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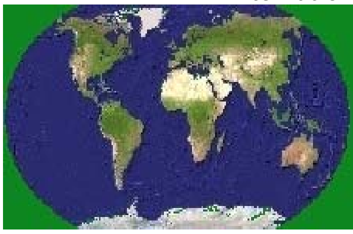

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Investigation of *Spirulina* Supplementation into Fresh Uht Milk Modified Soy milk for fermentation

Nguyen Phuoc Minh

Abstract
Through the process of experimentation, we have obtained some of the following results: in MRS broth, *Lactobacillus acidophilus* bacteria reached biomass after 15 hours of culturing, *Lactobacillus bulgaricus* bacteria reached biomass after 18 hours of culturing. In milk, *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* reached bacterial biomass after 15 hours of culturing. The enamel collection time of 6 hours incubated, the parameters of fermentation process: institutional distribution ratio between soymilk and UHT milk is 8:2; duration 3 hours fermentation, brewed at 43 °C; inoculate 10% fermented milk at the same rate with transplanted between *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* is 6:4; saccharose sugar supplements is 8%; additional algae spirulina concentrations of 6%, 5% gelatin; storage time for optimal product is 10 days at 4 °C.

Keywords: jackfruit juice, fermentation, yeast, soluble dry matter, acidity, fermented beverage

1. Introduction

Soybeans are considered one of humanity'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 the role of 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, according to the researchers the persistence use soymilk daily will reduce the blood cholesterol lowering drug, is blood pressure and prevent hardening of the arteries in older people. In addition to large amount of protein in soymilk also contains up to 8 types of amino acid important for human body (leucine, lysine, methionine, isoleucine, phenylalanine, threonine, tryptophan, and valine). Unsaturated fatty acids in soybeans also room against the germ of cancer such as: Bowman-Bird Inhibitor (BBI), protease inhibitor, phytate, phytosterols, isoflavones, and phenolic acid substances.

Milk is a nutrient rich foods, providing almost full of vitamins and minerals to the body. Thanks to the beneficial effects that milk and dairy products are very popular with everyone. The market of milk and dairy products are quite rich in variety, meet for people of all ages.

Yogurt, milk fermented product, one has to rely on the rule of natural survival struggle to enhance human health, lactic bacteria are introduced into the intestine will compete with bacterial rot and overwhelms the bacteria. According to studies, the yogurt is very beneficial for the body, it has anti-aging effects, reduces cholesterol in the blood, limiting intestinal disorders, relieve side effects due to the use of antibiotics, increases interferon, reducing stomach ulcers, osteoporosis prevention and etc.

Algae are the Group of micro-organisms capable of photosynthesis self-supporting, they participated in general primary living energy from sunlight. Spirulina has many advantages especially in terms o of some diseases. Spiru f nutritional value and contains many valuable substances in the prevention and treatment lina is used as a food source from thousands of years to provide nutrition and health improvements for people because Spirulina is rich in nutrients like protein, amino acid, essential fatty acids, vitamins, minerals.

Some studies about soymilk, *spirulina and yahourt* can be introduced as follows: Paul, P.B. *et al.* (1999) gave out commercial developments in microalgal biotechnology. A number of important advances have occurred in microalgal biotechnology 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 and 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.

Elina Tuomola *et al*. (2001) performed 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 foods 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. This article outlines the types of in vitro testing that can be used to ensure quality control of functional probiotic strains.

Gloria, Z.C. *et al*. (2004) 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.

Tadao Saito (2004) reviewed the selection of useful probiotic lactic acid bacteria from the *Lactobacillus acidophilus* group and their applications to functional foods. In the present review, a new mass screening system for selecting probiotic strains from *Lactobacillus* (L) 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 immunostimulatory oligo DNA motifs in L. gasseri strains are also described. Finally, the use of *L. acidophilus* LAB, selected by our 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 polyvinylidene-difluoride 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 our screening and contains antihypertensive peptides derived from whey proteins by protease digestion. In the future, superior functional foods containing more effective probiotic LAB are expected to be developed by the use of the proposed mass screening system.

