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Formulation and Evaluation of Probiotic Health Drink by using Carrot and Beetroot

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ABSTRACT

The purpose of this study was to develop a non-dairy probiotic beverage for persons who cannot consume dairy products owing to lactose sensitivity, dietary restrictions like vegetarianism, or other health conditions. The suitability of carrot juice and beetroot juice for the preparation of probiotic food with Lactobacillus acidophilus was explored in the search for an alternative probiotic carrier. Comparing the approximate composition of probiotic juice to that of fresh carrot and beetroot juice, it was found to contain 26% more protein and 17% fewer carbohydrates. The CCRD, RSM and statistical analysis determined that 6.5 and 37°C, respectively, were the ideal pH and fermentation temperature for the manufacture of probiotic carrot and beet root juice. The results of this study's data have revealed fresh information about the potential of fermented carrot-beet juice as a medium for the development of probiotics.

Keywords: Probiotic, Lactobacillus acidophilus, Culture media, CBL-Carrot and Beetroot Liquid.

INTRODUCTION

Probiotics are defined as “live microorganisms which when administered in adequate amount confer health benefits to the host” (FAO/WHO, 2002). Alternatively, probiotics have been defined as live microbial feed supplements that beneficially affect the host animal by improving its intestinal microbial balance (Fuller, 1989).

Probiotics were originally used to improve of both animals and humans through the modulation of the health of both animals and humans through the modulation of the intestinal microbiota. At present, several well-characterized strains of lactobacilli and bifidobacterial are available for human use to reduce the risk of gastrointestinal (GI) infections or treat such infections (Salminen et al., 2005).

However, the use of probiotics should be further investigated for their benefits and possible side effects, if any. As the knowledge about intestinal microbiota, nutrition, immunity, and genetics in health and disease has increased in the past years, such information could certainly help to develop new probiotic strains with disease-specific functions and could also facilitate the understanding of when to use probiotics and how they affect specific pathological states.

However, it is important that the probiotic strains for human use should undergo animal studies followed by human clinical trials in order to authenticate the suitability, safety, and benefits of probiotics for human consumption and development of functional foods.

Supplement of probiotics

A probiotic supplement can be consumed in a variety of ways. They can be found in many different forms, such as:

- Foods
- Drinks
- Tablets and capsules
- Powders
- Liquids

Supplements for probiotics and prebiotics can be taken together. Complex carbohydrates known as prebiotics provide food for the bacteria in your stomach. Prebiotics are

essentially the "food source" for the healthy bacteria. They assist in maintaining its health by providing food for the beneficial bacteria. Pectin, resistant starches, and inulin are examples of prebiotics. Symbiotics are nutritional supplements that contain both probiotics and prebiotics.

Health benefits

Health benefits of carrot

- Reduced risk of cancer
- Lower blood cholesterol
- Weight loss
- Improve eye health
- Reduced age-related macular degeneration

Health benefits of beetroot

- Lower blood pressure
- Increased exercise capacity
- Reduced kidney

Nutritional fact

The nutrition values are normal carrot and beetroot contained. therefore, that value are differs various type of soil produced the carrot and beetroot.

NUTRITION CONTENT	CARROT	BEETROOT
Calories	41cal	43cal
Water	88%	88%
Protein	0.9 gm	1.6 gm
Carbohydrate	9.6 gm	9.6 gm
Sugar	4.7 gm	6.8 gm
Fibre	2.8 gm	2.8 gm
Fat	0.2 gm	0.2 gm
Vitamins	Vitamin A, Vitamin B6, Vitamin K1	Vitamin B9, Vitamin C
Minerals	Potassium	Manganese, potassium, iron
Other compound	Carotenoids, Beta carotene	Betanin, inorganic nitrate

MATERIAL AND METHOD

MATERIAL

Raw material (carrot and beetroot) and water, Tryptone, NaCl, Yeast extract and agar powder, autoclave and Erlenmeyer flask, fermenter, UV laminar flow, incubator, filter paper and for juice purpose by using mixer grinder.

