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Microbiological Analysis of Vegetable Enriched Yoghurt on Long-term Storage

[1]Ankita Srivastava
[1]Assistant Professor
[1]Faculty of Biosciences, Institute of Biosciences and Technology, Shri Ramswaroop Memorial University,
Barabanki,
Uttar Pradesh, India—225003

Abstract : The advancement of functional foods is expressly designed to enhance health outcomes or mitigate the likelihood of disease onset. These foods are characterized by the inclusion of specific minerals, vitamins, fatty acids, or dietary fibers; however, their rapid degradation presents a significant challenge in the context of enriched food products. The objective of this research was to examine the impact of a 21-day storage period on the nutritional composition of fortified yogurts. We produced yogurts enriched with carrot, beetroot, and mint, and conducted a microbiological analysis at intervals of 7, 14, and 21 days of storage. The findings indicated that the vegetable-enriched yogurts exhibited a higher prevalence of yeasts and molds compared to standard yogurt across all storage intervals of 7, 14, and 21 days. The current research leads to the inference that yogurts enhanced with natural extracts (mint, carrots, and beetroot) may function as beneficial food products for health enhancement; however, their nutritional composition also fosters microbial proliferation, which gradually leads to the deterioration of the yogurts, thereby indicating potential consumer negligence and subsequent rejection. Enriched or fortified functional foods demonstrate a greater susceptibility to spoilage relative to conventional yogurt.

Index Terms - **Functional Foods, nutritional quality, microbiological analysis, long term storage, yoghurt,**

I. Introduction

Functional foods are defined as foods that offer health benefits beyond basic nutrition. These are formulated with ingredients like vitamins, minerals, dietary fibers, fatty acids, and biologically active substances such as phytochemicals or probiotics, which support specific physiological functions or help reduce disease risk (Ndife, J et al, 2014). One growing area of interest in functional foods is dairy products, especially fermented types like yoghurt, due to their role in gut health via probiotics and prebiotics. Probiotics are live microorganisms that enhance intestinal microbial balance, while prebiotics are non-digestible food components that stimulate the growth of beneficial gut bacteria (Seckin, K.A et al 2009). Yoghurt, one of the oldest and most widely consumed fermented dairy products, is made by fermenting milk with lactic acid bacteria like Streptococcus thermophilus and Lactobacillus delbrückii subsp. bulgaricus (Lee, W et al 2009). These bacteria ferment lactose into lactic acid, which decreases milk pH and promotes gel formation, resulting in yoghurt's unique texture (Peng, Y et al, 2010). According to FDA standards, yoghurt must contain at least 8.25% milk solids-not-fat (Tamime, A.Y, 2002). Yoghurt is also a carrier for probiotics and a rich source of nutrients such as protein, calcium, B vitamins, and potassium (Staffolo, M.D et al, 2004). It is often better tolerated by lactose-intolerant individuals because much of the lactose is already converted during fermentation (Heyman, M, 2000). Additionally, yoghurt has demonstrated benefits in improving gastrointestinal health and preventing antibiotic-associated diarrhea (Lourens-Hattingh, A et al, 2001). Fortification and enrichment are techniques used to improve the nutritional profile of foods. Fortification involves adding nutrients not originally present, while enrichment restores lost nutrients or boosts natural levels. Codex Alimentarius and WHO/FAO guidelines stress that fortified foods should address population-wide micronutrient deficiencies without causing excessive intake in subgroups. To enhance the functional profile of yoghurt, ingredients like beetroot, grape pomace, dietary fiber, and soy have been studied. Beetroot (Beta vulgaris), rich in betalains such as betanin and isobetanin, exhibits strong antioxidant properties by scavenging free radicals and protecting against oxidative stress (Ninfali, P et al, 2017 and Nora, M. A. A et al, 2018). Betanin in particular shows high antioxidant capacity due to its electron-donating ability (Czapski, J et al, 2009 and Frank, T et al, 2005). Beetroot is also used as a natural food colorant (E162). Carrots are another antioxidant-rich food, containing high levels of carotenoids like beta-carotene, which is a precursor to vitamin A. This helps maintain vision, immune function, and protects against age-related diseases like macular degeneration (Masih, D et al, 2019). Carrots also offer anthocyanins in purple varieties, contributing to antioxidant activity and visual appeal (Pandey, P et al, 2020). Mint, from the *Lamiaceae* family, is known for its antioxidant and anti-inflammatory properties, primarily due to compounds like rosmarinic acid. It is also a source of several micronutrients and is explored as a natural alternative to synthetic antioxidants in food preservation (Bajaj, S et al, 2016 and Nejad, J et al, 2014). In this study, functional foods such as yoghurt enriched with three ingredients beetroot, carrot, or mint has been prepared and their physiochemical properties has been evaluated.

