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DEVELOPMENT OF PROBIOTIC ENRICHED TOMATO ENERGY DRINK

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ABSTRACT:

Probiotics are isolated cultures of beneficial bacteria which when incorporated in food is known to produce lots of health benefits. In this *lactobacillus acidophilus*, a lactic acid fermenting bacteria is known for its significant health benefits and good tolerance over acidic pH. Tomato juice on the other hand is known to be an anti diabetic and it also acts as a good energy drink. It's rich in vitamins, minerals along with antioxidants. The aim of this work is to develop an energy drink combining the benefits of both *lactobacillus acidophilus* and tomato juice with an additional touch of jaggery and to evaluate its physical, chemical and biological characteristics. The tomato juice was initially prepared from freshly available tomatoes in the market, it was pasteurized and added with *lactobacillus acidophilus* inoculum and jaggery, after thorough mixing, incubated for a day, filled into airtight containers and refrigerated. The juice thus prepared was subjected to sensory analysis and the presence of *lactobacillus acidophilus* was tested by gram staining and antimicrobial testing. Further tests were done to estimate the nutritional value of the product, finding the levels of carbohydrate, fat, protein especially vitaminc and iron. The pH, acidity and the *lactobacillus acidophilus* countswere monitored at intervals of 2 days. The results show that the products sensory and nutritional characteristics were good and the *lactobacillus acidophilus* culture remained viable and were maintained above the needed levels for 10 days under refrigerated storage. Thus this work has resulted in the preparation and sensory evaluation of a probiotic energy drink with good health benefits as well as consumer acceptance.

KEYWORDS: Probioticsenergy drink, anti-diabetic, Tomato, antioxidants

INTRODUCTION:

Probiotics are live microbial food ingredients that have a beneficial effect on human health.The probiotic incorporation to food has been proven to have lots of health effects like anti diarrheal, anti diabetic, antipathogenic, anticholesterol, anticancer activities and so on. The probiotic mainly consist of groups of bacteria known as lactic acid bacteria (LAB). LAB have a long history of safe use and members of the genera *Lactococcus*and *Lactobacillus* have been given generally regarded as safe (GRAS) status. The antibacterial effect of LAB is a result of fermentation and is attributed to organic acids (particularly lactate and acetate), lowered pH, hydrogen peroxide, diacetyl, competition and nutrient depletion, altered redox potentials, deconjugation of bile acids and stimulation of the immune system, and it aid in the absorption of nutrients, which would otherwise, be indigestible or poorly digestible. In addition to this the bacteriocin obtained from these lactic acid bacteria was considered as natural preservatives*Lactobacillus acidophilus* is a lactic acid producing bacteria with significant health benefits like prevention of intestinal infections, immune enhancement, prevention of diarrhoeal diseases, prevention of hypercholesterolaemia, prevention of cancer, improvement in lactose utilization, prevention of upper gastrointestinal tract diseases, stabilization of the gut mucosal barrier and control of food allergy.*Lactobacillus acidophilus* have been found to be comparatively more tolerant to acidic conditions than most of the other probiotics.

Tomatoes which are neither a fruit nor a vegetable has proven health benefits like protection of skin health, prevention of cancer, prevention of diabetics, giving shiny hair, improving vision, prevention of cardiovascular diseases etc. Tomatoes have been found to be a good source of vitamin C, phenolics, flavonols and anthocyanins, and lipophilic antioxidants such as tocopherols, β-carotene and lycopene.Tomatoes are proven to act as an excellent energy drink and is referred to athletes over other energy drinks. Tomatoes are a big part of the famously healthy Mediterranean diet. Many Mediterranean dishes and recipes call for tomatoes or tomato paste or sauce. Some recent studies, including one from The University of Athens Medical School, have found that people who most closely follow the Mediterranean diet have lower death rates from heart disease and cancer. Researchers from the Harvard School of Public Health, who followed more than 39,000 women for seven years, found that consumption of oil and tomato based products particularly tomato and pizza sauce was associated with cardiovascular benefits.Tomatoes are known for a special compound known as lycopene.Lycopene (from the New Latin word *lycopersicum*, referring to the tomato species) is a bright red carotene and carotenoid pigment and phytochemical found in tomatoes and other red fruits and vegetables. Lycopene occurs in the human diet predominantly in tomatoes and tomato products. They provide over 85% of the total lycopene consumed. Experimental (*in vitro*) studies show that lycopene is an excellent singlet oxygen quencher and has antioxidant capacity. Lycopene has been shown to be effective in scavenging of NO radicals in lymphocytes, and increased consumption of tomatoes, a rich source of lycopene, had a positive effect on biomarkers of oxidative stress, *i.e*. DNA damage and lipid oxidation.The

