

Development of Probiotic Soy-Yogurt Fused with Goat Milk and Enhanced with Vitamin B-12

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Abstract: More consideration from the matured and vegans has been paid to soy-item because of its taste, simple absorbability, just as the relationship with wellbeing. Nonetheless, soy-item has an imperfection of low nutrient substance, principally the water-dissolvable vitamin B12. This study was to explore co-aging of glycerol and fructose in soy-yogurt to improve vitamin B12 creation by *Lactobacillus reuteri*. After a sequential mix analyses, the co-aging was affirmed to upgrade the development of vitamin B12 up to 4 µg/100mL. The two supplementations initiated the declaration of *cobT* and *cbiA* and worked to adjust the redox response. In the meantime, high substance of fructose supplementation diminished the creation of vitamin B12 and smothered articulation of *cobT* in microbes. It was demonstrated that the vitamin B12 content of this soy-yogurt is higher than other aged soybean based food and consequently can be filled in as elective nourishment for the matured and vegans. The fortness depicted under the kinds of food parts like carbs, proteins, dietary, strands, minerals and nutrients.

Keywords: Probiotics, Soy Milk, Goat Milk, Mango Kernel Powder, *Lactobacillus*

I. INTRODUCTION

Matured milk items are refined dairy items produced using skim, entire or marginally thought milk, that require explicit lactic corrosive microbes to foster their trademark flavor and surface. Aged milks are generally liquid or semi-liquid in nature and all contain lactic corrosive in differing extents. Aging of milk changes its properties coming about in drinks like yogurt; kefir and other refined dairy items.

Aged milks overall have a decent dietary benefit that contrasts well and that of milk from which they are made.

Yogurt and other refined dairy items are profoundly nutritious food varieties which in certain conditions likewise have restorative worth. Yogurt contains every one of the components of nourishment found in milk in additional absorbable structure. Yogurt is made all of the time from cow's or alternately buffaloes' milk, in spite of the fact that creation from goat's milk is monetarily more affordable than cows and buffaloes' milk.

In certain nations, goat's milk is polished off as liquid milk, even on a business premise and the parts of goat's milk are of impressive market revenue. Since the goat is the creature of needy individuals and its milk is drunk new, notwithstanding the chance of assembling dairy items from this milk, particularly being centered around the goat as a significant dairy creature in the family. The current research was expected to use goat's milk enhanced with soymilk in the production of yogurt similarly as with prompt goals to decide

1-The impact of expansion of soymilk to goat's milk at three distinct fixations on compound and organoleptic attributes and shelf life of the item.

2. To add mango kernel powder to the prepared yogurt in three different concentrations and analyse its organoleptic, chemical as well as shelf life.

II. MATERIALS & METHODS

A. Requirements:

Table 1: Requirements

Instrument	Glass wares	Other requirements
01. Incubator	01. Conical flask	01. Milk(soy, goat)
02. Autoclave	02. Pipettes	02. Soya bean
03. Balance	03. Beakers	03. Mango kernel powder
04. Distillation plant	04. Petri plates	04. Dabur honey
05. Laminar air flow	05. Micro pipettes	05. Plastic cup
06. Microscope	06. Micro pipettes tips	06. Distilled water
07. Centrifuge	07. Glass slides	07. Bacterial strains 7(a) <i>Bacillus megaterium</i>
08. Blender	08. Cavity slide	7(b) <i>Escherichia coli</i>
09. Refrigerator	09. Spreader	7(c) <i>Bacillus subtilis</i>
10. Vortex mixture	10. Thermometer	7(d) <i>Staphylococcus aureus</i>
11. pH meter	11. Mortar & pestle	
12. Magnetic stirrer	12. Test tubes	
13. Orbital shaker	13. Test tube stand	
14. Hot air oven	14. Centrifuge tube	
15. Spectrophotometer	15. Screw capped bottles	
16. Colony counter	16. Muslin cloth	
	17. Durham's tube	
	18. Cup borer	
	19. Forceps	
	20. Cotton swab	
	21. Whatman no.1 filter paper	