II. RESEARCH METHODOLOGY

2.1. Preparation of mint extract

Mint leaves were weighed (80grams) and then washed with clean water and cut with the help of knife. Mint was grinded into a smooth paste using a grinder with addition of a little distilled water. After grinding the mint paste was kept in a beaker, sealed with an aluminum foil and kept in the refrigerator until it was utilized for preparation of mint yoghurt.

2.2. Preparation of carrot extract

Carrots were washed and peeled with the help of a knife and cut into small cubes and then grinded into a small paste with the help of a grinder with addition of a little amount of distilled water to help the process. Upon obtaining the carrot smooth paste, 80grams of it was weighed into a beaker, covered with aluminum foil and kept in the refrigerator until preparation of carrot yoghurt.

2.3. Preparation of beetroot extract

Beetroot was washed and peeled using a knife and then cut into small pieces. The pieces were grinded with the help of a small amount of distilled water in a grinder. Upon obtaining the smooth paste, 80 grams was weighed into a beaker, sealed with aluminum foil and kept in the refrigerator until the preparation of beetroot yoghurt.

2.4. Preparation of yoghurts

1600 milliliters of refrigerated, full cream, pasteurized, packaged Amul Fresh cow's milk was heated at a temperature of about 150°C for about 5 minutes, then it was cooled and adjusted to 42°C. Inoculation was done with desirable proportion of starter culture (2.5%). (Nejad, J et al, 2014). Once the starter culture was completely mixed, the inoculated milk was divided into four portions, the first portion served as a control, the second portion was fortified with 80 grams of beetroot paste prepared prior, the third portion was fortified with 80 grams mint paste and the fourth portion was enriched with 80 grams smooth carrot paste previously prepared, the mixtures was poured in plastic cups which were prewashed with boiling water prior to usage labelled days 1, 7,14 and 21 for each of the four types of yoghurts such that there were 4 samples for each type of yoghurts in accordance to their day of analysis and the yoghurts were sealed using paraffin plastic for air tightness after to closing with the lid. The samples were incubated in an incubator until formation/coagulation of yoghurts (8-12 hours). The yoghurt samples were stored at about 4°C at refrigeration until each day of analysis 1, 7, 14, 21.

Figure 1: enriched yoghurts prepared for storage at 4°C. From left, beetroot, carrot and mint enriched yoghurts

