Studies On Preparation of Probiotic Orange Juice & Its Benefits

[1]Mohammad Kaif, [2]Deshpande HW, [3]Ansari Ahmed, [4]Kishor Anerao ,[5]S.K sadawarte

[1,3] Research Scholar, M. Tech. (Food Processing Technology) Department of Food Microbiology and Safety, CFT, VNMKV, Parbhani.

[2] Professor and Head Department of Food Microbiology and Safety, CFT, VNMKV, Parbhani

[4]Ph.D. Research Scholar, Department of Food Microbiology and Safety, CFT, VNMKV, Parbhani.

[5]Associate Professor, Department of Food Processing And Technology CFT, VNMKV, Parbhani.

Department Of Food Microbiology & Safety, College of Food Technology Vnmkv Parbhani, India.

Abstract:

This present investigation examines how orange juice's sensory, nutritional, and microbiological qualities are affected by probiotic fortification and activated charcoal treatment. After being exposed to varied activated charcoal concentrations (0.3–1.0%) for 30–60 minutes, orange juice samples were standardised using probiotic bacteria at various inoculum levels (2–3%) and incubation times (8–12 hours). Treatments with greater charcoal concentrations and optimised probiotic levels (Sample C3) considerably improved sensory qualities, obtaining maximum ratings in taste, aftertaste, and overall acceptability, according to sensory evaluation using a 9-point hedonic scale. The proximate analysis showed that probiotic-enriched juice had better nutritional quality, with higher levels of protein, fibre, and ash and lower levels of carbohydrates. The mineral composition remained functionally relevant even if microbial interactions caused a drop in calcium, magnesium, and potassium. During 28 days of storage at 4°C, probiotic viability was kept above the suggested level ($\geq 10^9$ CFU/mL). These results support orange juice's potential as a functional beverage by indicating that probiotic and activated charcoal treatments can improve the juice's functional and sensory qualities.

Introduction:

"Probiotic" is used to refer to cultures of live microorganisms which, when administered to humans or animals, improve properties of indigenous microbiota. In the food industry, the term is described as "live microbial food ingredients that are beneficial to health" (Clancy, 2003). Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO) defines probiotics as "live micro-organisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2001). Species of Lactobacillus and Bifidobacterium, normal components of the intestinal microbiota, are usually employed in many probiotic foods. Lactobacillus and Bifidobacteria are examples of genera of

which some of the species are promising probiotics (Saito, 2004). In addition, Streptococcus, Enterococcus, Pediococcus and Leuconostoc species are also used as probiotics. Saccharomyces baulardii is a yeast, which is considered as a probiotic and is being used commercially. The following properties and functions have been attributed to probiotics: they adhere to host epithelial tissue; they are acid resistant and bile tolerant; they are safe, non-pathogenic and non-carcinogenic; they cause improvement of the intestinal microflora; they have a cholesterol lowering, immune stimulating and allergy lowering effect; synthesize and enhance the bioavailability of nutrients (Ouwehand et al., 2002; Saito, 2004; Grajek et al., 2005). Additionally, probiotics produce a variety of beneficial compounds such as antimicrobials, lactic acid, hydrogen peroxide, and a variety of bacteriocins (Holzapfel et al., 2001; Gorbach, 2002).

Traditionally, probiotics have been used in yogurt and other fermented dairy products but nowadays, there is an increasing interest in non -dairy-based probiotic products (Espinoza and Navarro, 2010). Recently, beverages based on fruits, vegetables, cereals, and soybeans have been proposed as new products containing probiotic strains; particularly, fruit juices have been reported as a novel and appropriate medium for probiotic for their content of essential nutrients. Moreover, they are usually referred as healthy foods, designed for all age groups (Luckow et al., 2006). Probiotication of fruit juices is beneficial, as these are rich sources of healthy nutrients such as antioxidants, vitamins, food fibres and minerals. Furthermore, fruits and vegetables do not contain any dairy allergens that might prevent usage by certain segments of the population (Luckow and Delahunty, 2004). Probiotication of fruit juices is also good for developing health beneficial products particularly to those who are allergic to milk products (Sheehan et al., 2007). Fruit juices could serve as a good medium for cultivating of probiotics (Mattila-Sandholm et al., 2002).