METHOD

Preparation of juice

The freshly taken carrot and beetroot was clean and cut into small pieces. Each 650 grams of carrot and beetroot was weighed accurately. Next grind gently to collect the juice and filter to make 1000 ml. The juice was autoclaved at 15 lbs at 121℃ for 15 minutes.

Preparation of sample

The 1g of test sample (CBL) was taken using spread plate method. The 10^7 dilution was used to bacterial isolation.

Preparation LB Agar Medium

The medium was prepared by dissolving 1gm of Tryptone, 1gm of NaCl, 0.5gm of Yeast extract and 1.75 gm of agar powder of commercially available LB Agar Medium in 100ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. Then dissolved medium was kept at UV laminar flow for 10 minutes.

Inoculum of culture

In a laminar flow chamber, transfer approximately 1 ml of Lactobacillus acidophilus culture to the flask containing LB Agar medium. The culture was incubated at 37°C for 72 hours.

RESULTS AND DISCUSSION

Evalution

Physical evalution

Colour: Cherry red
Odour: Geosmin gives of a smell like freshly plowed earth
Taste: Earthy and mild bitter
pH: 4.56

Microbial content

LB Agar Medium

The medium was prepared by dissolving 1gm of Tryptone, 1gm of NaCl, 0.5gm of Yeast extract and 1.75 gm of the commercially available LB Agar medium in 100ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petri plates (25-30ml/plate) while still molten. The 10^7 dilution was plated on the LB agar medium by spread plate method and the plate was incubated at 37ºC for 24 hrs. After incubation, bacterial colonies were isolated and plated in to a fresh plate.

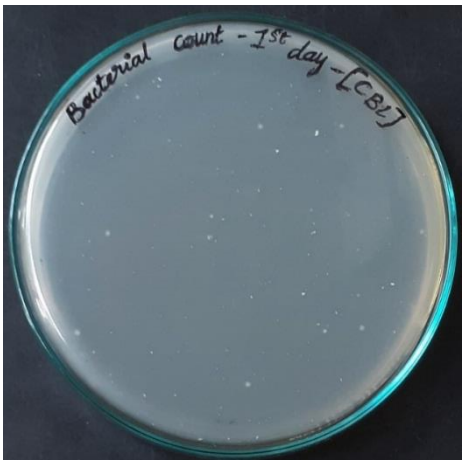


Fig 1: Enumeration of microorganism from Test sample

CFU (Colony forming unit)

The given sample (o.1ml-CBL) was taken using spread plate method. The number of colonies forming units (CFU) was determined for test samples by spread plate method using LB Agar cultured overnight at 37 °C in a bacteriological incubator.

$$\text{CFU/ml} = \frac{(\text{No. of colonies} \times \text{Total dilution factor})}{\text{Volume of culture plated in ml}}$$

$$= \frac{25\times10^{7}}{0.1} = 2.5 \times10^{9}\ \text{CFU/ml}$$

The Lactobacillus acidophilus was present in the extract sample (CBL) was found to be 2.5 $\times10^{9}$ CFU/ml.