Chemicals and reagents:

Table 2: Chemicals and reagents

Staining reagent	Biochemical test	Sugars
01. Gram's iodine	01. Hydrogen Peroxide (3%)	01. Glucose
02. Crystal violet	02. N, N, N', N'tetramethyl p phenylenediamine dihydrochloride	02. Galactose
03. Alcohol	03. NaCl	03. Fructose
04. Safranin	04. Phenol	04. Lactose
05. Malachite green	05. 10N HCl	05. Sucrose
06. Vaseline	06. 1N NaOH	

B. Sample collection:

Soya bean seeds were collected from the market of Vadodara city and goat milk was collected from the rural area of the city.

C. Preparation of soy milk:

Soymilk was prepared as follows: Beans (200 gm.) were absorbed one litre bubbling water, kept in a cooler short-term and homogenized in 1.4 litres bubbling water. The resultant slurry was sifted through cheesecloth to acquire the soymilk.

Preparation of samples for yoghurt manufacture:

Yogurt was prepared using soy milk incorporated with goat milk with the addition of Mango kernel powder. Samples were prepared as follows:

1. First sample was Control made with only Goat milk
2. Second sample was made with 60% soy milk and 40% goat milk with addition of mango kernel powder.
3. Third sample was made with 60% soy milk and 40% goat milk with addition of mango kernel powder.
4. Fourth sample was made with 50% soy milk and 50% goat milk with addition of mango kernel powder.

D. Yoghurt preparation:

Yoghurt samples were prepared as follows: Milk was heated to 90°C/30 min, followed by cooling to 45°C. Starter culture at the rate of 3% (1:1 combination of *L. bulgaricus* and *S. thermophilus*) was added. Soymilk and mango kernel powder was added after pasteurization. The mixture was poured into clean dry plastic cups (100- ml size), covered and incubated at 42.5°C/4 hr, the cups were then placed in the refrigerator at 4°C. The chemical and sensory analyses were carried out at 0, 3 and 6- day intervals.

E. Chemical Analysis of Milk and Yoghurt:

Fat Content:

Principle:

The customary standard reference technique for fat examination depends on one or the other weight or volumetric assurance. There are numerous scientific techniques for the assurance of the fat substance of milk; the Gerber test is broadly utilized from one side of the planet to the other.

The test is a volumetric technique where fat is separated from milk by radiating power. Sulphuric corrosive is utilized to break down the protein that shapes the film around the (fat

globules) and amyl liquor is added to work on the partition of fat from different solids.

Procedure:

10 ml of sulphuric corrosive were filled a spotless dry Gerber tube, then, at that point, 10.94 ml of milk or yogurt samples were added. Amyl liquor (1 ml) was added to the cylinder, followed by expansion of refined water (10 ml). The substances were completely blended till no white particles should have been visible. Gerber tubes were centrifuged at 1100 cycles each moment (rpm) for 4-5 minutes. The cylinders were then moved to water bath at 65°C/3 min. The fat segment was quickly read after expulsion from water bath.

Protein Content:

Principle:

The Kjeldahl methodology estimates the nitrogen content of an example. The protein content then, at that point, can be determined expecting a proportion of protein to nitrogen for the particular food being dissected. The Kjeldahl methodology can be fundamentally partitioned into three sections:

- (1) Processing
- (2) Refining
- (3) Titration

In the processing step, natural nitrogen is changed over to an ammonium within the sight of an impetus at around 370°C. In the refining step, the processed example is made antacid with NaOH and the nitrogen is refined off as NH₃. This NH₃ is "caught" in a boric corrosive arrangement. How much smelling salts nitrogen in this arrangement is evaluated by titration with a standard HCl arrangement. A reagent clear is brought through the investigation and the volume of HCl titrant expected for this clear is deducted from every assurance.

