IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

BIOCHEMCAL CHARACTERISATION OF POTENTIAL FRUITS AND VEGETABLES THAT CAN PRODUCE PROBIOTIC JUICES AND COMPARITIVE ANALYSIS OF PROBIOTIC JUICE FROM – APPLE, TOMATO, BEETROOT WITH YAKULT

[1.]Pavana H.M, [2.]Dr. Sindhu Anoop

[1.]Student, [2.]Assistant professor

[1.] Master of Biotechnology,

[1]Mount Carmel College, Bangalore, India

Abstract:

A healthy diet helps to have a healthy heart and blood vessels, strong bones and muscles, a sharp mind. That helps to improve the health and promote a good physical and mental well-being. These functional foods also add on the beneficial effects to improve the health status by preventing the nutrition-related disease. Different varieties of food have been fermented or enriched with probiotic by using the beneficial microorganisms that have been sold in the market. A variety of beneficial microbial species have been used Lactobacillus and Bifidobacterium have become the most commonly used probiotic strains in these food products, but others such as Saccharomyces cerevisiae (boulardii), Enterococcus, Bacillus, and Escherichia are also applied. One suxh food type is probiotic food, also a non-diary product. Here in this study, different fruits and vegetables – Apple, Tomato, and Beetroot were choose nans subjected for fermentation with the probiotic microorganisms isolated from the natural environment. In this study species like Spirulina, Lactobacillus Casei, Enterococcus were used. The fruit juice extracted from these fruits and vegetables were left for fermentation for 72 hours and pH, Acidity and other parameters were checked with the unfermented juices. Also a comparative analysis with commercially available Yakult was carried out. Also a biochemical tests were conducted for the comparative study.

Index Terms **– Probiotics, Fermentation, Gut Bacteria**

Introduction

The concept of food have been changed from providing energy for our body to diets that deliver physiological benefits in management and prevention of diseases. Foods have many roles such as satisfying hunger, providing necessary nutrients, improving health, promoting a state of physical and mental well-being as well as preventing or reducing nutrition-related diseases. According to a survey probiotic market have raised to worth $46.55 Billion in 2020, incorporating probiotics in different kind of food products (dietary supplements, functional foods, specialty nutrients, animal feed); in medicinal relevance (regular, therapeutic, preventive health care); or by any other convenient mode of application (Anon, 2016). Certain critical factors have been identified as the key reasons for enhanced trend towards the uptake of functional foods which includes health deterioration due to busy lifestyles increased awareness of the connection between diet and health, low

consumption of handiness foods and insufficient exercise, increased prevalence of self-medication, and a crowded competitive food market (Corbo et al., 2014). (1)

As defined by FAO/WHO (2001), probiotics are live microorganisms (mainly bacteria and few yeast strains) that confer a beneficial heath effect on the host if administered in appropriate amounts. Fermented milk products have been conventionally considered as the most excellent carriers for probiotics; however, the use of milk-based products may be also limited by lactose-intolerance, allergies, dyslipidemia and vegetarianism. A number of in vitro and in vivo studies are available which proves the benefits of probiotic foods for humans by maintaining or improving their intestinal microflora (2)

The probiotic strains produce various bioactive compounds, such as vitamins, antioxidants, amino acids and peptides, and when added to fruit juices may offer a synergy of health benefits from both sources. Such products can be considered as a new type of functional foods (3) The fermentation is considered as a low-cost process, which preserves the food and improves its nutritional and sensory characteristics. (4)Many cultures were used as starter cultures for fermented juices and have been recognized as probiotics A wide range of foods have been fermented or enriched in probiotics to be evaluated as possible carriers of these beneficial microorganisms and successfully placed on the market. Several species of *Lactobacillus* and *Bifidobacterium* have become the most commonly used probiotic strains in these food products, but others such as *Saccharomyces cerevisiae* (*boulardii*), *Enterococcus*, *Bacillus*, and *Escherichia* are also applied (5)(6)

Researches also bespoke that intake micronutrients such as calcium, retinol, vitamin E, nicotinic acid, folate, beta carotene in genome damage and repair showed positive effects which where bestowed by fruits (Fenech, 2005) (7).