protective, health beneficial effects of lycopene might be related to its antioxidant potential. In conditions associated with an increased exposure of free radicals (reactive oxygen species), such as smoking, UV (skin) exposure and inflammation, low serum lycopene levels have been reported. Besides its antioxidant properties, lycopene was demonstrated *(in vitro*) to have an effect on cell-cell communication, and cell growth/differentiation. Experimental and observational (epidemiological) studies indicate that consumption of tomato products, containing lycopene, are associated with lower cancer risk, especially in the case of prostate cancer.

Tomato is a good source of lycopene and studies have shown that the concentration of this compound increases as the tomato juice is more concentrated or heat processed. the standard levels of lycopene in tomato and tomato products are given below

Food	**Form**	**Lycopene concentration (µg/100g)**
Tomatoes	Fresh, raw	3100
Tomatoes	Fresh, cooked	3703
Tomatoes	Sauce, canned	6205
Tomatoes	Paste, canned	6500
Tomatoes	Juice, canned	8580
Tomatoes	Ketchup	9900

MATERIALS AND METHODS

MATERIALS

INGREDIENTS

- Tomatoes
  - The tomatoes which were cultivated in Tamil Nadu were purchased from the local market atAyanavaram area of Chennai.
- *Lactobacillus acidophilus* culture
  - L.acidophillus culture was obtained from Department of Biotechnology ,Madras Veterinary college , TANUVAS, Chennai-7.
- Jaggery
  - Jaggery was purchased from the local market at Ayanavaram area, Chennai, and was used as a growth supplement to microbes as well as to add iron content to the developed product.

PACKAGING MATERIALS

Small Poly ethylene terepthalate (PET) cups were used. These were purchased from local market and is generally used to store pickles. It can withstand acidic pH.

UTENSILS

Stainless steel vessels, Karachi, Filter, ladles and other serving crockery set were used for the study.

GLASSWARES

1. Pipette
2. Conical flask,
3. Volumetric flask
4. Measuring cylinder
5. Funnel
6. Beakers,
7. Petri dish
8. Crucible
9. Test tubes

EQIPMENTS USED FOR STUDY

1. Fruit mixer
2. Electronic weighing balance
3. Hot air oven
4. Autoclave
5. Incubator,
6. Laminar air flow chamber
7. Refrigerator
8. Muffle furnace
9. Vortex mixer

MISCELLANEOUS

1. Brushes (cleaning purpose)
2. Utensils cleaning powder etc.,

HEATING SOURCE

Liquid petroleum gas was used as heating source

METHODOLOGY:

Table :Composition of tomato juice:

S.No	INGREDIENTS	QUANTITY
1.	Tomato juice	1 litre
2.	Jaggery	100g
3.	Inoculum	2ml

Preparation of tomato juice :

1. Fresh tomatoes of 1.25Kg quantity was washed in tap water peeled and cut into small pieces and crushed in fruit mixer until the pulp is well smashed to give juice.
2. This juice thus obtained was then filtered to remove off the seed and other suspensions
3. From this 1litre of juice was taken and added with 100g of jaggery.
4. The juice was pre heated to 35^{o}c
5. This was then pasteurized at 72^{o}c for 15seconds in a heater
6. Cooled to 37^{o}c
7. The pasteurized juice was cooled and added with 2% of inoculum
8. Stirred well and filled into cups
9. inoculated at 37^{o}c for 24hrs
10. Cooled and stored in refrigerator for further analysis