D. O. Otieno *et al*. (2005) 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 wk 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 CR and Vijayalakshmi G. (2008) 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 profile like 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 soya milk 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.

Because of the reasons above, the purpose of the study "investigation of spirulina supplementation into fresh cow milk modified soymilk for yaourt fermentation" in order to create new products generating diverse sensory and nutritional effects.

2. Material & Method
2.1 Material
Soybean is collected in Tra Vinh province, Vietnam. Sterilized milk (non-sugar) is supplied from Vina milk JSC, Vietnam. Spirulina is purchased from Japan. Refine sugar is bought in super market in Tra Vinh province, Vietnam. Strain *Lactobacillus acidophilus*, *Lactobacillus bulgaricus* are originated from Pasteur Institute, HCM City, Vietnam.

2.2 Research method
2.2.1 Growth curve of *Lactobacillus acidophilus*, *Lactobacillus bulgaricus* on medium
Just like *Lactobacillus acidophilus*, *Lactobacillus bulgaricus* bacteria in the environment MRS agar chain transplants through the environment MRS loose, incubated at 37oC in the interval 0, 5, 10, 15, 20 hours. The number of bacteria is determined by the method of disk dump.

2.2.2 Growth curve of *Lactobacillus acidophilus*, *Lactobacillus bulgaricus* on milk (soymilk: fresh milk, 1:1)
Just like bacteria in the environment MRS agar chain transplants through the environment MRS loose, incubated at 37oC in optimum time. Take the bacteria in the environment MRS liquid to the milk sugar-free environment, incubated at 37oC for 4 h. Bacteria from the environment fresh milk to be transplanting to raw milk and pasteurized milk mixed with a ratio of 1: 1, incubated at 37 ºC across time periods and determines the number of bacteria. The number of bacteria is determined by the method of pouring plates.

2.2.3 Stock fermented milk
Bacteria from the environment MRS loose chain implantation through the environment fresh milk UHT and incubated at 43 ºC in 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 ºC during the period of disposition as above. Sensory product surveys over time.

2.2.4 Fermentation
2.2.4.1 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.

2.2.4.2 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 fresh milk varies according to the distribution scale parameter: mode between soy milk and UHT milk as follows: 4: 6, 5: 5, 6: 4.

2.2.4.3 Effect of saccharose supplementation
Preparation of soymilk from results of experiments is executed. In turn we supplemented the saccharose 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.

2.2.4.4 Effect of bacteria inoculation ratio
Preparation of soy milk results of experiments. To change the ratio of seed to plant *Lactobacillus acidophilus* and Lactobacillus ratio 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.

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

2.2.6. Effect of fermented milk preservation
The product is stored at a temperature of 4oC; 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.

2.3 Analyzing method
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 phenolphthalein 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 statgraphic7.0.

3. Result & Discussion
3.1. Growth of *Lactobacillus acidophilus, Lactobacillus bulgaricus* on MRS broth
3.1.1 Colony morphology on MRS agar
Colony morphology on MRS agar Stray bacteria *L. acidophilus* on the environment MRS agar has arounded, size 2-5 mm, convex, white opaque, and not color. Stray bacteria *L. bulgaricus* on the environment MRS agar flat with yellowish, diameter 2-3 mm.

