibrium phase lasts from 18 to 30 hours, during this phase the reproduction continues to take place in some of the cells in the medium, the total number of new cells are born equal to the total number of dead cells are lost, the nutrients in the environment started running out, along with the accumulated lactic acid which can cause inhibition of the growth of the bacteria. After 30 hours of cell density diminishing, brewing started decline, due to the amount of nutrients in the environment is exhausted, the competition for nutrients by bacteria takes place, along with the product of the metabolic processes have inhibited the growth of bacteria that should the number of cells produced less than the number of cells lost.

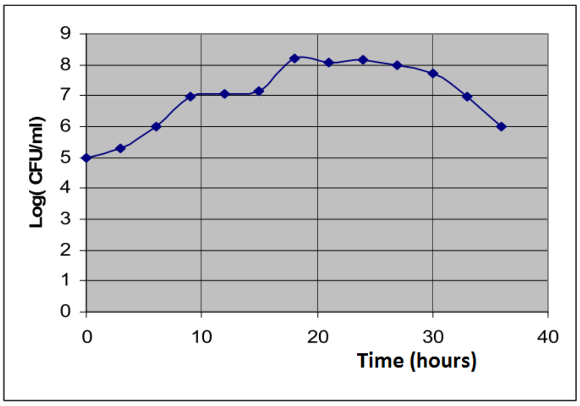

Figure 4. Growth curve of *L. bulgaricus* in MRS broth.

From the growth curve of bacteria *L. acidophilus* it can be noticed that the potential phase of *L. bulgaricus* also lasts for about 2-3 hours early. In 3-4 hours followed by a level number. And maximum cell number in 18 hours. Equilibrium phase takes place in 18-30 hours. After 30 minutes the decline phase of bacteria. The biomass of bacteria *L. acidophilus* was collected after 15 hours of culturing and collect biomass *L. bulgaricus* after 18 hours of culture in MRS broth.

Growth curve of Lactobacillus acidophilus, Lactobacillus on milk (soymilk and UHT milk with ratio 1:1)

The experiment continued to build the growth curve of *Lactobacillus acidophilus*, *Lactobacillus bulgaricus* in milk fluid environment in order to survey the number of microorganisms in the environment room for milk in the range of time to put into cultivation as the yeast for the production of yoghurt.

Table 2. Growth of *L. acidophilus* by culturing in soymilk.

Time (hour)	Bacteria density of *L. acidophilus*, Log (CFU/mL)	pH
0	7.86	6.3
5	8.92	5.9
10	10.69	5.1
15	11.36	4.2
20	10.26	4.0
25	9.08	3.8

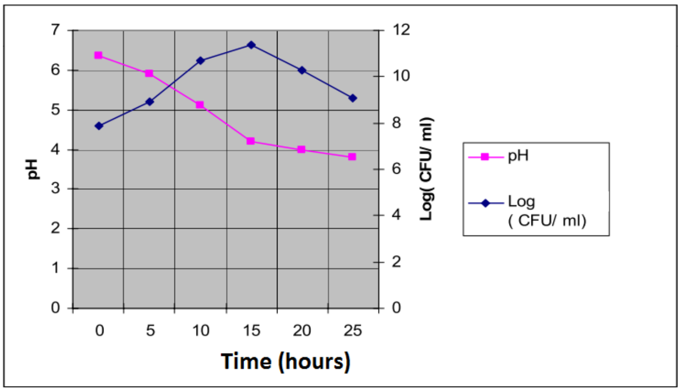

Figure 5. Growth curve of *L. acidophilus* in milk medium.

Table 3. Growth curve of *L. bulgaricus* by culturing time in milk.