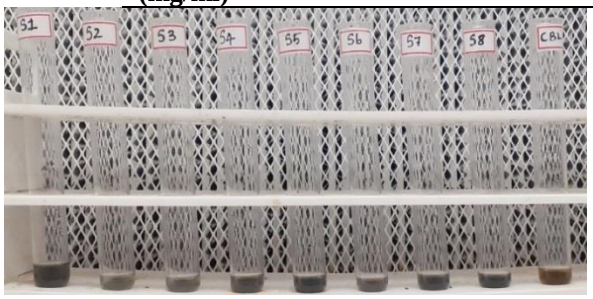
Nutritional content

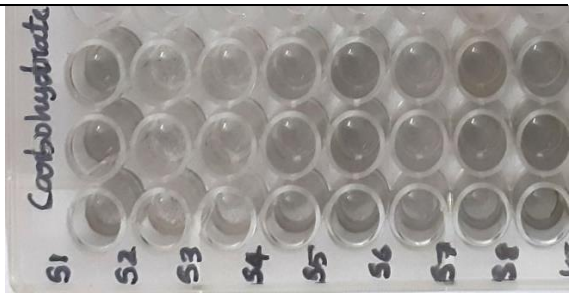
Carbohydrate

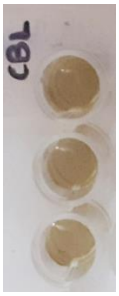
Pipette out into a series of test tubes different volumes of glucose solution from the supplied stock solution (50 mg/ml) and make up the volume to 1 ml with distilled water. To take 100 µl of test sample mix with 200 µl of 75% H_2SO_4. To each tube add 400 µl of the anthrone reagent (supplied) and mix well by vortex and cool the tubes. Cover the tubes with Caps on top and incubate at 90° C for 17 minutes or boiling water bath for 10 minutes. Cool to room temperature and measure the optical density at 620 nm against a blank. Prepare a standard curve of absorbance vs glucose.

Standard

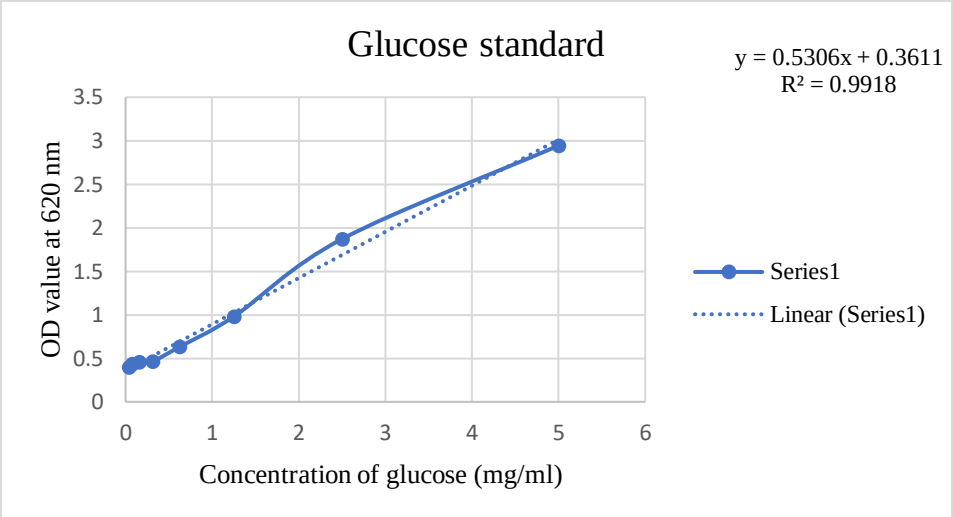
Standards	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**
Dist.H2O (µl)	900	500	500	500	500	500	500	500
Serial dilution of Glucose (µl)	100 from the stock	500	500	500	500	500	500	500
Conc. of standards (mg/ml)	5	2.5	1.25	0.625	0.312	0.156	0.078	0.039







Glucose mg/ml	OD at 620 nm (in triplicates)			Mean value
5	2.996	2.928	2.91	2.944
2.5	1.853	1.875	1.885	1.871
1.25	0.999	0.989	0.954	0.98
0.625	0.645	0.644	0.61	0.633
0.312	0.471	0.456	0.472	0.466
0.156	0.469	0.454	0.442	0.455
0.078	0.444	0.429	0.42	0.431
0.039	0.404	0.388	0.392	0.394



Name of the sample	OD value at 620 nm	Total Carbohydrate content	Mean value of total Carbohydrate content (gm)
CBL	0.524	0.30701	0.3955899
	0.639	0.52375	
	0.55	0.35601	

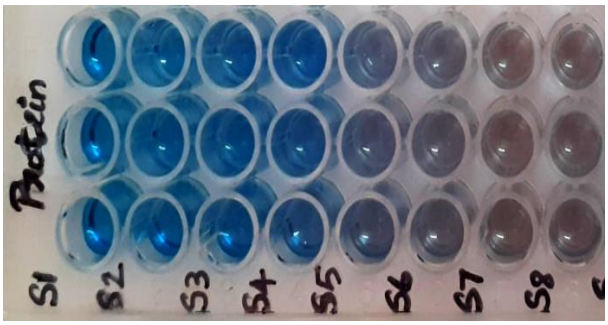
The total Carbohydrate content was present in the extract sample (CBL) was found to be 0.39 gm.