Materials and methods

Preparation of Bacterial cultures

1. Culturing of Spirulina
   - Spirulina medium was prepared by the method suggested by CFTRI by mixing the solution A and B after autoclaving.100ml medium was dispensed into pre-sterilized 250ml conical flask and covered with loose cotton plug. The conical flask were inoculated with stock culture of spirulina, 1ml each by using sterile pipette. The inoculated conical flask were provided with illumination and inoculated for 15 days (8)

2. Bacillus CULTURE PREPARATION
   Soil sample were collected from the grounds of Mount Carmel College, Bengaluru. Sample was then serially diluted. Then transferred to nutrient agar plates using pour plate technique. Incubation was done for 24 hours at 37°C. Colonies were further subjected to streaking. After multiple streaking, colonies were observed on the nutrient agar plate. Single colonies were then transferred to Nutrient Broth and kept in incubator shaker for 24 hours at 37°C. (9)

3. Lactobacillus Casei culture preparation
   For culturing the bacteria yakult was taken as a sample. It was subjected to serial dilutions. Then the samples were inoculated in MRS Agar. Incubated for 48hours at 37°C. Then colonies were streaked on MRS Agar medium. Then single colonies were taken and inoculated to MRS Broth and placed in incubator shaker for 24 hours at 37°C.

Collection and preparation of the of the sample juices

Apple(Malus domestica), Beetroot(Beeta Vulgaris), Tomato(Solanum lycopersicum) were collected from the local market near Vijayanagar, Bangalore . These fruits and vegetables were hopped and grinded in a mixer by adding water. And filtered using the filter paper and stored in a glass jar bottle and preserved for further estimations.

Inoculation of the juice sample:
The juices were then inoculated with the desired bacterial cultures. Broths along with the desired bacteria were prepared. Then to obtain the biomass for inoculation, 15ml of the broths were taken in centrifuge tubes and centrifuged at 5000rpm for 5mins.The supernatant was discarded and the pellet was dissolved in the juices and kept for fermentation for 72 hours.

Estimation of pH of the samples:
This was done using a pH meter by standardizing with buffer tablets. The pH for the raw juices without any bacterial inoculation and juices inoculated with E. coli, Bacillus, Lactobacillus with cell density 105 cfu/ml and 107cfu/ml was measured.

Estimation of the acidity of the samples by using the Titration method:
0.1M NaoH solution was prepared. Phenolphthalein was used as an indicator and NaoH was titrated against the 10ml sample.

BIOCHEMICAL TEST FOR SAMPLE ANALYSIS

1. Estimation of reducing sugar by DNS method

2. Standard stock solution for glucose was prepared.
   Using the DNS reagent glucose concentration of raw juices, fermented juices and yogurt was found out. 1 ml of each of the sample was added to different test tubes. 3ml of DNS Reagent was added to each of the test tube, they were heated in water bath at 100°C for 10 minutes. Then they were allowed to cool and 6ml of distilled was added to it. Absorbance was read at 540nm. A standard graph was prepared by plotting absorbance in Y- axis and the concentration in the X- axis.(Marisa Garriga1 et al,2017)

3. Estimation of Protein by Lowey's method
   Standard stock solution of protein was prepared. 1 ml of raw , inoculated and yogurt sample was taken in test tubes. To this 2.5ml of Lowry Reagent was added along with 1ml to Folin's Reagent. It was incubated in dark for 30 minutes. The absorbance was measured at 670nm. A standard curve was plotted by taking absorbance on Y- axis and concentration of BSA in the X-axis.(Lowry, et al,1951)

4. Estimation of Phenolics - The phenolic content was determined using Folin-Ciolteau Method.

5. Determination of Vitamin C in the sample
   1ml of the sample was added to 25ml of 0.5% oxalic acid was mixed properly. This solution was titrated against DCIP solution till light pink color was obtained.(Khattak, K.F and Rahman, T.U,2017)

Biochemical test for Bacterial samples

1. GRAM STAINING - This is used to differentiate gram negative and gram positive bacteria from each other.

2. CATALASE TEST
   The broth containing the desired microbial culture was added to a sterile Eppendorf tube. To it a few drops of hydrogen peroxide solution were added and results were observed.
3. OXIDASE TEST
   A filter was taken and moistened with distilled water then with a loop a smear was made on the filter paper of the desired microbial culture. To the smear 2-3 drops of Kovac's Reagent was added. Colour change to blue was observed within 10-30 seconds.