Procedure:

Ten millilitres of milk (10 gm. yoghurt) were poured into a clean dry Kjeldahl flask and 2 gm. Kjeldahl tablest (CUSO₄) were added as catalyst. Twenty five milliliters of concentrated sulphuric acid were added to the flask, which was then heated until a clear solution was obtained, the flask then was left for another 30 minutes, after which the flask was removed and allowed to cool. The digested sample was poured in a volumetric flask and diluted to 100 ml with distilled water. Five millilitres were distilled with 10 ml of 40% NaOH. The distillate was received in a conical flask containing 25 ml of 2% boric acid plus three drops of indicator (bromocresol green + phenolphthalein red). The distillation was continued until the volume in the flask was 75 ml, then the flask was removed from the distillator. The distillate was titrated with 0.1 N HCl until the end point (red Color) was obtained.

The protein content calculated from the following equation:

$$N (\%) = \frac{T * 0.20 * 20 * 0.014}{W} * 100$$

W

Protein content = N (%) × 6.38 Where:

T = titration figure

W = weight of the original sample Total Solids Content:

Principle:

Absolute solids content is the whole build-up left after complete vanishing of water from milk. This incorporates fat protein, lactose and mineral matter. These strong constituents exist in milk in a mechanical blend.

Procedure:

Three grams of samples (milk or yoghurt) were weighed into a dry clean flat-bottomed aluminium dish, and heated on a steam bath for 10-15 minutes. The dish was then placed in an oven at 70°C overnight, cooled in a desiccator and weighed quickly. Heating and weighing were repeated until the difference between the two successive weighing, was less than 0.1 mg. The total solids content was calculated from the following equation:

$$\text{Total solids (\%)} = \frac{W1}{W0} * 100$$

Where:

W1 = Weight of sample after drying

W0 = Weight of sample before drying

Principle:

The ash content is a proportion of the aggregate sum of minerals present inside a food, while the mineral content is a proportion of how much explicit inorganic parts present inside a food, like Ca, Na, K and Cl. Assurance of the debris and mineral substance of food sources is significant for various reasons:

Dietary naming. The focus and sort of minerals present should regularly be specified on the mark of a food. Quality. The nature of numerous food sources relies upon the fixation and sort of minerals they contain, including their taste, appearance, surface and strength. Microbiological soundness. High mineral substances are at times used to impede the development of specific microorganisms.

Sustenance. A few minerals are crucial for a solid eating regimen (e.g., calcium, phosphorous, potassium and sodium) while others can be poisonous (e.g., lead, mercury, cadmium and aluminium).

Handling. It is regularly essential to know the mineral substance of food sources during handling since these influences the physicochemical properties of food sources.

Debris is the inorganic build up staying after the water and natural matter have been taken out by warming within the sight of oxidizing specialists, which gives a proportion of the aggregate sum of minerals inside a food. Insightful strategies for giving data about the complete mineral substance depend on the way that the minerals can be recognized from the wide range of various parts inside a food in some quantifiable way. The most generally utilized techniques depend on the way that minerals are not obliterated by warming, and that they have a low unpredictability contrasted with other food components. The three fundamental kinds of logical methodology used to decide the debris content of food sources depend on this rule: dry ashing, wet ashing and low temperature plasma dry ashing. The technique picked for a specific examination relies upon the justification for doing the investigation, the kind of food broke down and the gear accessible. Ashing may likewise be utilized as the initial phase in planning tests for examination of explicit minerals, by nuclear spectroscopy or the different customary techniques depicted underneath. Debris substance of new food sources seldom surpasses 5%, albeit a few handled food sources can have debris substance as high as 12%.

Procedure:

Five grams of the sample were weighed into a suitable crucible and evaporated on a steam bath to dryness, then placed into a

muffle furnace at 550°C for 3 hr., cooled in a desiccator and weighed. The ash content was calculated as follows:

$$\text{Ash content (\%)} = \frac{W1}{W2} * 100$$

Where:

W1 = Weight of sample after drying
W0 = Weight of sample before drying

Analysis of Titrable Acidity:
Take 10 gm. curd/milk in jar. Add 5 ml distilled water and blend completely and add not many drops of phenolphthalein as end point indicator. Titrate against 0.1N NaOH till light pink end point. Note down the volume of 0.1N of NaOH required.

