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Procreation and quality analysis of soy & soy-cow curds by using starter culture as fermented rice rinsed water

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Abstract
Lactic acid bacteria are a diverse group of bacteria that produce lactic acid as their major fermentation product. LAB is widespread in nature and is beneficial probiotics in our digestive systems. Soya bean curd contains high quality protein that can be easily digested and cholesterol free. The production of soya curd and soy-cow curd by fermentation of soya milk and cow's milk with lactobacillus plantaram which isolated from rice rinsed water was studied. The efficacy of using rice rinsed water of 10%, 15%, 20%, 25% of prepared LAB solution is added to soya milk (S1, S2, S3, S4)as a starter culture. Fermentation of soy-cow milk done by using 15%of LAB solution which is noted as SC1, C2, SC3 show imparted beneficial effects of sensory, physiochemical, nutrient and microbial properties of curd. The overall acceptability was greatest for S1 in soy curd and SC3 in soy-cow curd. Higher crude protein content (4g), low fat (3.8g) and low level of lactose (0.4g) were recorded for soy-cow curd of (50:50).So this type of curd is recommended for weight watcher and lactose intolerance persons.

Keywords: lactic acid bacteria, cow's milk, soya milk, fermentation, starter culture, lactose intolerance

1. Introduction
LAB is used as natural or selected starters in food fermentations in which they perform acidification due to production of lactic and acetic acids flavour. Protection of food from spoilage and pathogenic microorganisms by LAB is through producing organic acids, hydrogen peroxide, and diacethyl, antifungal compounds such as fatty acids or phenullactic acid and/or bacteriocins (Vugst, 1994). Probiotics are defined as "Living organisms which upon ingestion in certain number exert health benefits beyond inherent basic nutrition". LAB are useful for human being and animals in many aspects these include, prevention of diarrhoea, effects in lactose intolerance, treating ulcer, stimulation of immunity both at intestinal and systemic level, food preservation effects, antifungal activity, role in infectious diseases prevention, role in allergy, effects on the incidences of colon cancer and slow the progression of cancer, and produce many valuable dairy products (Masood, 2011) [6]. Soybean is a rich source of protein and economical protein food. Soy-based foods may have some health benefits to consumers due to their hypolipidemic, anticholesterolemic and antiatherogenic properties as well as due to reduced allergen city. It also contains isoflavones, which can reduce the risk of most hormone-associated health disorders. Soymilk obtained by aqueous extraction from whole soybean, is a well-known food product that is growing in popularity in many areas of the world (Ghorbania, 2012) [3]. Soy curd has many nutritional benefits including reducing cardiovascular disease, reducing menopausal symptoms, weight loss, arthritis and brain function. It contains phytochemicals such as isoflavones, saponins, phytosterols that promote health. Addition of probiotic culture enhances functional attribute of soy curd (Velasquez *et al.*, 2007) [8]. Yogurt is considered as healthy food due to its high digestibility and bioavailability of nutrients and also can be recommended to the people with lactose intolerance, gastrointestinal disorders such as inflammatory bowel disease and irritable bowel disease, and aids in immune function and weight control. Because of these health benefits associated with yogurt consumption, there is an increasing trend for yogurt and is the fastest growing dairy category in the market in particular, standard yogurt and yogurt drinks (Mckinely, 2005). Global interest on rice and its fermented product is increasing due to their caloric value, unique quality, and high acceptability. Rice is a good source of carbohydrates (77–89 percentage) and energy (1460–1560 KJ).It also provides a moderate amount of protein (6.3–7.1 percentage), though it is devoid of lysine There are many popular rice fermentation procedures used to make it more nutritious (i.e., enrichment with essential amino acids and removal of phytic acid, a major anti-nutrient in rice), easily digestible (as microbial enzymes predigest it), and acquire therapeutic properties (antimicrobial peptides, antioxidants, etc.) and symbiotic properties (Steinkraus 2002) [7].

2. Materials and Methods
2.1 Preparation of Rice Water
Take 150 gm of rice and 400ml of drinking water in a vessel. Soak the rice in this measured quantity of the water in a bowl. Kept it for half an hour. After half an hour take filter the rice rinsed water and rice water separately. Make sure that there is no rice in this collected rinsed water.