Time (hours)	Bacteria density of *L. bulgaricus*, Log (CFU/mL)	pH
0	7.56	6.36
5	8.85	6.10
10	10.00	5.15
15	10.60	4.40
20	9.90	4.10
25	9.48	3.90

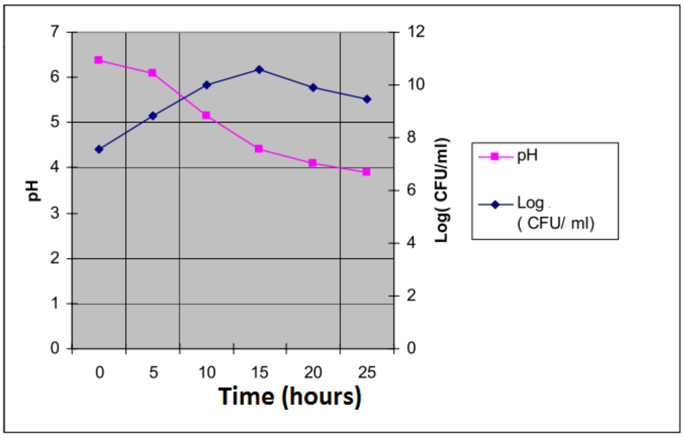

Figure 6. Growth curve of *L. bulgaricus* in milk medium.

From the growth curve of *L. acidophilus* and *L. bulgaricus* it can be observed that the number of bacterial cells at the time of implantation into the environment room > 10^7 CFU/ml milk. The number of cells increases rapidly in the next 15 hours combined with the significantly reduced pH value caused by bacterial lactic acid produced during metabolism. The number of bacterial cells reached in 15 minutes. After 15 hours of reduced cell number due to the concentration of nutrients in the environment decreases and the amount of lactic acid in many environments that inhibit the growth of bacteria. In a medium of milk, bacteria still grow and flourish, the number of bacterial cells > 10^7 CFU/ml during the survey period to ensure probiotic give the body enough.

Stock femented milk

Table 4. pH of of fermented milk under *L. acidophilus* and *L. bulgaricus* by time.

Time (hours)	pH of fermented milk by *L. acidophilus*	pH of of fermented milk by *L. bulgaricus*
0	6.33	6.34
2	6.23	6.20
4	6.09	6.13
6	5.98	5.90
8	5.67	5.64
10	5.14	5.15

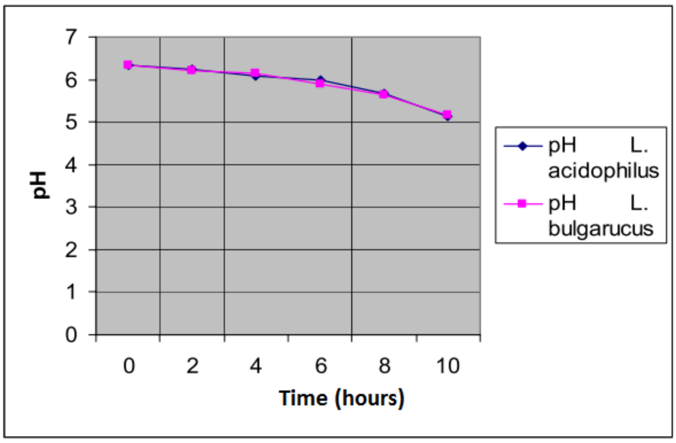

Figure 7. pH of of fermented milk under *L. acidophilus* and *L. bulgaricus* by time.

Table 5. Description of fermented milk by time.

Time (hours)	*L. acidophilus*	*L. bulgaricus*
0	Liquid structure White opaque Smell the beans and fatty odour	Liquid structure White opaque Smell the beans and fatty odour
2	Not yet frozen, liquid	Not yet frozen, liquid
4	Gelatinous structure bottom pots, light be ans smell, sour andmuch fat.	Yet the sour smell of occasional freezing, and peas smells
6	Condensed product structure. Smooth surface, be combined with the beans.	Gelatinous product structure, not as dense, strong smell of sour milk, sour mix with the beans.
8	The product is split a layer of water on the surface.	Condensed product structure, sour odor an d smell like soy that is damaged.
10	The product is separated completely.	The product is separated completely.