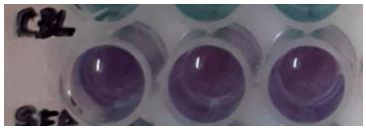
Protein

Added 10 µl of each standard solution and test samples to the Elisa titter plate. Then, 100µl of Bradford's reagent was added to the standards and test samples. All the samples and standard were done in triplicates to avoid any error. The plate was incubated for a minimum of 10 minutes at dark. The absorbance was measured at 595nm in a microplate reader. From this the value of unknown concentration is found out.

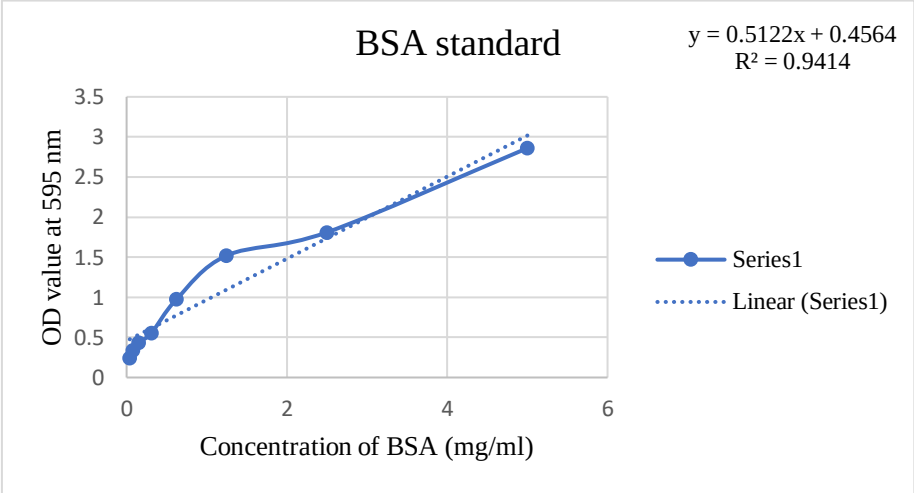
Standard preparation

Standards	S1	S2	S3	S4	S5	S6	S7	S8
Dist.H2O (µl)	900	500	500	500	500	500	500	500
Serial dilution of BSA (µl)	100 from the stock	500	500	500	500	500	500	500
Conc. Of standards (mg/ml)	5	2.5	1.25	0.625	0.312	0.156	0.078	0.039





BSA mg/ml	OD at 595 nm (in triplicates)			Mean value
5	2.842	2.825	2.914	2.86
2.5	1.814	1.814	1.806	1.811
1.25	1.523	1.542	1.501	1.522
0.625	0.982	0.972	0.981	0.978
0.312	0.571	0.518	0.582	0.557
0.156	0.442	0.451	0.417	0.436
0.078	0.321	0.366	0.338	0.341
0.039	0.273	0.241	0.231	0.248



Name of the sample	OD value at 595 nm	Total protein content	Mean value of total protein Content (gm)
CBL	0.499	0.08317	0.04959
	0.423	0	
	0.49	0.0656	

The total protein content was present in the extract sample (CBL) was found to be 0.049 gm.

Fibre

The material was ground and 1 g of it is extracted with petroleum ether to remove fat (initial boiling temperature 35-38°C and final temperature 52°C) and dried at 80qC to constant weight.