Calculation: % Acidity as lactic acid = 0.09 * volume of 0.1N NaOH

Sensory evaluation:
Yoghurt samples were subjected to sensory evaluation using 10 untrained panellists at 0, 3 and 6 day intervals. The test was done in a duplicate.

pH content:

Principle:

A pH meter is a logical instrument that actions the hydrogen-particle action in arrangements, showing its corrosiveness or basicity (alkalinity) communicated as pH esteem. The guideline of pH meter is the centralization of hydrogen particles in the arrangement for example it is the negative logarithm of a hydrogen particle. The pH scope of arrangements changes between 1 to 14, where 1 is the most elevated in acidic nature, and 14 is the most noteworthy in alkalinity.

Procedure:

- Ensure the temperature of the Liquid being examined to 200-250C.
- Immerse the glass electrode in the liquid to be examined.
- Turn off the knobs to pH Checking & note.
- When measuring the pH above 10, ensure that the electrode is suitable for use under alkaline conditions & apply any correction that is necessary.
- Record the pH of the solution used to standardize the meter and electrodes at the end of a set of measurements. If the difference between this reading and the original value is greater than 0.05, the set of measurements must be repeated.

Moisture content:

Principle:

Moisture content assurance exactness for substance tests recognizing a bigger mass volume will be pretty much as exact as the agent test quality. While picking material examples from a group, the area of test choice is significant. For reproducible experimental outcomes, a test should be really illustrative of the whole homogenous cluster being tried. For instance, a mass powder blending process should be totally mixed prior to removing a delegate test. In the mass blending process a typical condition happens where as a more prominent centralization of moisture exists away from the surface and edges of the material. A test assembled from the top won't be a genuine portrayal of the group.

Ideal example weight goal is significant for moisture content assurance while utilizing the thermo gravimetric drying

process. A test's weight and arrangement can impact the exactness and test process duration. To downplay test process duration, a little example weight ought to be utilized. Assuming that an example size is inordinate, a bigger volume of mass should be disintegrated diminishing the test execution and by and large exactness. Test loads are connected with repeatability. Similarly as with over the top example size, a too little example will impede repeatability results because of a moment for each centage centralization of moisture in the reference test.

The key for fruitful moisture assurance, effective usefulness, and prevalent item quality is quick test assurance without extensive stops underway cycles. Fruitful moisture investigation implies testing at the purpose in handling, and making changes before item honesty is affected.

Procedure:

- I. Dry the empty dish and lid in the oven at 105°C for 3 h and transfer to desiccator to cool. Weigh the empty dish and lid.
- II. Weigh about 3 g of sample to the dish. Spread the sample to the uniformity.
- III. Place the dish with sample in the oven. Dry for 3 h at 105°C.
- IV. After drying, transfer the dish with partially covered lid to the desiccator to cool.

Reweigh the dish and its dried sample Calculation

$$\text{Moisture (\%)} = \frac{(M1-M2)}{CC (M1-M)} \times 100$$

Where;

M = mass in g of empty dish

M1 = Initial mass in g of dish + material taken for analysis

M2 = Final mass in g of dish after drying

Microbial investigation:

D. For specification of *Lactobacillus*:

Plan sequential weakening of curd (10^{-1} to 10^{-7}). Mark the reasonable weakening (OK) on MRS agar plate. Brood at 37°C for 24 hrs. After brooding notice province ascribes and play out gram's staining. Count the settlement and work out CFU. B. For specification of Yeast and Mold:

Plan sequential weakening of curd (10^{-1} to 10^{-2}). Mark the legitimate weakening (OK) on PDA plate (pH 3.5). Brood at 28°C for 48 hrs. After brooding notice province ascribes and play out gram's staining. Count the settlement and work out CFU.

III. RESULTS

A. Microscopic Examination

1) Gram's staining

By performing gram's staining, purple colored, short rod gram positive bacteria were observed against colorless background.

2) Endospore Staining:

There was no trace of spore observed by performing Endospore staining (Schaeffer and Fulton's method).