From sensory evaluation it was found that for fermented milk, *L. acidophilus* in the time of 6 hours of incubation reached in terms of sensory values structure and smell, but its not very acidic (pH = 5.98). Also as for the glazes bacteria *L. bulgaricus*, at the time of 6 hours of sensory brewed on the structure did not meet but reach of smell, due to this bacterium is born in producing yoghurt. When combining pH and sensory products, growth curve of bacteria in the milk fluid environment, fermented milk was chosen for application in the production of yoghurt is the yeast bacteria in 6 hours of incubation.

Fermentation

Yoghurt fermentation process will be affected by many factors. Surveys were conducted of the following factors: the time of fermentation, the ratio of institutional coordination between the soy and milk, the ratio of seed to plant (the ratio between *L.acidophilus* and *L. bulgaricus*), saccharose sugar added.

Fermentation time

Table 6. pH of of fermented milk by time.

Time (hours)	0	1	2	3	4	5	6
pH	6.45	6.30	5.92	5.36	5.02	4.73	4.63

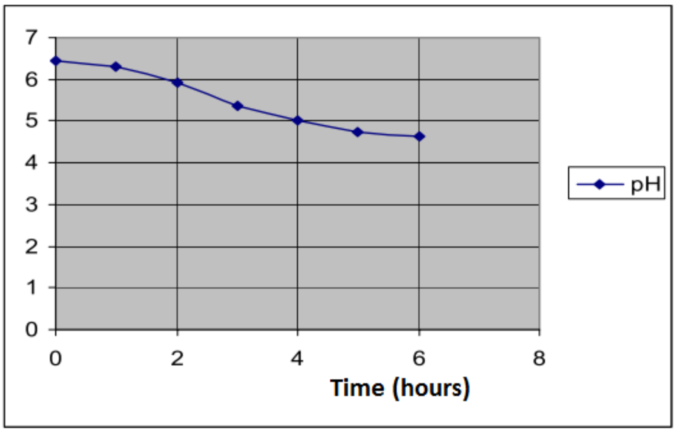

Figure 8. pH of fermented milk

Table 7. Sensory description of fermented milk.

Time (hours)	Product characteristics
1	Not yet frozen, liquid
2	Jitters began to structure the bottom jar, pale blue of algae, light sour smell, taste the sweet peas and more
3	Condensed product structure, its surface, pale blue, sour smellstrong, mild sour, the beans and the fat lot
4	The product is split a layer of surface water, pale blue, sour smell strong, mild sour, the beans and the fat lot
5	The product was separated from surface water and the bottom of the jar, sour smell strongly associated with the smell of rancid of soy, beans and sour power, reduced fat
6	The product is separated completely, the sour smell of rancid of soy

From sensory evaluation it was noticed that the following products 3 and 4 hours of fermentation that perception of smell and taste the same but different product structures. Combine the 3 elements: pH, and growth curve of bacteria in the milk fluid environment the products of fermentation time 3 hours were chosen.

According to Gloria, *et al* [3], adding algal biomass of *S. platensis* in the milk will promote lactic fermentation.The Panel is therefore the period of fermentation of yoghurt, soya *Spirulina* (3 hours) shorter than the duration of fermentation of other yoghurt products (4-6 hours).

On the other hand, the pH of the soymilk and spirulina (5.36) is much higher than with the conventional dairy products (4.0-4.6) we accept the pH value as if the pH of the product in the course of this survey if it will produce <5 fail structurally. Why does this phenomenon occur can be explained as follows: pH equal power of soy protein in the range 4.2-4.6, the ability of low-protein is non-water for pH equal to their power, so the pH of the product as possible progress on the pH value equal to this electrical products as separated water.

Effect of mixture ratio between soymilk and fresh milk non-sugar

D: To reduce the smell of soy, further experimentation changed the ratio of institutional coordination between the soy milk and pasteurized milk with the aspect ratio as follows
E: D: 4 ratio of soy milk and fresh milk-part 6
F: E: 6 ratio of soy milk and fresh milk-part 4
G: F: 8 ratio of soy milk and fresh milk part 2

From sensory evaluation results, and results of statistical analysis are shown in Table 8.