2.2 Preparation of Lab Solution
Fill a clean glass jar about two by third full with rice rinsed water. Cover the mouth of the jar with breathable cloth (such as muslin) or paper (not plastic) and secure with rubber bands or ties to keep out pests. Store at room temperature away from direct light. Be careful not to shake

or move the jar while it ferments. After 5 days, LAB will multiply and give off a slightly sour odour. There will be a mat of semi-solid material floating on the top of the cloudy liquid in the jar. Collect only the cloudy liquid (fermented rinse- water) by pouring off and discarding the mat layer. Measure one part of fermented rinse-water and add 10 parts of milk to fill your jar 2/3 full. As in the next step, cover the mouth of the jar with cloth or paper and secure with rubber bands or ties to keep out pests. Store at room temperature away from direct light. Be careful not to shake or move the jar while it ferments. After 2 to 3 days, the contents of the jar will separate into a floating solid fraction and a yellow liquid fraction. It may take longer in cooler climates. The yellow liquid is the LAB culture. Pour off the liquid fraction, being careful not to mix any solids back into the LAB culture. The preparation of LAB solution procedure was presented in PLATE I.

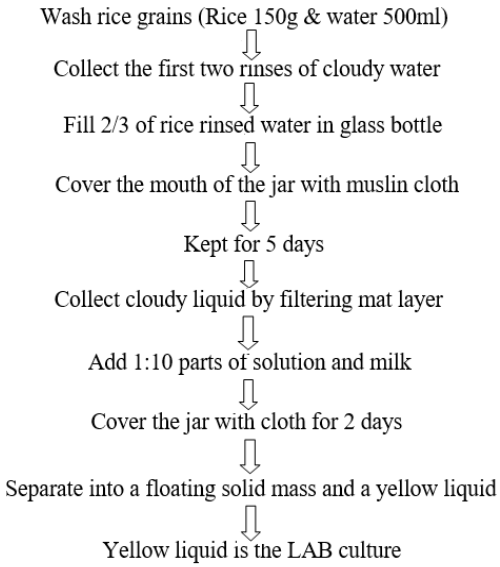

Plate 1: Flow Chart for Preparation of Lab Solution

2.3 Preparation of Soya Milk from Soyabean

Soybean was cleaned and soaked for 24hours overnight. Remove the outer skin completely from the soya then soybean was finely grinded by adding little amount of water. Filter the milk from soya bean twice with muslin cloth. The filtered milk was kept it for boiling and then cooled to 40ºc.

2.4 Preparation of Fermented Soy Milk

Soya bean was used to extract the soymilk and it is boiled at 100ºc,keep the milk to room temperature about 40º.To that add four different quantity of LAB solution as the starter culture as S1,S2,S3,S4 variations of soy milk respectively. Then kept it for overnight for normal fermentation of soy milk to curd.

2.5 Preparation of Fermented Cow's Milk

Pure cow's milk is selected for the preparation curd. Cow's milk is boiled at the 100ºc.after that kept it in room temperature till it reaches 40ºc. Then kept it for overnight for normal fermentation of cow's milk to curd at room temperature.

2.6 Preparation of Soy-Cow by Using Soy-Cow Milk

Combination of soymilk and cow's milk is mixed properly in the three different combinations of SC1, SC2, and SC3. In this soy-cow curd was prepared by mixing of soy milk and cow's milk in ratio of 25:75, 75:25, 50:50 respectively. To this various mixtures of soy milk and cow milk were inoculated with 15% of LAB solution (fermented rice rinsed water) as a starter culture. Then kept it for normal fermentation to formulate the curds. The procedure for preparation of preparation of soya curd from LAB solution was presented in PLATE II.

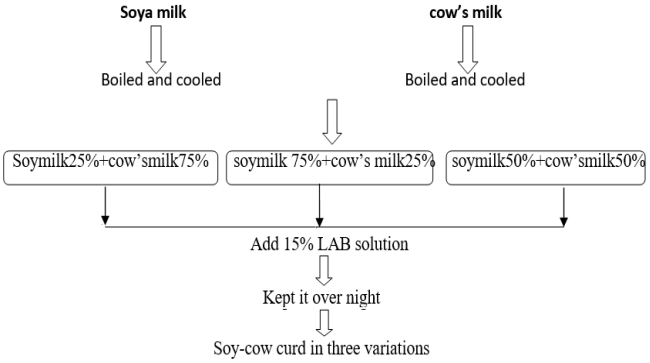

Plate 2: Soy-cow curd from fermented rice rinsed water

2.7 Organoleptic Evaluation of Fermented Curds

The evaluation of sensory attributes like colour, flavour, taste, texture and overall acceptability was done using the score card. The soy and soy-curd variations which is formulated were evaluated using organoleptic evaluation by 25 semi trained panel members. The most desired character to the particular quality carried the maximum score 5 and least desired character minimum score of 1. The five point of hedonic rating scale was use sensory evaluation.