FLOW CHART FOR DEVELOPMENT OF PROBIOTIC ENRICHED TOMATO ENERGY DRINK

TOMATO JUICE

⇩

ADDITION OF 10% JAGGERY

⇩

PRE HEATING AT 35^{O}C

⇩

PASTEURIZATION AT 72^{O}C FOR 15seconds

⇩

COOLING TO 37^{O}C

⇩

INOCULATION

⇩

MIXING

⇩

FILLED INTO CUPS

⇩

INCUBATION AT 37^{O}C FOR 24hr

⇩

COOLING AND STORAGE UNDER REFRIGERATION

The probiotic tomato drink developed

ANALYSIS OF PRODUCT

Preparation of Inoculum:

Isolated culture of *Lactobacillusacidophilus* were obtained and a loopful of this culture was inoculated into MRS (de Man, Rogosa and Sharpe Medium) broth at 30^{o}c for 24hrs and this was used as inoculum.

Physical analysis:

Measurement of total lactic acid:

Acidity was measured in percentage of lactic acid as follows

1. 2ml of sample was taken in a flask
2. Diluted with 20ml of distilled water
3. Added with 3 drops of phenolphthalein indicator
4. Titrated against 0.1N NaOH
5. End point is the appearance of pink colour.
6. Calculation was done based on the formulae

$$\text{Total lactic acid} = \frac{V*N*BM\text{lactic acid}*100}{B * 1000}$$

Where,

V= volume of NaOH

N= normality of NaOH solution

B= volume of sample

BM lactic acid= 90

Measurement of ph:

pH was measured using pH meter at 22^{o}c.

Sensory analysis:

The hedonic scale was used to measure the consumer acceptance of food products. The product would be standardized using the sensory evaluations. All the samples were organoleptically evaluated by a panel of responds for acceptability on a scale using of 9 point ranging from like extremely to dislike extremely.

9 - Like extremely

8 - Like very much

7 - Like moderately

6 - Like slightly

5 - Neither like or dislike

4 - Dislike slightly

3 - Dislike moderately

2 - Dislike very much

1 - Dislike extremely

Chemical analysis:

The chemical analysis viz., moisture, protein, fat, carbohydrate, ash, iron, vitamin - c, energy value where analyzed by the following methods,

Determination of moisture content:

Objective:

To determine the moisture content present in the given sample using hot air oven method.

Principle:

Moisture content was determined by drying the sample in hot air ovenand by calculating the loss in weight after drying. This method relies on measuring the mass of water in a known sample. The moisture content is measured by finding the mass of the food before and after drying whose difference gives the mass of water.

Procedure:

1. Initial weight of empty dish was weighed and noted as W
2. Approximately 5g sample was weighed was weighed in the moisture dish, previously dried in the oven and weighed(W2)
3. The dish was placed in the oven and maintained at 105±1^{o}c for 4hrs
4. The process of drying, cooling and weighing was repeated at every 30 minutes interval until constant weight was obtained. The final weight was noted (W3).

Calculation:

$$\%\text{Moisture content} = \frac{W2 - W3}{W1} \times 100$$

W = weight of empty dish

W1 = weight of sample (W2-W)

W2 = weight of sample with dish before drying

W3 = final weight of sample after drying

Determination of carbohydrate:

Thecarbohydrate content of food can be determined by calculating the percent remaining after all the other components have been measured:

Available carbohydrates = 100 - (%moisture+%ash+%crude protein+%crude fat)

Determination of protein:

Objective:

To determine the protein content in the given sample by Kjeldhal method.

Principle:

The sample is digested with concentrated sulphuric acid (an oxidizing agent which digests the food),anhydrous sodium sulfate (to speed up the reaction by raising the boiling point) and a catalyst such as copper, titanium, selenium or mercury (to speed up the reaction). Digestion converts any nitrogen in the food(other than that which is in the form of nitrates or nitrites) into ammonia and other organic matter to CO_2 and H_2O. ammonia gas is not liberated in an acid solution because the ammonia is in the form of ammonia ion(NH_4^+) which binds to the sulfate ion (SO_4) and thus remains in solution.

$$\text{N (food)} \longrightarrow (NH_4)_2SO_4$$

The solution in the digestion flask is then made alkaline by addition of sodium hydroxide, which converts the ammonium sulfate into ammonium gas.

$$(NH_4)_2SO_4 + NaOH \longrightarrow 2NH_3 + 2H_2O + Na_2SO4$$

The liberated ammonia from the alkaline solution of ammonium sulphate is distilled into a known volume of standard acid. The amount of nitrogen is determined by back titrating the acid with standard alkali. The protein is calculated by multiplying nitrogen value by a suitable factor.