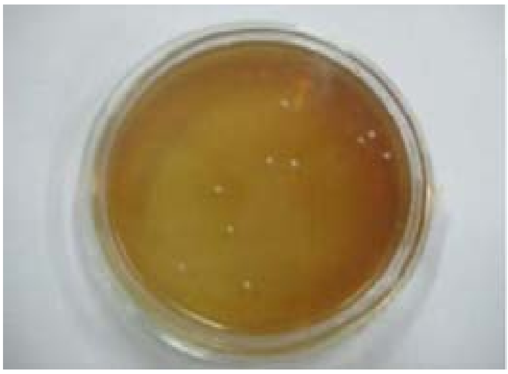

Fig 1: Morphology of *L. acidophilus*

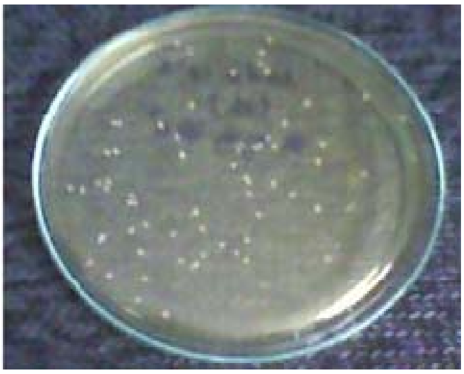

Fig 2: Morphology of *L. bulgaricus*

3.1.2 Growth curve of *Lactobacillus acidophilus, Lactobacillus bulgaricus* on MRS broth

We proceeded to erect the growth curve of bacteria *Lactobacillus acidophilus*, *Lactobacillus bulgaricus* on the environment MRS broth, aimed at qualitative research micro-organisms over time and determine the time of maximum cell density from which continue to proliferate in the soy milk. The bacteria are cultured in MRS 37oC temperature, pH = 5.7. Just be transplanted directly from the tube holding the jelly-like tilt.

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

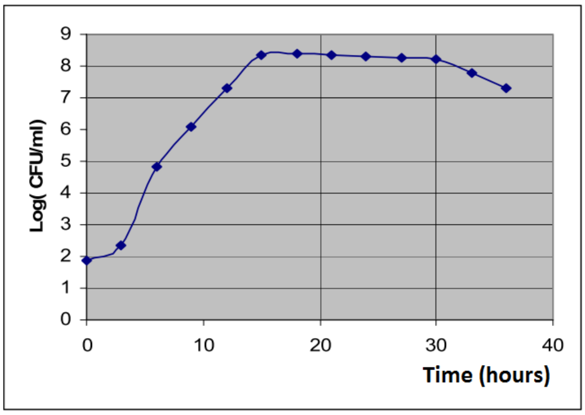

Fig 3: Growth curve of *L. acidophilus* in MRS broth.

From the growth curve of bacteria *L. acidophilus* we noticed: a potential play lasts around 2-3 hour 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 much lactic acid can cause inhibition of the growth of the bacteria. After 30 hours of cell density diminishes, started brewing decline, due to the amount of nutrients in the environment is exhausted, the competition of 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.

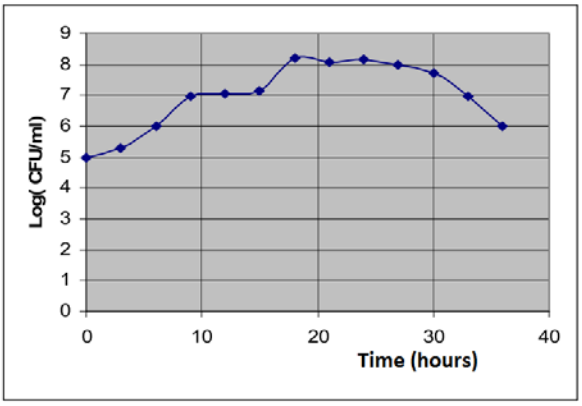

Fig 4: Growth curve of *L. bulgaricus* in MRS broth

From the growth curve of bacteria *L. acidophilus* we notice: 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. We collect biomass bacteria *L. acidophilus* after 15 hours of culturing and collect biomass *L. bulgaricus* after 18 hours of cultured in MRS broth.

3.2. Growth curve of *Lactobacillus acidophilus,* Lactobacillus on milk (soymilk and fresh milk with ratio 1:1)

We proceed 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 yogurt.