The highly acceptable cow and soy curd be prepared by using 20%, 10% of LAB solution (fermented rice rinsed water) respectively. So that average of these two percentage 15% was used for the preparation of soy-cow curd combination.

2.8 Physio-Chemical Analysis of Selected Curds from Different Milks

The physiochemical characteristics such as moisture, Titrable acidity, Ph were analysed initially in the selected curds that formulated. All the works are done in the Food Science Laboratory of Dr. N.G.P Arts and Science College, Coimbatore. All the tests are done according to the AOAC method

2.9 Nutrient Analysis of Selected Curds from Different Milk

The nutrients like energy, protein, fat, fiber, calcium content were analysed initially by using standard procedure. All the works are done in Dr. N.G.P Arts and Science College. All the tests are done according to the AOAC method

2.10 Microbial Analysis of Selected Curds from Different Milk

The Total bacterial count, yeast mould count, bacteria type, lactic acid bacteria growth, MALDI TOFF test all these was

analysed in microbiology laboratory of Dr. N.G.P Arts and Science college, Coimbatore.

2.11 Statistical Analysis of Selected Curds Prepared from Fermented Rice Rinsed Water.

The data of each parameter were statistically analysed. The result obtained was tabulated and statically tools like mean and standard deviation were used to interpret the results.

3. Results and Discussion

3.1.2 Sensory Evaluation of Formulated Soy Curds Prepared by Using Lab Solution

The table I revealed that attributes such as colour, flavour, texture, taste and overall acceptability were evaluated by panel list. Soy curd variation S1 was rated high in terms of colour (4.04), flavour (3.72), taste (3.68), texture (4.12) and overall acceptability (4.08). Variation S1, S3, S4 shows low in terms of flavour due to the beany flavour associated with soy beans. There be observed difference in terms of overall acceptability of variation S1 among other soy curds. Other show least range acceptability in soy curds. It is worth noting that the protein and no cholesterol and small level of saturated fatty acid found in soy milk will help in boosting immune system and also useful for people who are lactose intolerant or allergic to animal milk.

Table 1: Sensory Evaluation of Formulated Soy Curds Prepared By Using Lab Solution

Criteria	S1 (10%)	S2 (15%)	S3 (20%)	S4 (25%)
Colour	4.04± 0.84	2.80±0.81	2.48±0.50	2.2±1.9
Flavour	3.72±1.54	2.96±0.84	2.92±0.94	2.68±1.06
Texture	4.12±1.20	3.01±0.86	3.12±1.16	2.40±1.19
Taste	3.68±1.28	2.24±1.22	2.08±1.28	1.52±0.71
Overall acceptability	4.08±0.86	2.84±0.85	2.22±1.11	2.12±0.72

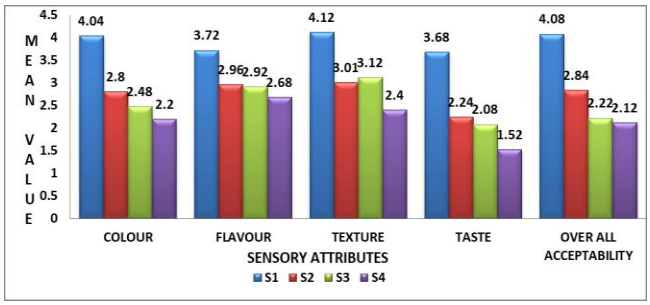

Plate 3: Over All Acceptability of Selected Soy Curd

3.1.3 Sensory Evaluation of Formulated Soy-Cow Curds Prepared by Using Lab Solution

The table II showed that the panellist tasted and rated the variations on the 5 point hedonic scale on their degree of liking for the 3 variations of curds presented. The overall acceptance was determined by the assessment of colour, taste, texture, flavour and overall acceptability. Most of the panellist accepted variation SC3 by giving very good point 4.04 giving the least point 2.84 as fair. Thus there seem to be a slight preference for the 50:50combination of soy milk and cow' milk equal proportion for curd preparation in all the sensory quality attributes.

Addition of soy milk to cow's milk in 50:50 combination curd SC3 as in the mean score of 4.04. However, data emanating from the present study depicts that soy curd could be helpful in meeting a significant portion of daily needs of these nutrients.