Table 8. Average score of sensory evaluation by modification of soymilk and fresh milk.

	Ratio of modification		
Sensory evaluation	D	E	F
Total score of align comparision	71	63	46
Average sensory evaluation (±0,32)	-0.31[a]	-0.03[a]	0.39[b]

Note: in the same row, the number of the same letters, the difference doesn't make sense in 95% reliability according to test LSD (P > 0.05).

From Table 8, some minor remarks and conclusions can be drawn. Institutional distribution rate between the soy and milk with different effects mean to sensory evaluation results of the product at the 95% confidence level (P < 0.05). And tested formula F (8 rate the soy milk and fresh milk part 2) have averaged the highest sensory and meaningful difference in reliability 95% according to test LSD (P < 0.05) than the two remaining knowledge should formula F (8 rate the soy milk and fresh milkpart 2) be chosen as fixed parameters for subsequent experiments.

Effect of saccharose supplementation

Additional sugar affects the fermentation and its by-product, and this study investigated the influence of sugar added to the perception of the product.
G: additional sugar is 6% (w/v)
H: additional sugar is 8% (w/v)
I: additional sugar is 10% (w/v)
From sensory evaluation results and statistical analysis results are shown in Table 9.

Table 9. Average score of sensory evaluation for fermented milk by saccharose supplementation.

Sensory evaluation	Saccharose supplementation		
	G	H	I
Total score of align comparision	72	42	66
Average sensory score (±0.3)	-0.34[a]	0.51[b]	-0.17[a]

In the same row, the number of the same letters, the difference doesn't make sense in a 95% reliability according to test LSD (P >0.05).

From Table 9, the following minor observations were made. Addition of sugar in milk very meaningfully influences the results of sensory evaluation of the products at 95% confidence levels (P <0.05). Experience formula H (sugar added to is 8%) had the highest average score for sensory and meaningful difference in reliability 95% according to test LSD (P < 0.05) than the two remaining. Formula F (added 8%sugar) was thus chosen as a fixed parameter for the next experiment.

Effect of bacteria inoculation

Alterations to transplanting seedlings ratio (ratio between *L. acidophilus* and *L. bulgaricus*) were made as follows:

J: 4 percent seedlings, *L. acidophilus* and *L. bulgaricus*, varieties in 6%, 10% seed implanted in milk (v/v).

K: 5% of the varieties of *L. acidophilus* and *L. bulgaricus* in like 5%, 10% seed implanted in milk (v/v).

L: 6% same *L. acidophilus* and *L. bulgaricus*, varieties in 4%, 10%seed implanted in milk (v/v).

Data from sensory evaluation results and statistical analysis results are presented in Table 10.

Table 10. Fermented milk sensory evaluation by ratio of bacteria inoculation.

	Bacteria inoculation		
Sensory evaluation	J	K	L
Total score by align comparision	60	63	57
Average sensory score (±0.36)	-0.56[a]	-0.85[a]	0.85[a]

Transplanting seedlings ratio (ratio *between L.acidophilus* and *L. bulgaricu*s) was then changed as follows:

J: 4% seedlings, *L. acidophilus* and *L. bulgaricus*, varieties in 6%, 10% seed implanted in milk (v/v).

K: 5% of the varieties of *L. acidophilus* and *L. bulgaricus* in 5%, 10% seed implanted in milk (v/v).

L: 6% same *L. acidophilus* and *L. bulgaricus* varieties in 4%, 10% seed implanted in milk (v/v).

Results from sensory evaluation and statistical analysis are shown in Table 10 above.

Effect of algae supplementation

In order to examine the influence of algae supplement amounts to the value perception of the product, the algae supplement milk with the formula as follows was tested.