Procedure:

1. Approximately 2g of the homogenized sample was taken into a 300ml kjeldhal flask without wetting the walls of the flask.
2. 25ml of concentrated sulphuricacid , 10g of anhydrous sodium sulphate and .2 g of copper sulphate was added.
3. The contents in the flask was digested at 80^{o}c for 5 hours.
4. The contents of the flask were allowed to cool.
5. The sides of the flask were washed by a fine jet of distilled water and finally 50ml of distilled water was added and the salts were dissolved completely. The contents were cooled and quantitatively transferred into a 1000ml distillation flask using not more than 300ml distilled water.
6. The cooled contents were distilled till all ammonia passes over into acid.
7. The distillate was then titrated with 0.1N NaOH.
8. The end point was the disappearance of pink colour and the titration value was noted.

Calculation:

$$\text{Protein estimation} = \frac{\text{Blank value - Titre value x 0.14 x 6.25}}{\text{Weight of the sample}}$$

Determination of fat

Objective:

To determine the lipid content of the given sample using soxhlet extraction method.

Principle:

Semi-continuous solvent extraction methods are commonly used to increase the efficiency of lipid extraction from foods, the basic principle is based on the fact that they are readily soluble in organic solvents when compared to other components like carbohydrates, proteins etc.

Procedure:

1. Approximately 2g of dried sample was weighed into a dried thimble, with porosity permitting the rapid flow of petroleum ether.
2. The initial weight of dried flat bottom flask was noted.
3. The flask was filled with petroleum ether
4. Assemble the boiling flask with condensing unit and continue the extraction by heating solvent in boiling for 4 hrs.
5. After extraction the boiling flask was driedwith extracted fat in hot air ovenat 100^{o}c for 30 minutes, cooledto room temperature in desiccator and the final weight was noted.

Calculation:

$$\%fat = \frac{B-A}{W} \times 100$$

Where,

W= weight of sample in gram.

A= initial weight of flat bottom flask before extraction.

B= final weight of flat bottom flask after extraction and drying.

Determination of ash content

Objective:

To determine the ash content of given food material using dry ashing procedure.

Principle:

This method is based on the fact that minerals are not destroyed by heating and that they have a low volatility compared to other food components. Water and other volatile materials are vaporized at high temperatures and organic substances are burned in the presence of oxygen in air to CO_2, H_2O and N_2. Most minerals are converted to oxides ,suphates, phosphates, chlorides or silicates. Although most minerals have fairly low volatility at these high temperatures, some are volatile and may be partially lost.

Procedure:

1. Approximately 5g is taken in crucible which was previously dried in the oven and weighed.
2. The crucible was placed in a muffle furnace at 550^{o}c for 3 hours.
3. It was then placed in the desiccator, cooled to room temperature and weighed.

Calculation:

$$\%Ash = \frac{\text{weight of ash}}{\text{Weight of sample}} \times 100$$

Determination of iron

Objective:

To determine the iron content in the given sample using spectrophotometric method.

Reagents required:

1. Standard ferrous solution
2. 1,10 phenanthroline
3. Hydroxylamine hydrochloride
4. Sodium acetate(0.1M)
5. Acetic acid(0.1M)
6. Acetic acid - sodium acetate buffer(Ph = 4.5)

Procedure:

1. Pipette out 1, 2, 3, 5, 10, 15 and 20ml of the standard ferrous solution into a series of 100ml standard flasks labeled from 1 to 7.
2. In another flask labeled “sample” add 10 ml of the unknown sample.
3. To another 100ml standard flask, labeled “blank”, add about 20ml of distilled water to prepare the blank solution.
4. To each of the above flasks add 1ml of Hydroxylamine hydrochloride and 5ml of 1,10phenanthroline.
5. Buffer each solution by adding 8ml of acetic acid or sodium acetate buffer.
6. Allow atleast 15 minutes after the addition of the reagents for full colour development (the colour once developed is stable for hours) dilute each solution to 100ml mark with distilled water and mix well.
7. The standard solution so obtained correspond to 0.1, 0.2, 0.3, 0.5, 1.0, 1.5and 2 ppm respectively.
8. Record the absorption spectrum for the 2.0 ppm standard solution against the reagent blank in the range of 400 to 700nm.
9. Select the wavelength which gives the maximum absorption. The reported value is 508nm,
10. Measure and record the absorbance of all the standard solutions at 508nm.
11. In the same way record for sample also.
12. Draw a graph with absorbance at y axis and concentration at x axis.
13. With the help of this calibration curve the value for sample is determined

14. Calculate the ferrous ions present in the sample solution by accounting for the dilution factor and report the value.