3) Motility Test:

By hanging drop method there was no bacterial motility observed under microscope which clearly shows the isolated bacteria were non-motile.

B. Microbial Analysis of Curd

For the microbial examination of curd, the example was plated on various agar mechanism for specification of coliforms, yeast cells and lactic corrosive microorganisms. We utilize distinctive agar plate for the development of microorganisms and count the settlement. The CFU include of every life form displayed in the table 18. No sign of settlement found on MacConkey's agar shows there was no pollution of coliforms; no province on PDA demonstrated that there was no defilement of yeast cells. What's more province are seen on MRS plate demonstrates presence of lactic corrosive microorganisms.

Table 3: Microbial analysis of curd

Agar media	Cow	Buffalo	Goat	Coconut
MRS	1.8×10^6	2.5×10^6	1.5×10^6	2×10^6
Mac Conkey's	0	3×10^5	1×10^5	0
PDA	3×10^5	6×10^5	4×10^5	1×10^5

C. % acidity of curd:

By using titrimetric method, the % acidity of different curd was measured after every 30 min time intervals. The acidity increased gradually which is mentioned in the below table. (goat milk/soy milk).

Table 4: % acidity of curd

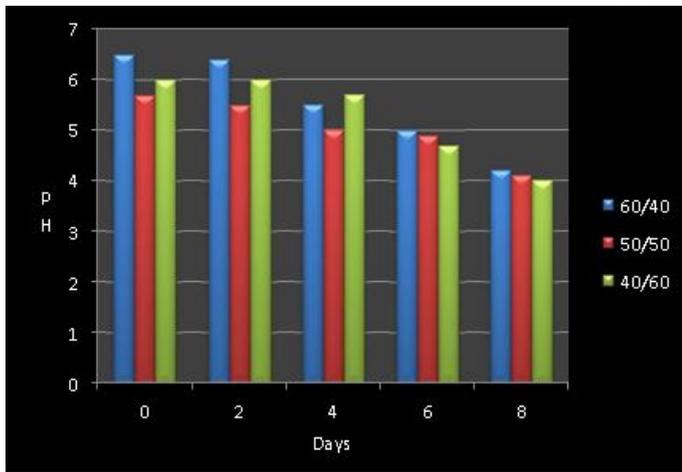
Time (Min)	Control	60/40	50/50	40/60
0	0.17	0.16	0.17	0.22
30	0.24	0.18	0.23	0.28
60	0.25	0.21	0.27	0.33
90	0.31	0.23	0.33	0.38
120	0.45	0.36	0.33	0.58
150	0.61	0.44	0.36	0.66
180	0.73	0.57	0.51	0.73

pH content:

By performing pH test at the gap of 2 days following results were obtained:

Table 5: pH content of prepared curd

Days	60/40	50/50	40/60
0 day	6.5	6.1	6
2 days	6.4	5.9	6
4 days	5.5	5.5	5.7
6 days	4.8	4.9	4.7
8 days	4.2	4.1	4



Graph: pH content of prepared curd Biochemical tests:

By performing various biochemical tests following results were obtained:

Table: Results of biochemical tests

Composition (%)	Control	40/60	50/50	60/40
Moisture	85%	77 ± 1 %	88 ± 1 %	86 ± 0.5 %
Ash	1 ± 0.2 %	1.16 ± 0.9 %	1.6 ± 0.8 %	2.04 ± 0.10
Fat	5.8 ± 0.2 %	5.23 ± 0.010%	5.64 ± 0.2 %	6.04 ± 0.4
Protein	12.7 ± 0.3 %	11.36 ± 0.4 %	11.88 ± 0.20 %	12.4 ± 0.6
TSS	32.6 ± 0.57 %	29.4 ± 0.90 %	30.26 ± 0.40 %	35.8 ± 0.77

Vitamin B-12 Composition:

(Goat milk/soy milk)

Composition	Average need	40/60	50/50	60/40
Vitamin B-12	2.4 µg	9.6 ± 1.4 µg	12 ± 1 µg	14.1 ± 1.6 µg

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