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

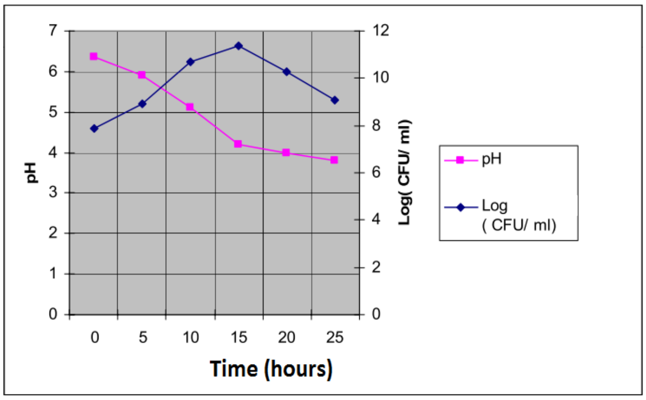

Fig 5: Growth curve of *L. acidophilus* in milk medium

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

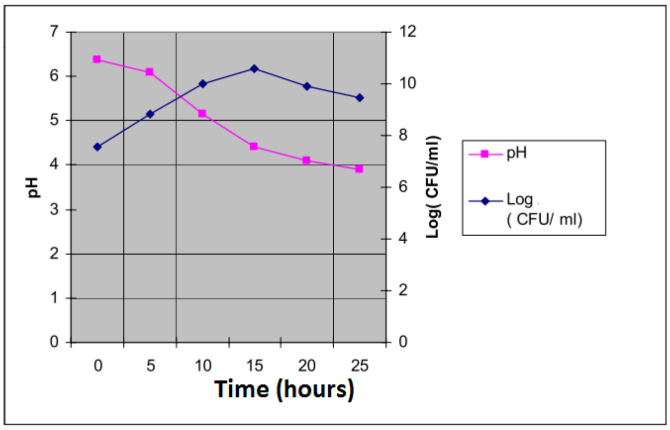

Fig 6: Growth curve of *L. bulgaricus* in milk medium

From the growth curve of *L. acidophilus* and *L. bulgaricus* we noticed: the number of bacterial cells at the time of implantation into the environment room > 107 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 room of milk, bacteria still grow and flourish, the number of bacterial cells > 107 CFU/ml during the survey period to ensure probiotic give the body enough.

3.3 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

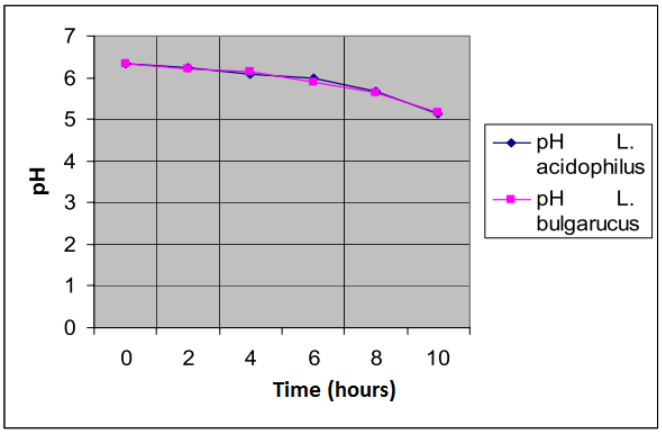

Fig 7: pH of of fermented milk under *L. acidophilus* and *L. bulgaricus* by time

By sensory evaluation, we found that for fermented milk, *L. acidophilus* in the time of 6 hours of incubation reached in terms of sensory values structure and the 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 yogurt. When combining pH and sensory products, growth curve of bacteria in the milk fluid environment, we chose fermented milk that for application in the production of yogurt is the yeast bacteria in 6 hours of incubation.

3.4 Fermentation

Yogurt fermentation process will be affected by many factors. In this topic we conducted surveys 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.

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 beans 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 and smell like soy that is damaged.
10	The product is separated completely.	The product is separated completely.