Determination of vitamin c

Volumetric method:

Ascorbic acid otherwise known as vitamin c is an antiscorbutic. It is a water soluble and heat liable vitamin. The method described below is easy, rapid and a large number of samples can be analyzed in short time.

Principle:

Ascorbic acid reduces the 2,6dichlorophenols indophenols to a colorlessleuco base. The ascorbic acid gets oxidized to dehydroascorbic acid. Though the dye is a blue coloured compound, the end point is the appearance of pink colour. The dye is pink coloured in acid medium.

Materials:

1. Oxalis acid 4%
2. Dye solution: weigh 42g sodium bicarbonate into a small volume of distilled water. Dissolve 52mg 2,6dichlorophenols indophenols in it and make upto 200ml distilled water.
3. Stock standard solution: dissolve 100mg ascorbic acid in 100ml of 4% oxalic acid solution in a standard flask (1mg/ml)
4. Working standard: dilute 10ml of the stock solution to 100ml of 4% oxalic acid. The concentration of working standard is 100µg/ml

Procedure:

1. Pipette out 5ml of working standard solution into a 100ml conical flask**.**
2. Add 10ml of 4% oxalic acid and titrate against the dye (V1 ml).end point is the appearance of pink colour which persist for few minutes. The amount of dye consumed is equal to the amount of ascorbic acid.
3. Extract the sample (0.5 - 5g depending on the sample) in 4% oxalic acid and make up to a known volume(100ml) and centrifuge.
4. Pipette out 5ml of this supernatant, add 10mlof 4% oxalic acid and titrate against the dye (V2 ml)

Calculation:

Amount of ascorbic acid mg/100g sample,

= (0.5mg/V1ml) x (V2/5ml) x 100ml/wt of sample x 100

Microbial analysis:

***Lactobacillus acidophilus*:**

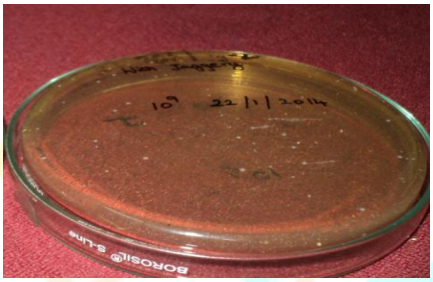
Apparatus required:

Pipettes, dilution blank, petri dish, test tubes

Media used :MRS agar

Procedure:

1. Mix the sample thoroughly and transfer 1ml of sample to 9ml of dilution blank (first dilution).
2. Transfer 1ml of first dilution to 9ml of second dilution blank this gives second dilution , like this do up to 9 dilutions and transfer 1 ml of the 9^{th} dilution to the petriplate.
3. Add 10 to 15ml of MRS agar over it.
4. Incubate the plates at 37^{o}c for 24 hrs.
5. *Lactobacillus acidophilus* Count/g = no of colonies x dilution factor



Growth of L.*acidophilus* culture in MRS agar

Anti-microbial activity :

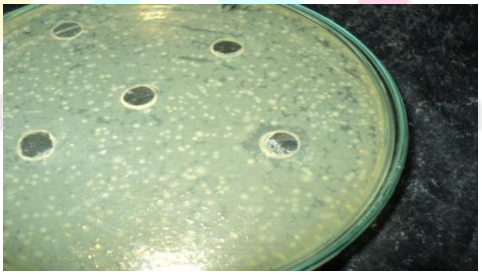
Apparatus required:

Pipettes, dilution blank, petri dish, test tubes, cork borer

Media used :MH broth, MH agar

Procedure:

1. Transfer a loopful of culture to the MH broth and incubate at 37^{o}c for 24 hrs.
2. From this culture add 0.5ml of inoculums into preset and dried plates of MHagar and spread it evenly.
3. In this cut 5 wells of 8mm dia with a sterile metal corkborer .
4. Fill the wells with 20µl of the diluted sample(10^{-9}dilution).
5. Incubate at 37^{o}c for 24hrs.
6. Observe for clear zones near wells and measure the dia.