A: additional algae concentrations of 6% (v/v)

B: additional algae concentrations of 8% (v/v)

C: additional algae concentrations of 10% (v/v)

Results from sensory evaluation and statistical analysis are illustrated in Table 11.

Table 11. Fermented milk sensory evaluation by ratio of algae supplementation.

	Algae supplementation		
Sensory evaluation	A	B	C
Total score of align comparision	44	60	76
Average sensory score (±0,3)	0.45[a]	0[b]	-0.45[c]

Note: in the same row, the numbers bring the different lettersmean difference in reliability 95% according to the lsd test (p >0.05).

Figure 9. Fermented milk supplemented with algae (a: 6%; b: 8%, c: 10%)

From Table 11, the following may be concluded. Algae content added to fermented milk had meaningful impact on the sensory evaluation results products at 95% confidence levels ($P < 0.05$). And A knowledge test (additional algae concentrations of 6%) had the highest average score for sensory and meaningful difference in reliability 95% according to test LSD ($P < 0.05$) than the two remaining.

In order to examine the influence of algae supplement amounts to the value perception of the product, various formulae of algae supplemented milk were tested as follows.
A: additional algae concentrations of 6% (v/v)
B: additional algae concentrations of 8% (v/v)
C: additional algae concentrations of 10% (v/v)
These sensory evaluation and results of statistical analysis are shown in Table 11.

Preservation time for fermented milk

The product was maintained at 4^{o}C for 16 days. During the storage time the norms of pH, the acid and the number of lactic acid bacteria in products were followed. The results are as follows.

Table 12. pH, acidity, bacteria by preservation time.

Time (days)	Log bacteria (CFU/ml)	pH	Acidity (%)
0	8.18	5.2	0.28
2	8.53	5.13	0.3
4	9.08	5.0	0.31
6	9.64	4.9	0.35
8	9.83	4.88	0.4
10	9.85	4.84	0.42
12	9.91	4.8	0.5
14	9.80	4.75	0.53
16	9.70	4.7	0.59

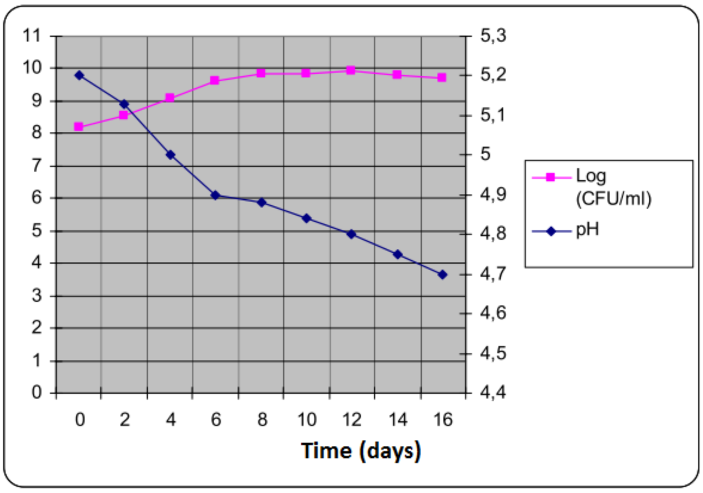

Figure 10. pH and count of lactic bacteria by preservation time.

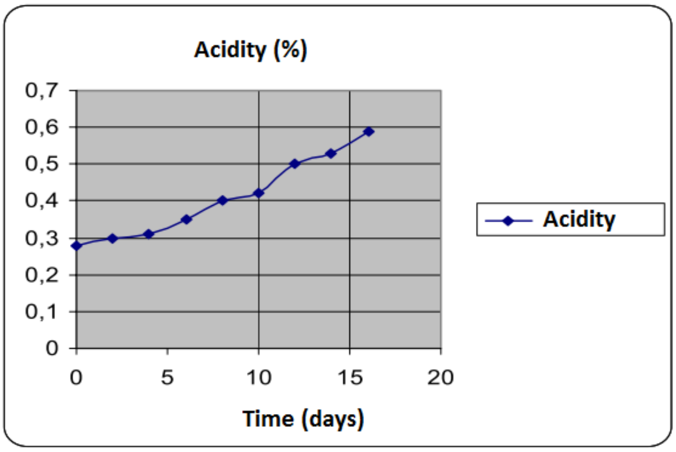

Figure 11. Acidity of sample by preservation time.