3.4.1 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

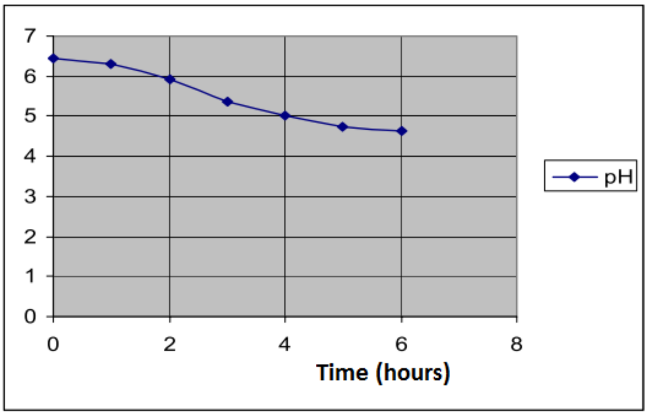

Fig 8: pH of fermented milk

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 smell strong, 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

By sensory evaluation we noticed 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 we choose products of fermentation time is 3 hours.
According to Gloria and Associates (2004), when adding algal biomass of S. platensis in the milk will promote lactic fermentation. The Panel is therefore the period of fermentation of yogurt, soya spirulina (3 hours) shorter than the duration of fermentation of yogurt products other (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

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

D: To reduce the smell of soy, we conducted survey, 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 we draw 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). Through table 8, we draw out some minor remarks and conclusions: 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 we choose to experience formula F (8 rate the soy milk and fresh milk part 2) as fixed parameters for subsequent experiments.

3.4.3 Effect of saccharose supplementation

Additional sugar affects the fermentation and its by-product, in this study we investigated the influence of sugar added to the perception of the product. We conduct the following additional.

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 we draw out 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)	-0.34ᵃ	0.51ᵇ	-0.17ᵃ

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). Through table 9 we draw out some minor remarks: Additional sugar in milk vary meaningfully influence to 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 knowledge should we choose to experience formula F (added 8%sugar) as a fixed parameter for the next experiment.

3.4.4 Effect of bacteria inoculation
We conduct changed transplanting seedlings ratio (ratio between *L. acidophilus* and *L. bulgaricu*) as follows:
J: 4 percent seedlings, *L. acidophilus* and bulgaricus, l. varieties in6% 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 bulgaricus, l. varieties in 4% 10%seed implanted in milk (v/v)
From sensory evaluation results and statistical analysis results we draw are 10 Tables.

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)	-0.56ᵃ	-0.85ᵃ	0.85ᵃ

3.5 Effect of alga supplementation
In order to examine the influence of algae supplement amounts to the value perception of the product, we conduct the algae supplement milk with the formula 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)
From sensory evaluation results, and results of statistical analysis we draw are 11 Tables

Table 11: Fermented milk sensory evaluation by ratio of alga supplementation

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

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

Fig 9: Fermented milk supplemented alga (a: 6%; b: 8%, c: 10%)

Through our 11 Table have commented and concluded the following: algae content added to fermented milk meaningful impact to 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 knowledge should we choose to experience consciousness at a fixed parameter do for the next experiment.
In order to examine the influence of algae supplement amounts to the value perception of the product, we conduct the algae supplement milk with the formula 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)
From sensory evaluation and results of statistical analysis we draw are 11 Tables

3.6 Preservation time for fermented milk
We conduct maintained at 4 ºC in 16 days. During the storage time we follow the norms of pH, the acid and the number of lactic acid bacteria in products and 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