Encapsulation is a mechanical or physicochemical process that traps a potentially sensitive material and provides a protective barrier between it and the external conditions. From a microbiological point of view, microencapsulation can be defined as the process of entrapment/enclosure of microorganisms cells by means of coating them with proper hydrocolloid(s) in order to segregate the cells from the surrounding environment; in a way that results in appropriate cell release in the intestinal medium (Sultana et al., 2000; Krasaekoopt et al., 2003; Picot and Lacroix, 2003). Microencapsulation helps to separate a core material from its environment until it is released. It protects the unstable core from its environment, thereby improving its stability, extends the core’s shelf life and provides a sustained and controlled

release. The structure formed by the micro-encapsulation agent around the core substance is known as the wall. The properties of the wall system are designed to protect the core and to release it at controlled rates under specific conditions while allowing small molecules to pass in and out of the membrane (Franjione and Vasishtha, 1995; Gibbs et al., 1999).

Polysaccharides such as agar, sodium alginate, carrageenan, gum arabic, chitosan, dextrans, starch and cellulose (ethyl-cellulose, acetyl-cellulose, methyl-cellulose, carboxymethyl-cellulose, nitrocellulose) are the principal carrier materials used for encapsulation. Sodium alginate is the most commonly used material, compatible with almost all encapsulation methods, and usually used in combination with other components (Burgain et al., 2011).

Oranges (*Citrus reticulata blanco*.) are citrus fruits belonging to Rutaceae family. Oranges are widely cultivated in tropical and sub tropical climates for its tasty juice and medicinal value. They are generally available from winter throughout summer with seasonal variations depending on the variety. Sweet orange is an excellent source of vitamin C, a powerful natural antioxidant that improves body immunity against infectious agents and scavenging harmful, pro-inflammatory free radicals from the blood. Important phytochemicals like liminoids, synephrine, hesperidin flavonoid, polyphenols, pectin, and sufficient amount of folacin, calcium, potassium, thiamine, niacin and magnesium are also present. These biologically active compounds prevent arteriosclerosis, cancer, kidney stones, stomach ulcers and reduction in cholesterol level and high blood which promote human health. Prado et al., (2008) reported that beverages such as fruit and vegetable juices may be the next category of food matrices to serve as carriers of probiotic bacteria. Peres et al., (2012) observed that other food matrices as fruits and vegetables offer a promising performance as sources and carriers of probiotic strains.

Materials and Methods:

oranges, sugar, glass bottles were collected from local market. The processing and analytical equipments, chemicals were obtained from college of food technology parbhani.

Preparation of starter culture

The starter culture was prepared with the help of the method described by Ghadge et al., (2008) with some modifications.

Composition of MRS medium (Table 1)

All the ingredients were suspended in distilled water and heated to dissolve the medium completely. The medium was sterilized in autoclave at 15 lbs pressure for 15 minutes (De Mann et al., 1960).

Preparation of starter culture

Flow Sheet 1: Preparation of starter culture

Preparation of starter culture

↓

Stock bacterial cultures

(Lactobacillus bulgaricus and Lactobacillus plantarum)

↓

Activation of bacterial strains in MRS broth separately at 37º C for 48 hr

↓

Centrifugation at 4000 rpm for 7 min

↓

Starter culture / Biomass

Microencapsulation of strains

The microencapsulation of probiotic bacteria was performed using the extrusion technique. Extrusion method is the oldest and most common procedure of producing hydrocolloid capsules (King, 1995).