Table 2: Sensory Evaluation of Formulated Soy-Cow Curds Prepared by Using Lab Solution

Criteria	SC1(25:75)	SC2(75:25)	SC3(50:50)
Colour	3.60±1.00	3.28±0.73	3.60±0.91
Flavour	3.68±1.31	2.28±1.02	4.28±1.30
Texture	4.40±0.76	3.08±0.70	3.40±1.22
Taste	4.52±0.77	2.24±0.66	4.56±0.76
Overall acceptable	3.64±0.90	2.84±0.85	4.04±0.78

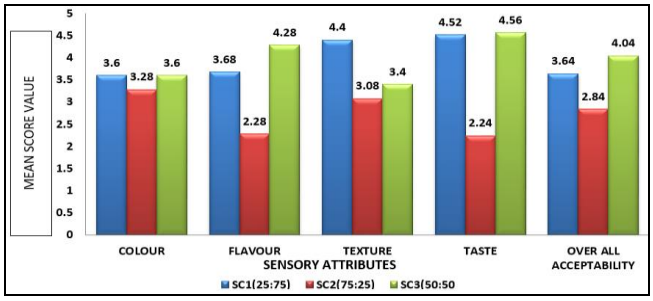

Plate 4: Overall Acceptability of Selected Soy-Cow Curd

3.2 Physio-Chemical Analysis of Selected Soy, Soy-Cow Curds Inoculated by Lab Solution

The table III presents the value of physiochemical parameters in the selected, soy-cow curd and soy curd samples. The kid of probiotic culture and incubation temperature, time period significantly affects physicochemical characteristics of these samples. Ph, tritable acidity and moisture were measured in selected curds. Soy curd pH indicates acidity. It was observed that soy–cow curd (4.9). By the fermentation effect of lactic acid bacteria that converted the lactose in the soy milk into lactic acid. The higher acidity of the soy curd makes it a poor breeding site for pathogenic microorganism.

Titrable acidity followed an increasing trend with increased composition of cow's milk for curd preparation. Titrable acidity was seen in soy curd 0.74%. There was significant difference in the level of moisture content in soy, soy-cow curd. Soy, soy-cow shows about 94.2%, 91.2 respectively. Higher level of moisture content in the curd says that there is low shelf life and easily detoriated because of the microbial growth.

Table 3: Physio-Chemical Analysis of Selected Soy, Soy-Cow Curds Inoculated by Lab Solution in 100 G

Parameters	S1 (10%)	SC3(50:50)
Ph	5.1	4.9
Moisture (%)	94.2	91.2
Tritable acidy (%)	0.74	1.26

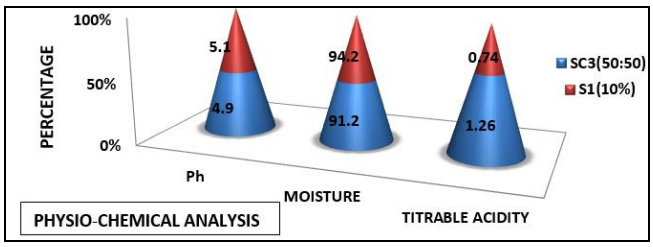

Plate 5: Physio-Chemical Analysis of Selected Soy, Soy-Cow Curds Inoculated by Lab Solution

3.3 Nutrient Analysis of Selected Soy, Soy-Cow Curds Inoculated by Lab Solution

Table IV shows that the nutrient analysis of the selected two

types of curds which are inoculated with fermented rice rinsed water as the starter solution. While the higher crude protein in soy curd showed about 4.0g. Because of it contain good nutritional quality and a superior protein efficiency ratio, which shows a potential source of low cost vegetable protein for human consumption. It is interesting to note that the fat content of soy curd (3.8g) show much lower than that of soy-cow curd of about 4.6g.The fat obtained by Shuhong (2013) study got the same of about 3.6g in soy curd. So that soy curd can decreases triglyceride, total cholesterol and LDL cholesterol level in the human.

The total fiber content was higher in soy curd (2g) compared to soy- cow curd (1.4g). So it is clear that soy curd is a good dietary fiber which can reduce blood fat, blood pressure, lower the level of cholesterol in blood by that ion. It prevents coronary heart diseases and constipation. It also regulates diabetic's blood sugar level by high content of fiber in soy curd. The mineral content calcium was higher in soy curd about 125 mg. Soy-cow curd estimated about 110mg. So this soy curd can be recommended for the lactating, pregnant mother. And also for the person suffering from osteoporosis. There was significant difference in the level of lactose content in two selected curds that inoculated with fermented rice rinsed water. In this study soy curd show very least level of lactose content about 0.4g.so that it is a suitable economical substitute for cow curd and an ideal nutritional supplement for lactose-intolerant population.