4. METHYL RED/ VP TEST
   The desired microbial cultures were added to test tubes to which 5 drops of Methyl Reagent and 5 drops of VP Reagent was added it. Color change to red or yellow was noted and results were found out.

Test to check the survival of the micro-organisms in the Gastro intestinal tract

To check if the micro-organisms in inoculated in the juices made could survive in the gastrointestinal tract or not, they were subjected to gastrointestinal conditions. For this the juices with the inoculated micro-organisms were subjected to gastrointestinal conditions that was created artificially in the lab environment.

Result

Probiotic foods have become lot in demand these days due to the increase in the importance of functional foods. There are several products that are available in the market which are mostly dairy products like Yakult, which claim to be different from regular yogurt as the bacteria and the culture used is different. These diary product may not be suitable for lactose intolerance to consume, which is a major throwback. So to overcome this, as an alternative fermented fruits and vegetable juice could be used with the probiotic micro-organism. This paper shows how the easily vegetables and fruits can be used along with easily available micro-organisms to create the probiotic juice which is also a cost effective compared to the commercial one.

Conclusion

Probiotic Juices from sources like fruits and vegetables good be a good alternative for the people for maintaining a healthy diet and lifestyle. It can also be very useful for metabolising lactate in the people who are cannot directly consume it. It can be concluded that the aim of the study was successfully achieved and it generated results that proved that juices if subjected certain good micro-organisms could help to increase the essential nutrients content.

References

1. Patel, Ami. (2017). Probiotic fruit and vegetable juices- recent advances and future perspective.. International Food Research Journal. 24. 1850-1857.
2. Fuller R. Probiotics in man and animals. J Appl Bacteriol. 1989 May;66(5):365-78. PMID: 2666378.
3. Jalal Bohlouli, Iman Namjoo, Mohammad Borzoo-Isfahani, Mohammad Ali Hojjati Kermani, Zakiyeh Balouch Zehi, Amir Reza Moravejolahkami
Heliyon. 2021 Jan; 7(1): e05925. Published online 2021 Jan 10. doi: 10.1016/j.heliyon.2021.e05925

4. Holzapfel WH, Haberer P, Snel J, Schillinger U, Huis in't Veld JH. Overview of gut flora and probiotics. Int. J. Food Microbiol. (1998) 41, 85–101. 10.1016/S0168-1605(98)00044-0 [PubMed] [CrossRef] [Google Scholar]
5. Bao Toan Nguyen, Erika Bujna, Noemi Fekete, Anh T. M. Tran, Judit M. Rezessy-Szabo, Ram Prasad, Quang D. Nguyen
Front Nutr. 2019; 6: 54. Published online 2019 May 9. doi: 10.3389/fnut.2019.00054
6. https://www.hindawi.com/journals/jchem/2020/5790432/
7. Vasudha, S. and Mishra, H. N.; 2013.; "Non dairy probiotic beverages."; International Food Research Journal 20(1): 7-15 (2013).

8. Nyabuto, Dorothy & CAO, Kewei & Mariga, Alfred & Kibue, Grace & He, Meilin & Wang, Ch. (2015). Growth performance and biochemical analysis of the genus Spirulina under different physical and chemical environmental factors. African journal of agricultural research. 10. 10.5897/AJAR2015.10210.
9. Zhenxiang LU, Weina GUO, Chang LIU J Vet Med Sci. 2018 Mar; 80(3): 427–433. Published online 2018 Jan 23. doi: 10.1292/jvms.16-0572

10. Perricone, M.; Bevilacqua, A.; Corbo, M.R.; Sinigaglia, M.; 2014.; "Technological characterization and probiotic traits of yeasts isolated from Altamura sourdough to select promising microorganisms as functional starter cultures for cereal-based products."; Food Microbiol. 2014, 38, 26–35