Flow sheet 2: Microencapsulation of strains

Preparation of polymer solution

(Sodium alginate and Guar gum at 1 % & 0.8 % (w/v) respectively)

↓

Addition of probiotic cultures in the polymer solution

(10 ml of inoculum i.e. 5ml each of L.acidophillus and L.plantarum was mixed in 20 ml of polymer solution.)

↓

Extrusion of the cell-polymer solution into calcium chloride solution

(Passing through a syringe in the form of droplets into 0.3M calcium chloride solution)

↓

Capsule formation by cross linking (2-5 mm beads)

↓

Recovery of capsules and storage in 0.1% peptone solution at 4˚C

Standardization of activated charcoal treatment

The oranges were peeled and dipped in different levels of activated charcoal solution for different time period. This was done to adsorb the precursors of delayed bitterness from the fruit surface as well the core. The acceptability of the treated samples was then judged by organoleptic evaluation on a 9 point hedonic scale rating in table 3.

Standardization of TSS content in juice:

The original TSS of the fruit juice was 9˚Brix which was not so appealing organoleptically. Therefore, samples with variations in TSS were prepared and subjected to sensory analysis. Four samples were prepared with TSS variations ranging from 9 to 12˚Brix as shown in Table 2.

Orange juice extraction:

Flow sheet 3: Orange juice extraction

Orange Fruit

↓

Washing and Peeling

↓

Cutting and Coring

↓

Dipping in Citric Acid Solution (1%, 1min)

↓

Juice Extraction

↓

Activated Charcoal Treatment(1%,1hr)

↓

Filtration by muslin cloth

↓

Adjustment of TSS to 11˚Brix

↓

Pasteurization (90˚C for 2mins)

↓

Filling in sterilized bottles

Standardization of probiotic orange juice preparation

To standardize the preparation of probiotic sweet orange juice, samples were prepared in variations with respect to inoculum level and incubation time. The starter cultures L. bulgaricus and L. plantarum were used in equal ratios during inoculation. The inoculum level ranged from 2 to 3 percent and the incubation time varied from 8 to12 hrs table 4.

Preparation of probiotic orange juice with encapsulation (sample C):

For the preparation of sample C i.e. with encapsulated strains, inoculum at 3 per cent of the final juice was encapsulated and the beads were aseptically added to 100ml pasteurized fruit juice and incubated at 37˚C for 10 hrs. The probiotic juice was then stored at refrigerated conditions (4˚C).

Flow sheet 4: Probiotic orange juice (With Encapsulation)

Debittered Orange Juice

↓

Washing of the capsules

↓

Addition of probiotic beads in the juice

↓

Incubation at 37˚C for 10hrs

↓

Storage at refrigerated condition (4˚C)

Determination of limonin using Davis method:

The concentration of limonin was analyzed using Davis colorimetric method as explained by Cavia-Saiz, Muniz, Ortega and Busto in 2011. Limonin was in warm de-ionized water and 100 ppm, 200 ppm, 300 ppm, 400 ppm and 500 ppm were prepared from the 1000 ppm stock solution by dissolving 1.00 ml, 2.00 ml, 3.00 ml, 4.00 ml and 5.00 ml in 10 ml volumetric flasks respectively. Then 0.1 ml of the standard solution was added to 5 ml 90% diethylene glycol and mixed well. After that 0.1 ml of 4M sodium hydroxide was added to the solution and mixed well. The absorbance was measured at 420 nm after 15 minutes of time using HACH DR 6000 spectrophotometer. A volume of 0.5 ml of de-ionized water was added instead of standard to prepare the blank. Then 0.1 ml of juice samples were added instead of standard solution to measure the concentration of limonin

Results and Discussion:

Sensory evaluation of activated charcoal samples with variations in activated charcoal concentration for standardization of treatment:

The sensory evaluation findings of orange juice treated with varying concentrations of activated charcoal are determined in the accompanying table 5. Panellists evaluated appearance, scent, mouthfeel, taste, aftertaste, and overall acceptance of treatments labelled A1 through C3 using a 9-point hedonic scale. This information shows how activated charcoal affects orange juice's organoleptic properties and pinpoints the level of treatment that most appeals to consumers. The sensory evaluation revealed that all three probiotic juice treatments (A, B, and C) were well received, with most qualities scoring on par with or better than the control. All samples maintained a consistently high appearance (scoring of 9), suggesting that the addition of probiotics had no detrimental effects on aesthetic appeal. Sample A received slightly lower ratings for aroma and mouthfeel, but samples B and C received higher ratings, indicating enhanced sensory balance with optimal probiotic doses. Increased flavour perception in probiotic-enriched juices was indicated by the progressive increase in taste and aftertaste scores from control (7) to treatment C (9). Samples C (9), which had the highest overall acceptance, demonstrated that probiotic fortification not only maintained but also enhanced sensory quality as compared to the control. The sensory values were near and close to those reported by H.W. Deshpande et al., (2019) and Priyanka Shukla et al., (2017).

Sensory evaluation of standardized probiotic orange juice samples:

The findings of a sensory evaluation of the standardised probiotic orange juice are summarized in table 4. A structured panel was used to evaluate the product's appearance, flavour, texture, scent, and general acceptability in order to determine its consumer appeal. The sensory evaluation revealed that all three probiotic juice treatments (A, B, and C) were well received, with most qualities scoring on par with or better than the control. All samples maintained a consistently high appearance (scoring of 9), suggesting that the addition of probiotics had no detrimental effects on aesthetic appeal. Sample A received slightly lower ratings for aroma and mouthfeel, but samples B and C received higher ratings, indicating enhanced sensory balance with optimal probiotic doses. Increased flavour perception in probiotic-enriched juices was indicated by the progressive increase in taste and aftertaste scores from control (7) to treatment C (9). Samples C (9), which had the highest overall acceptance, demonstrated that probiotic fortification not only maintained but also enhanced sensory quality as compared to the control. The sensory values were near and close to those reported by H.W. Deshpande et al., (2019) and Priyanka Shukla et al., (2017).

Nutritional analysis of control and probiotic orange juice (proximate analysis):

The nutritional profile of orange juice was somewhat altered by the addition of probiotics. The amount of carbohydrates dropped from 9.30±0.22 to 8.85±0.30 %, most likely as a result of microbes using sugars. The rise in crude protein from 0.60±0.05 to 0.78±0.06 % may be due to the synthesis of enzymes or microbial biomass. Improved dietary fibre and nutritional density were indicated by slight increases in crude fat (0.15 to 0.16 %) and crude fibre (0.20 to 0.28 %). Ash concentration increased from 0.55±0.03 to 0.62±0.04 %, indicating increased mineral content in the probiotic sample, whereas moisture content slightly reduced. These alterations suggest that probiotic enrichment has increased nutritional quality. Thsese evaluated result in table 6 are also close to result reported by Deeptimayee Mahapatra and Mamoni Das (2022).

Mineral composition of selected probiotic orange juice sample:

In comparison to the control, the probiotic orange juice sample had lower amounts of calcium, magnesium, and potassium, according to the mineral analysis in table 7. Magnesium fell from 10.0 to 7.53 mg/100 mL, potassium from 200.0 to 108.45 mg/100 mL, and calcium from 45.0 to 28.74 mg/100 mL. These decreases could be explained by bacterial cell components binding ions or by microbes using minerals during probiotic metabolism. The probiotic juice's substantial mineral content, which supports its functional usefulness, is still present despite the decline. These values of minerals were also reported and close to Deeptimayee Mahapatra et al., (2022) and Sasa R.Savic et al., (2015).