Antimicrobial activity of L.*acidophilus* on E.*coli* strain

Coliform count :

Apparatus required:

Pipettes, dilution blank, petri dish, test tubes

Media used :violet red bile glucose agar

Procedure:

1. Mix the sample thoroughly and transfer 1ml of sample to 9ml of dilution blank (first dilution).
2. Transfer 1ml of first dilution to 9ml of second dilution blank this gives second dilution , transfer 1ml of the 2^{nd} dilution to the petriplate.
3. Add 10 to 15ml of VRBGA agar over it.
4. Incubate the plates at 37°c for 24 hrs.
5. coliform Count/g = no of colonies x dilution factor

Yeast and mould:

Apparatus required:

Pipettes, dilution blank, petri dish, test tubes

Media used :potato dextrose agar

Procedure:

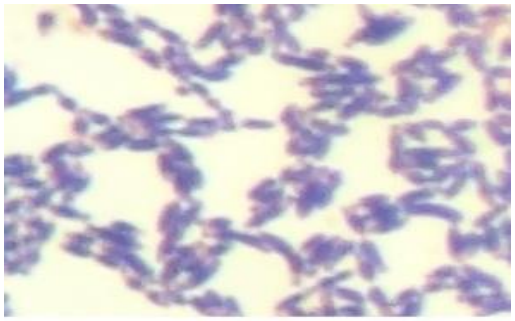
1. Mix the sample thoroughly and transfer 1ml of sample to 9ml of dilution blank (first dilution).
2. Transfer 1ml of first dilution to 9ml of second dilution blank this gives second dilution , transfer 1ml of the 2^{nd} dilution to 9ml of third dilution blank then 1ml from2^{nd} and 3^{rd} to the petriplate.
3. Add 10 to 15ml of potato dextrose agar over it.
4. Incubate the plates at 37°c for 24 hrs.
5. Yeast and mouldCount/g = no of colonies x dilution factor

Gram straining:

It was designed by Christian Gram in 1884. The staining involves a series of orderly steps. The primary stain is crystal violet and counter stain is safranine.

Staining method:

1. Prepare and heat fix the smears of culture in glass slide.
2. Place the slide on a staining bridge(made of two long glass rods).
3. Flood the smear with Gram's crystal violet for one min.
4. Wash with water.
5. Flood with Gram's iodine for one min.
6. Wash with water.
7. Destain with drop- wise addition of ethyl alcohol for 30 sec.
8. Wash with water.
9. Counter stain with safranine for one min and air dried.



Gram positive rods in MRS plates

RESULTS

Sensory evaluation

Assessing the acceptability of the probiotic tomato juice was done. The quality of any food is assessed by means of human sensory organs. The evaluation is said to be sensory of subjective or organoleptic. The quality contributes via colour, flavor, taste, consistency and overall acceptability of food. The analysis was done by untrained panel members. The results are presented below

Table :Sensory evaluation

S.No	Criteria	Probiotic juice value
1	Colour and appearance	8±0.258
2	flavour	6±0.147
3	Taste	6±0.012
4	Consistency	8±0.365
5	Overall acceptability	7±0.000
N=6 trails		

The sensory analysis was done initially after incubation and when the product was at the room temperature. The results show that the sensory qualities are acceptable but yet a lot of improvements has to be done in the product to improve its sensory properties so that the product is liked by little children also. The product was bright red in colour, was of good juice consistency and with a sweetsour taste.