2.5. Microbiology Analysis: Yeast and mold count

15.6 grams of Potato Dextrose Agar (PDA) IN 400ml of distilled water, this was heated to boiling to dissolve the media completely then the media was sterilized by autoclaving 15lbs (pressure) at 121degrees Celsius for 15 minutes. 1.9 g of peptone powder was dissolved in 200ml distilled then heated to dissolve and sterilized by autoclaving at 15lbs, 121 degrees Celsius for 15 minutes. The autoclaved media was poured in plates (about 15 ml) then the media was dried overnight. One gram of each cultured yoghurt samples was diluted with 9 ml of 0.1% sterile peptone water. Ten-fold serial dilution (10-2) was prepared in 9 ml of sterile peptone water (Houghtby, G.A et al, 2004). Yeast and mold counts were conducted on each of the yoghurt samples. The serial dilutions were placed on potato dextrose agar (PDA). Enumeration was done using the spread plate technique Aseptically pipette 0.1 ml of each dilution on pre- poured, solidified PDA agar plates and spread inoculum with a sterile, plastic spreader. Plates were rotated to spread the sample homogenously on the plate and the plate was open and let dry for a few minutes then closed. Plates were then incubated upright under anaerobic condition (using Gas-pack system, at 35 °C and remained undisturbed for a period of 48 hours. All yoghurt samples were plated on days 1 and 7. The number of Colony Forming Units (CFU) on plates containing 15 to 300 colonies were calculated per gram of samples as shown below;

Number of CFUs per ml of sample=
(Number of colonies per plate×Dilution factor of the counted plate)/(Volume of innoculant plated)

III. RESULTS AND DISCUSSION

Yeast and mold were counted in all the samples during on the first and seventh days of storage. Diluted samples were inoculated onto PDA agar, supplemented with by spread plate method as mentioned earlier. The plates were incubated at 35°C for 48 . Visible colonies were counted and calculated as the total yeast and mold and recorded as CFU/g. Total count of molds and yeast (fig. 2 and fig. 3) The occurrence of yeasts in dairy products is significant because they can cause spoilage, effect desirable biochemical changes and they may adversely affect public health. While fermentative and spoilage activities of yeasts at elevated temperatures are well known in many food and beverage commodities, little attention has been given to the specific occurrence and significance of yeasts in dairy products at these temperatures. Since yeasts play a substantial role in the spoilage of commercial fruit yoghurts, especially when cold storage practices are neglected, the deterioration of yoghurt samples obtained from the manufacturers were evaluated at different temperatures for a period of 30 days during this study. Total yeast was enumerated, isolated and identified from the yoghurt samples as shown in tables 1 and 2. The highest number of yeast populations, up to >300 colony counts, was found. Most of the colonies were too many to count and very clustered thus resulting in a lot of errors. Molds were observed in commercial yoghurt probably owing to the earlier date of manufacture. Among the self-made yoghurts; control yoghurt had the least CFU/g, mint yoghurt had the least colony counts than the other fortified yoghurts and carrot had the highest. This is due to the antifungal properties of mint as stated by (Milanović, V. et al, 2021) on the inhibitory effect of peppermint essential oils on spoilage of food by yeast and molds, therefore indicating the inhibitory effect of mold and yeast growth in the yoghurts. Carrots and beetroot are said to improve fermentation in wine which is mainly by yeast (*Saccharomyces cerevisiae*) means it encourages the growth of yeast thus having a high yeast count and zero mold count in the yoghurt. As shown in tables 1 and 2 there was higher CFU/g in day 7 than on day 1, because the longer the duration of storage the higher the risk of contamination by yeasts and molds.

Table 1: Represents colony forming units/g of yeast and molds in all yoghurt samples after 7 days of storage

Yeast and mold count in yoghurt samples					
Sample	**Hours of incubation**	**Volume of inoculant plated**	**dilution of original culture**	**Number of colonies**	**CFU/g**
Control/Plain	48	0.1	10	13	**1.30E+03**
Control/Plain	48	0.1	100	3	**3.00E+03**
Control/Plain	120	0.1	10	54	**5.40E+03**
Control/Plain	120	0.1	100	16	**1.60E+04**
Mint enriched	48	0.1	10	67	**6.70E+03**
Mint enriched	48	0.1	100	15	**1.50E+04**
Mint enriched	120	0.1	10	152	**1.52E+04**
Mint enriched	120	0.1	100	45	**4.50E+04**
Carrot enriched	48	0.1	10	187	**1.87E+04**
Carrot enriched	48	0.1	100	52	**5.20E+04**
Carrot enriched	120	0.1	10	261	**2.61E+04**
Carrot enriched	120	0.1	100	102	**1.02E+05**
Beetroot enriched	48	0.1	10	164	**1.64E+04**
Beetroot enriched	48	0.1	100	44	**4.40E+04**
Beetroot enriched	120	0.1	10	220	**2.20E+04**
Beetroot enriched	120	0.1	100	47	**4.70E+04**
Branded	48	0.1	10	304	**3.04E+04**
Branded	48	0.1	100	75	**7.50E+04**
Branded	120	0.1	10	397	**3.97E+04**
Branded	120	0.1	100	97	**9.70E+04**