From Figures 10 and 11, it can be observed that the number of lactic acid bacteria in products is on 10^8 CFU/ml during the time survey, make sure the number of probiotic bacteria gives body (> 10^7 CFU/ml). The pH of the product is the drastic reduction in 8 days early to preserve, at the same time as the increase of the acid, which can explain this as follows: maintained at 4°C works speed limits growth and development of bacteria rather than fully suppressive of growth, on the other hand there are also bacterial adaptation to new environmental conditions should the number of cells increase, the amount of lactic acid produced during metabolism that they reduce pH and acid level increases.

However, due to the pH of the soy protein equal to about 4.2-4.6, sensory observations product during maintenance it was noticed after 10 days had maintained separate phenomena of water surface. So the optimum storage time of the product of 10 days was selected.

In order to determine the degree of consumer acceptance for soymilk and *Spirulina*, sensory evaluation methods were applied for sample tastes between the two points as follows:

Model 206: yogurt with soy milk and spirulina Vinamilk 207Samples:

Results evaluated 30 tastes who try out are shown in Table 13.

Table 13._ Consumer preference of fermented milk.

Parameter	Colour preferred	Aroma preferred	Taste preferred	Structure preferred	General
Sample 206	7.5	6.8	6.73	7.03	7.03
Sample 207	7.8	7.47	8.13	8.17	7.97

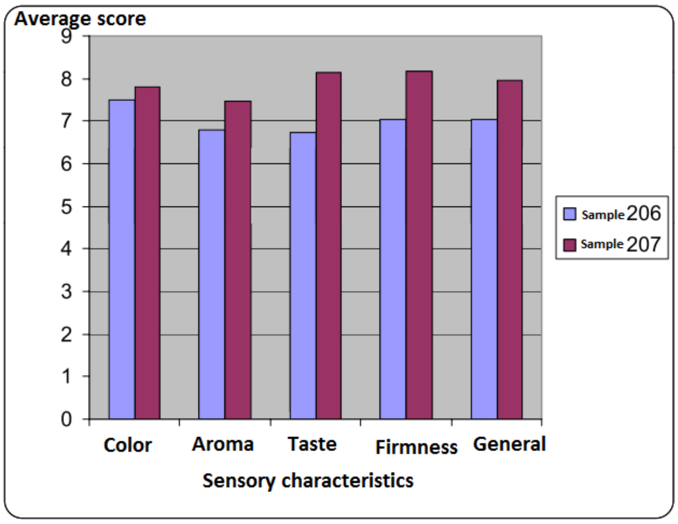

Figure 12. Consumer preference for the two products.

For product research, indicators of colour are the most preferred test and low indicators about the smell and taste. The average score for the norms of research products ranged from 6-7. As such, the level of interest of people who try to research samples in the range somewhat like to like. Indicators about the smell and taste is the lowest, lower than dairy products on the market, this may be due to the product still smells of beans The bacteria *L. bulgaricus* flavour compounds was not sufficient to eliminate odour completely. Beans are characteristic of the soy protein will be split more sour if the product water so should study the type of stabilizer to make product research is improved.

Overall, both products are reaching the point 6, which means that people try to also accept both products are research and products that are popular in the market

.

Conclusion

Due to limitations in terms of time, as well as the equipment, it was not possible to apply the results for further quality improvement It is suggested that further research should be conducted to follow product viscosity over time fermentation and study the effects of stabilizer added to the products that might improve both taste and texture. Further research could also try some additional flavourings and sour milk to lower the effect of soy odour.

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