Chemical analysis

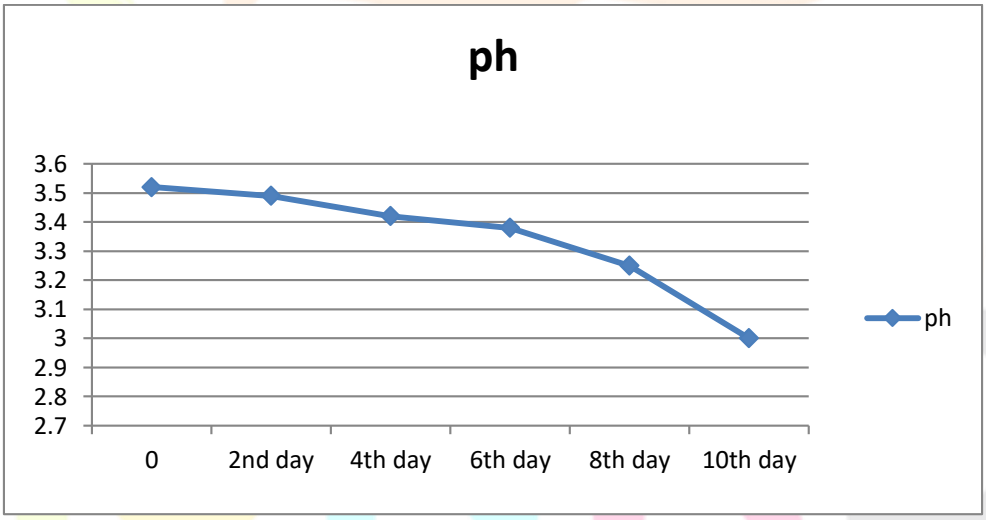
Table :Nutrition value per 100g of probiotic tomato juice

S.No	Nutrients	Quantity(g)
1	Moisture	90.17±0.073
2	Carbohydrate and sugars	8.633±0.025
3	Protein	0.773±0.007
4	Fat	0.029±0.006
5	Ash	0.386±0.001
6	Vitamin c	0.012±0.0004
7	Iron	0.028±0.000
8	Energy value	37.89±0.0042
N=6		

The tomato juice developed has been found to be with very low fat and also contain good levels of readily available energy givers like carbohydrate and sugars. The vitamin c and iron is also found to present and also in levels that would partially satisfy the daily recommended levels in the diet. Thus the product is one step ahead of other dairy based probiotics with very low fat and with presence of iron and vitamin c.

pHanalysis

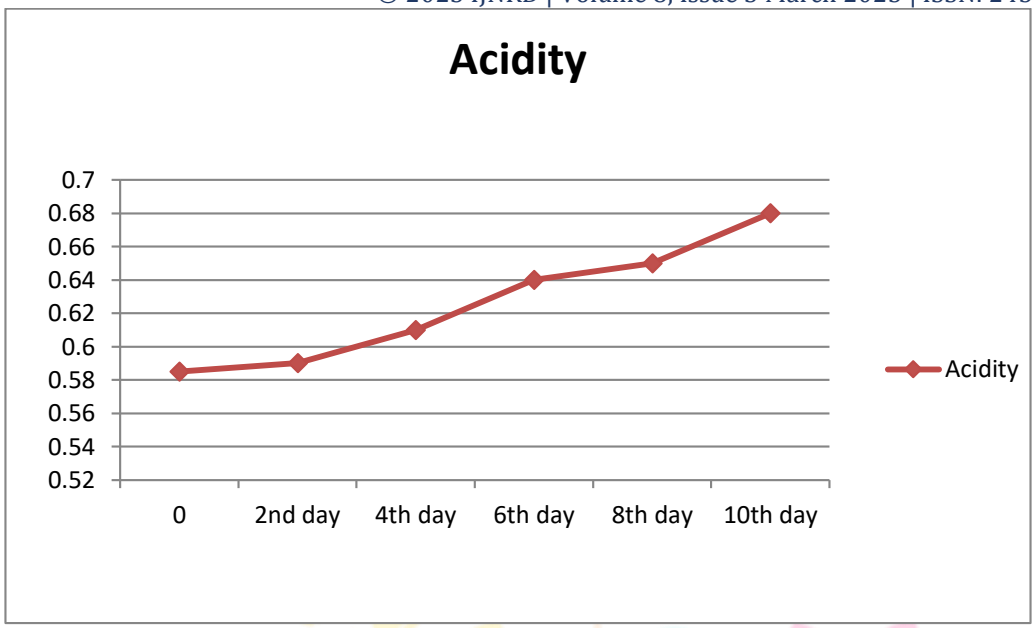
The **pH** was measured at every 2 days and the range varied from 3.52 to 3.20 at 10 days.