Table 2: Represents colony forming units/g (CFU/g) of yeast and molds in yoghurt samples after 7 days of storage.

Yeast and mold count in yoghurt samples					
Yoghurt Sample	**Hours of incubation**	**Volume of inoculant plated**	**dilution of original culture**	**Number of colonies**	**CFU/g**
Control/Plain	48	0.1	10	282	**2.82E+04**
Control/Plain	48	0.1	100	121	**1.21E+05**
Control/Plain	120	0.1	10	316	**3.16E+04**
Control/Plain	120	0.1	100	231	**2.31E+05**
Mint enriched	48	0.1	10	302	**3.02E+04**
Mint enriched	48	0.1	100	257	**2.57E+05**
Mint enriched	120	0.1	10	387	**3.87E+04**
Mint enriched	120	0.1	100	274	**2.74E+05**
Carrot enriched	48	0.1	10	497	**4.97E+04**
Carrot enriched	48	0.1	100	307	**3.07E+05**
Carrot enriched	120	0.1	10	513	**5.13E+04**
Carrot enriched	120	0.1	100	326	**3.26E+05**
Beetroot enriched	48	0.1	10	483	**4.83E+04**
Beetroot enriched	48	0.1	100	304	**3.04E+05**
Beetroot enriched	120	0.1	10	505	**5.05E+04**
Beetroot enriched	120	0.1	100	317	**3.17E+05**
Branded	48	0.1	10	582	**5.82E+04**
Branded	48	0.1	100	316	**3.16E+05**
Branded	120	0.1	10	675	**6.75E+04**
Branded	120	0.1	100	370	**3.70E+05**

Figure 2: represents yeast and mold growth plated on day 1 of storage from left; Commercial, control, mint, Beetroot and carrot yoghurt samples. Top represents dilution 1 (10^{-1}) and bottom represents dilution two 10^{-2}).

Figure 3: represents yeast and mold growth plated on day 7 of storage from left; Commercial, control, mint, Beetroot and carrot yoghurt samples. Top represents dilution 1 (10^{-1}) and bottom represents dilution two 10^{-2}).

IV. CONCUSION

This study aimed at developing probiotic yoghurt enriched with mint, carrots and beetroot respectively while monitoring its physiochemical properties during 21 days of storage. All the enrichments used in the study are well known for their antioxidant capacities; antioxidants are responsible for acting as free radical scavengers and prevent oxidative damage to the human body. Additionally, probiotic contributes to health benefit in the prevention of gastral intestinal tract disorders, immunity enhancement and improvement of lactose intolerance disorders. Taking into consideration the above benefits, thus incorporating the antioxidant rich extracts to the probiotic yoghurt is beneficial, as it results in additional value. Incorporation of antioxidant rich mint, carrot and beetroot to the probiotic yoghurt is of advantageous nutritionally, as it contributes to the health benefit to the human body. Generally, beetroot-enriched yoghurt was more acceptable as it has the highest percentage oxidation inhibition compared carrot and mint enriched yoghurts, respectively. Furthermore, on storage the physical-chemical characteristics; pH, showed a significant decrease as the storage time increased while titratable acidity increased. Moisture content, syneresis and TSS increased with storage.

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