If fat content is below 1%, extraction is not required. Dried material (2 g) was boiled with 200 ml of 1.25% (w/v) sulphuric acid for 30 min with bumping chips, filtered through muslin cloth and washed with boiling water until washings are no longer acidic. Then, the material was boiled with 200 ml of 1.25% (w/v) sodium hydroxide solution for 30 min, filtered through muslin cloth again and washed with 25 mL of boiling 1.25% (w/v) H2SO4, 50 ml x 3 portions of water and 25 ml alcohol.

The residue was removed and transferred to ash dish (pre weighed dish W1). Again, the residue was dried for 2 h at 130±2°C. Then, the dish was cooled in a desiccator and weighed (W2), ignited for 30 min at 600±15°C. Finally, the material was cooled in a desiccator and reweighed (W3).

$$\text{Crude fibre} = \frac{(W2-W1)\,(W3-W1)}{W}$$

$$= \frac{(1.19-0.11)\,(0.21-0.11)}{2.5} = 0.04\text{gm}$$

Where,
W is the mass of sample.
The total Crude fibre content was present the extract sample (CBL) was found to be 0.04gm.

LIPID

Preparation of standard solution

Prepare the solvent, chloroform: methanol is 2:1. Mix cholesterol in solvent at predetermined concentration, for instance 5mg/ml or 10 mg/ml. Vary volume of the standard sample to assign different amount of cholesterol in different tubes.

Standard preparation

Standards	S1	S2	S3	S4	S5	S6	S7	S8
Chloroform and Methanol (2:1) (µl)	-	500	500	500	500	500	500	500
Serial dilution of cholesterol (µl)	1000 from the stock	500	500	500	500	500	500	500
Conc. of standards (Mg/ml)	10	5	2.5	1.25	0.625	0.312	0.156	0.078

Prepare the samples

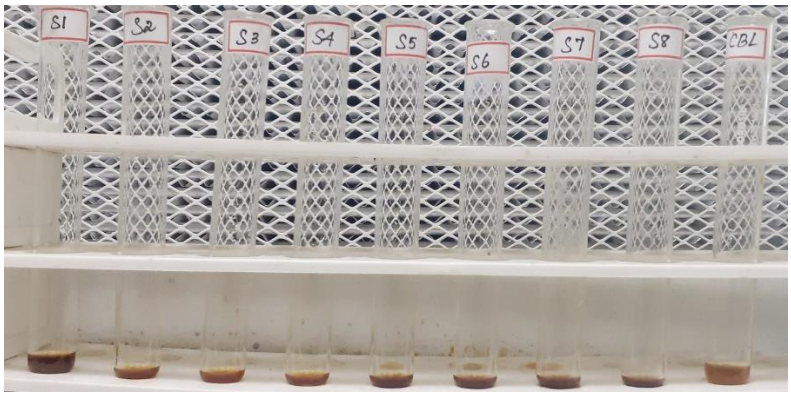
Dissolve the samples in water at a predetermined concentration. Vary the volume of mucins to assign different amounts of samples in different tubes.

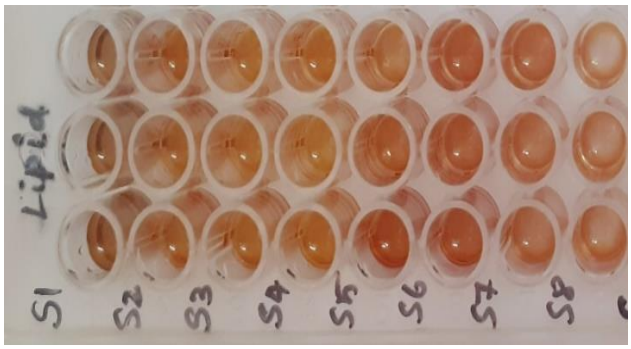
Measure background absorbance

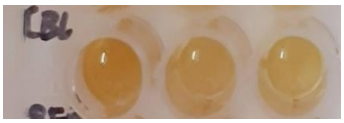
Add 100 µl concentrated sulfuric acid into each tube and incubating at 90 C for 10 min (on a dry heating bath). Cooling to room temperature and measuring background absorbance at 540nm.