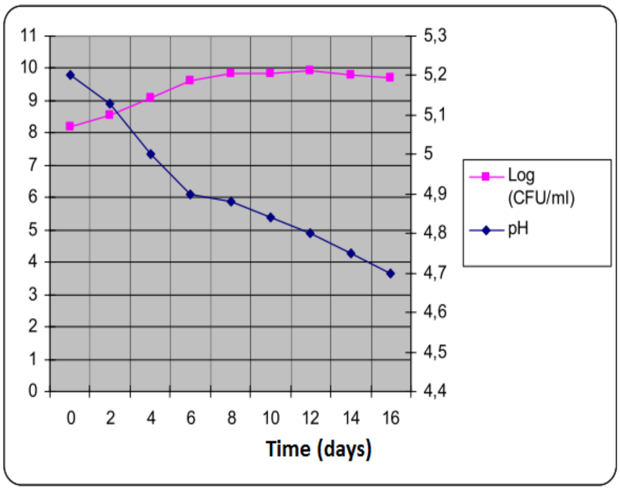

Fig 10: pH and count of lactic bacteria by preservation time

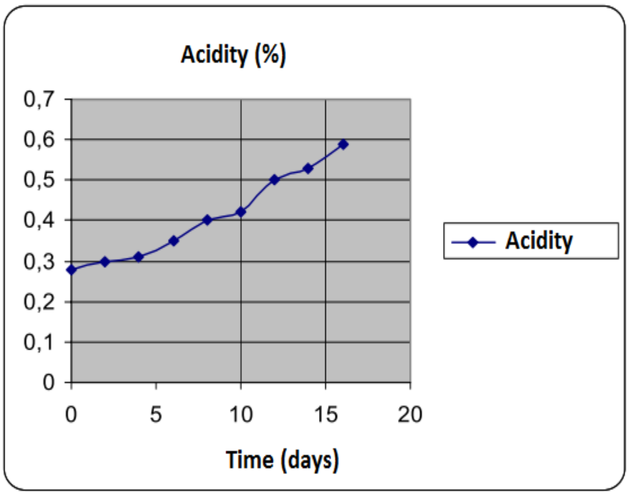

Fig 11: Acidity of sample by preservation time

Through figure 10 and Figure 11 we have commented as follows: the number of lactic acid bacteria in products is on 108 CFU/ml during our time survey, make sure the number of probiotic bacteria gives body (> 107 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 increases, 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 power equal to about 4.2-4.6, sensory observations product during maintenance we noticed after 10 days had maintained separate phenomena of water surface. So we select the optimum storage time of the product is 10 days.

In order to determine the degree of consumer acceptance for soymilk and spirulina, we conducted for sensory evaluation methods for sample tastes between the two points is as follows:

Model 206: yogurt with soy milk and spirulina Vinamilk 207 Samples: Results evaluated 30 tastes who try out are shown in Table 13.

Table 13: Customer favourist by fermented milk

Parameter	Color preferred	Aroma prefered	Taste prefered	Structure prefered	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

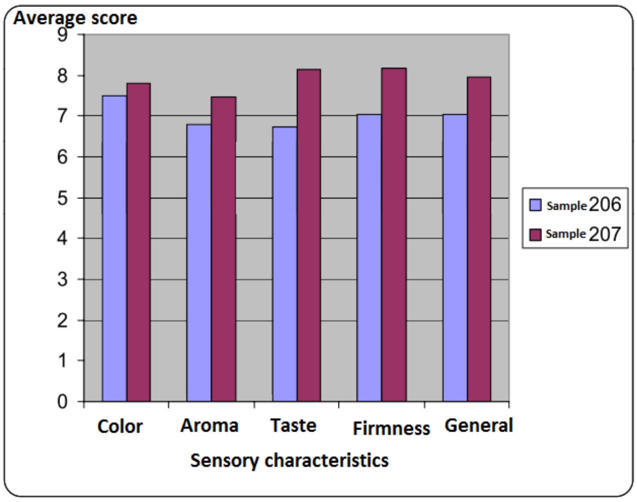

Fig 12: Customer favourist for two products

For product research, indicators of color are the most preferred test and low of 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 and tomatoes have not done, the bacteria *L. bulgaricus* flavour compounds was not sufficient to eliminate odor 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 on more.

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

4. Conclusion

Due to limitations in terms of time as well as the equipment, so we cannot survey off the relevant experiments in order to for better-quality products, we have some of the following suggestions: follow product viscosity over time fermentation; Study on stabilizer added to products that improve the taste and texture; The survey adds some additional flavorings and sour milk to soy odor.

5. References

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