Table 4: Nutrient Analysis of Selected Soy, Soy-Cow Curds Inoculated by Lab Solution in 100g

Parameters	S1 (10%)	SC3(50:50)
Protein (gm)	4.0	3.1
Fat (gm)	3.8	4.6
Fiber (gm)	2.0	1.4
Calcium (mg)	125	110
Lactose (gm)	0.4	3.2

3.4 Microbial Analysis of the Selected Soy, Soy-Cow Curds Inoculated by Lab Solution

3.4.1 Total Plate Count

In total plate count here we examined the total bacterial count, total yeast mould count in the selected curds from soy-cow, and soy curds which are inoculated with fermented rice rinsed water as the starter culture. The results are shown in the TABLE V.

Table 5: Total Plate Count

Selected Curd	Total Bacterial Count	Total Yeast Count	Total Mould Count
SC3	14 x 10^{-7} cfu/g	68 x 10^{-5}cfu/g	68 x 10^{-5}cfu/g
S1	34 x 10^{-7}cfu/g	35 x 10^{-5}cfu/g	35 x 10^{-5}cfu/g

Table V shows the counts of probiotic bacteria of the samples. The highest Count of probiotic bacteria was observed in the soy curd of about 34x10^{-7} cfu/g and incubated at 37ºc overnight. And results demonstrated that the soy-cow curd have almost same lower count of 14x10^{-7}cfu/g respectively. From the above table that yeast count and mould count found to be same soy curd show the least yeast and mould count of about 35x10^{-5}cfu/g. And the highest yeast and mould count be noticed in the soy-cow curd of 68 x 10^{-5}cfu/g.

3.4.2 Morphological Identification of Bacteria

The nine genera of bacteria were isolated from the samples. Three of bacteria are isolated from each selected curd of soy, soy-cow curd. And it is kept it for 37ºc for one day. The bacteria were isolated on nutrient agar and examined for colonial and morphological characterization.

Table 6: Morphological Identification of Bacteria

Type of Curds	Isolates	Size	Shape	Colour	Microcopy Identification
Soy Curd	Soy 10^{-5}	Large	Round	Golden yellow	Bacilli
	Soy 10^{-6}	Large	Round	White	Bacilli
	Soy 10^{-7}	Large	Round	Golden yellow	Bacilli
Soy-Cow Curd	Soy-cow10^{-5}	Large	Round	Pink	Bacilli
	Soy-cow10^{-6}	Large	Round	White	Bacilli
	Soy-cow10^{-7}	Large	Round	Pink	Bacilli

Table VI shows that results of morphological identification of nine genera that isolated from the selected curd samples. And it is kept it for 37ºc for one day. The nine genera were lactobacillus. The morphological characterization indicates that all the isolates were round and large in size. It also showed that nine genera of isolates are rod like bacteria, which means bacillus bacteria.

3.4.3 Identification of Bacterial Type by Using Gram Staining

The bacterial type of LAB isolated from naturally fermenting soy-cow curd and soy curd were tabulated in TABLE VII. It showed the bacteria isolated and their probable identity. By using gram staining method, all the curd isolates were gram positive was identified clearly.

Table 7: Identification of Bacterial Type by Using Gram Staining

Selected Curds	Gram Reaction
SC3	Gram +ve
S1	Gram + ve

4. Conclusion

This study revealed that a combination of soy milk and Cow milk (50:50) could be used for the preparation of high protein, less cholesterol and small level saturated fatty acid in this curd. Soy curd will help in boosting immune system and also useful for people who are lactose intolerant or allergic to animal curd. There who are avoiding the soy curd because of its flavour can be recommended by this combination curd of soy cow's milk without any nutritional changes and it is a perfect alternative food than the usual cow curd. The formulated Fermented rice rinsed water (LAB solution) is rich in CHO, antioxidants, minerals, B vitamins, vitamin E, and trace amount of pitera. It helps to improve blood circulation, prevent age related spots, inflammation in skin, hair protection and beautifying effect. The formulated fermented rice rinsed water which have 6 months of shelf life, so that this LAB solution can be used as a starter culture for curd preparation were curd is not easily available or for fermentation. This method of preparing LAB culture from rice grain be used for commercial purpose instead of using gelling agents for

making commercial curds it will gave more jelly like curd without any food additives and side effect.

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