Measure the absorbance after colour development

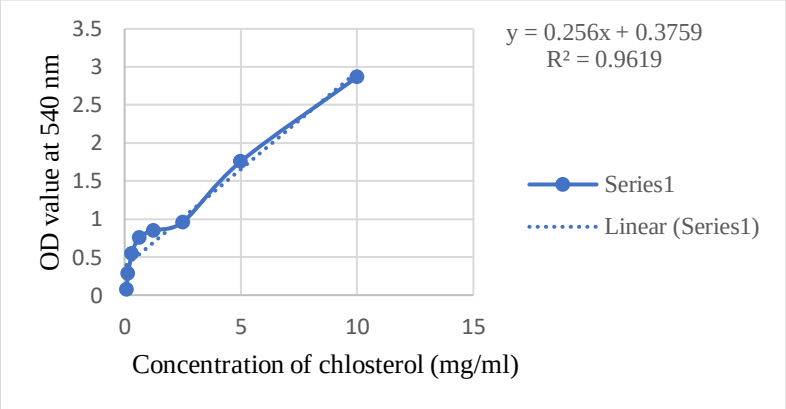
Prepare the sulfo-phosphoric-vanillin acid agent: 0.2 mg vanillin per ml 17% phosphoric acid) for colour development. Add 50 µl sulfo-phosphoric-vanillin acid agent for colour development. Measuring absorbance at 540 nm after 5 min of colour development.







Cholesterol mg/ml	OD at 540 nm (in triplicates)			Mean value
10	2.891	2.831	2.881	2.867
5	1.778	1.701	1.797	1.758
2.5	0.94	0.956	0.987	0.961
1.25	0.806	0.899	0.848	0.851
0.625	0.781	0.783	0.718	0.76
0.312	0.531	0.54	0.562	0.544
0.156	0.293	0.286	0.284	0.287
0.078	0.092	0.071	0.076	0.079



Name of the sample	OD value at 540 nm	Total Lipid content	Mean value of total Lipid content (gm)
CBL	0.527	0.59023438	1.16835938
	0.574	0.77382813	
	0.924	2.14101563	

The total Lipid content was present in the extract sample (CBL) was found to be 1.16 gm.

Energy

Note the amount of protein, carbohydrates, and fat contained in the item. When assessing a food's nutritional value, should look at 3 things: protein, carbohydrates, and fat. These macronutrients account for all of the calories in the item (aside from calories from alcohol). As a result, the exact amount of each macronutrient indicates what proportion of the total calories they make up.

S. NO	MICRO NUTRIENTS	WEIGHT (gm)	CALORIC EQUIVALENT	WEIGHT× CALORIC EQUIVALENT
1.	Carbohydrate	0.39	4	1.56
2.	Protein	0.049	4	0.196
3.	Fat	1.16	9	10.44
			TOTAL	**12.196 kcal**

The energy content was present in the extract sample (CBL) was found to be 12.196 kcal.

Further research was conducted using lactobacillus acidophilus after earlier trials showed that it could thrive on pasteurised carrot and beet juice without any special nutrient requirements. After observing the strain's viability at various pH levels, it was determined that at pH 4.56, there were more than 2.5 10^9 CFU/ml of bacteria present. The CBL sample contained the following amounts of carbohydrate-0.39 gm, fibre-0.04 gm, protein-0.049 gm, lipid-1.16 gm, and energy-12.196 kcal. For the creation of probiotic carrot and beetroot juice, the analysis determined the ideal pH and fermentation temperature to be 4.56 and 37°C, respectively. pH 6.5 was chosen as the final product development pH at 37°C.

CONCLUSION

The study on probiotic potential of carrot and beetroot juice came to the conclusion that both juices can be used as probiotic supplements to strengthen the immune system and improve digestive health. They have shown to include good bacteria like lactobacillus acidophilus which can assist in restoring the guts bacterial balance. Consuming these juices can also aid in improving digestion and reducing inflammation. Therefore, adding carrot and beetroot juice to a healthy diet can be beneficial.

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