The ph levels varied only slightly during the observation period and also remained within limits for consumption.

Acidity

The acidity measured at every 2 days and % lactic acid ranged from 0.5 to 0.68



The acidy was also only slightly varied during the storage period.

Shelf life

Organisms	**0th day**	**2nd day**	**4th day**	**6th day**	**8th day**	**10th day**
Coliforms	nil	nil	nil	Nil	Nil	nil
Y and M	nil	nil	nil	Nil	nil	nil

The coli forms and yeast and moulds were absent during the entire observation period, thus determining the shelf life at 10 days for the product.

Keeping quality

Physical character	Under refrigerated conditions						
Days	0	2	4	6	8	10	12
Colour	ok	ok	ok	ok	ok	Ded	Detd
Taste	ok	ok	ok	ok	ok	ok	Detd
Texture	ok	ok	ok	ok	ok	Ded	Dend

Ded - Deviated

Detd - Deteriorated

Dend - Denatured

Even though the no of probiotic cells remained above the required limit (10^9) even after 10 days the shelf life of the product was limited to 10 days owing to the keeping quality deterioration as it happens in all fermented product. But it remained good until the 10th day after incubation.

Monitor of counts of *Lactobacillus acidophilus*

Days	No of organisms
0	6.53×10^9
2	5.68×10^9
4	6.00×10^9
6	4.56×10^9
8	4.75×10^9
10	3.58×10^9
N = 6	

The *Lactobacillus acidophilus* counts remained above the recommended levels although the analysis with slight variations. This reveals that the probiotic drink will be effective and would accomplish its role to act as a probiotic supplement to the consumer.

Antimicrobial studies and result for gram staining

These tests were carried out only to confirm the presence of *Lactobacillus acidophilus* strain initially after incubation.

The diameter of the clear zone for antimicrobial activity against E.coli was 12 mm.

The gram staining showed gram positive Y shaped rods.

These tests confirmed the presence of the particular strain in the product and proved devoid of other contaminations.

DISCUSSION

Probiotic drinks are mainly dairy based and the dairy based probiotics was a well established section. studied the lowering trend of accepting dairy based probiotic drinks by the consumers due to their fat content and allergens like lactose and also stated in his study that people are ready to accept non dairy based probiotic drinks based on their functional property alone. The tomato based probiotic drink developed was added with 2% culture and present study reported a viability of probiotic culture for the specific period of 10 days which is in accordance with the author. The initial confirmation of the probiotic organism was also done by gram staining and antimicrobial studies and the tests confirmed the presence of the particular strain of *Lactobacillus acidophilus*only.

The nutritional analysis were done according to AOAC method and gave results showing low levels of fat and protein but high levels of carbohydrates which is an direct energy source. There were also significant levels of Vitamin c (0.012±0.0004g) and iron (0.028±0.000g).The sensory quality of the product was also good but with slight fruity flavor which was not liked by all but the product remained acceptable and graded as good while keeping for 10 days. studied the viability of cells in tomato juice the cells remained above the needed levels upto the 4th week of storage but in the product developed, due to the addition of jaggery the cells have multiplied faster

increasing the pH and acidity which reduced the viable counts that were found decreasing within the 10th day itself.Hence the purpose of developing a tomato based energy drink was successfully accomplished with added goodness of probiotics.

CONCLUSION AND FUTURE PERSPECTIVE

In this study there was an attempt made to produce a probiotic drink with tomato juice as base. The influence by the numerous health benefits of tomato juice as well as the *Lactobacillus acidophilus* organism has been exploited for health benefits. The product was well differentiated from commercially available dairy based probiotics due to its fat free nature and enriched with the presence of Vit C and iron in the product. The viability of probiotic cells was determined and also the proximate composition of the product was determined which indicate its nutritive value.

Further studies on

- The health effects of this drink on selected human subjects.
- Development of effective packaging material to increase shelf life of this product and
- Studies on incorporation of other probiotics other than *Lactobacillus acidophilus* in tomato juice can be done and evaluated for additive effect.

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