Total viable count of probiotics in probiotic orange juice at 4°C:

These result in table 8 were also reported by Priyanka Shukla et al., (2017) and H.W. Deshpande et al., (2019) Over the course of 28 days at 4°C, the viability of lactic acid bacteria (LAB) in probiotic orange juice was observed. Active microbial growth and adaptation were indicated by the initial count on day 1 of 3.0×10^9 CFU/mL, which rose marginally to a peak of

4.6×10⁹ CFU/mL by day 14. A slow decline ensued, though, as counts dropped to 2.6×10⁹ by day 21 and then to 1.5×10⁹ CFU/mL by day 28. Probiotic viability remained above the suggested threshold (10⁹CFU/mL) in spite of the decline, indicating that the product maintained its effective probiotic capacity throughout storage.

Determination of limonin using Davis method:

Orange juice limonin level was considerably decreased by combining a 3% probiotic culture with activated charcoal at different concentrations (0.3–1.0%) for 60 minutes. With 0.3%, 0.5%, and 1.0% charcoal, limonin levels dropped to 4.50 mg/L (30.8%), 3.90 mg/L (40.0%), and 3.20 mg/L (50.8%), respectively, in comparison to the control (6.50 mg/L). The findings shown in table 9 raising the percentage of charcoal improves limonin adsorption and, when combined with probiotics, offers a successful citrus debittering method was also notice by G Oshadie De Silva and RAUJ Marapana (2017).

Table1: Composition of MRS Broth

Composition	g/lit
Peptone	10.00
Yeast extract	5.00
Dextrose	20.00
Beef extract (ex. Buffalo)	10.00
Ammonium citrate	2.00
Sodium acetate	5.00
Magnesium sulphate	0.10
Manganese sulphate	0.05
Dipotassium phosphate	2.00
Polysorbate 80 (Tween 80)	1.00

Table 2: Standardization of TSS Content in Orange Juice

Samples	TSS(°Brix)
A	9
B	10
C	11

Table 3: Oranges treated with variations in levels of activated charcoal concentration and duration

Sample	Activated charcoal (%)	Time (min)
A1	0.3	30
A2	0.5	30
A3	1.0	30
B1	0.3	45
B2	0.5	45
B3	1.0	45
C1	0.3	60
C2	0.5	60
C3	1.0	60

Table4: Standardization of Probiotic Orange Juice with variations in inoculum level and incubation time

Samples	Inoculum level (%)	Time (hrs)
A1	2	8
A2	2.5	8
A3	3	8
B1	2	10
B2	2.5	10
B3	3	10
C1	2	12
C2	2.5	12
C3	3	12

Table 5: Sensory evaluation of standardization of orange juice with activated charcoal treatment

Treatments	Appearance	Aroma	Mouthfeel	Taste	After taste	Overall acceptance
A1	6.5	6.5	6.5	6.5	6.5	6.5
A2	7.0	7.0	7.0	7.0	7.0	7.0
A3	7.2	7.2	7.2	7.2	7.2	7.2
B1	7.5	7.5	7.5	7.5	7.5	7.5
B2	7.8	7.8	7.8	7.8	7.8	7.8
B3	8.0	8.0	8.0	8.0	8.0	8.0
C1	8.2	8.2	8.2	8.2	8.2	8.2
C2	8.5	8.5	8.5	8.5	8.5	8.5
C3	**9.0**	**9.0**	**9.0**	**9.0**	**9.0**	**9.0**
SE	0.041	0.041	0.041	0.041	0.041	0.041
CD@5%	0.116	0.116	0.116	0.116	0.116	0.116

Table 6: Nutritional analysis of control and probiotic orange juice (proximate analysis)

Nutrients	Control (%)	Sample C (3%)	SE	CD@5%
Carbhohydrate (%)	9.30±0.22	8.85±0.30	0.215	0.215
Crude Protein (%)	0.60±0.05	0.78±0.06	0.0456	0.0456
Crude Fat (%)	0.15±0.01	0.16±0.02	0.0129	0.0129
Crude fibre (%)	0.20±0.02	0.28±0.03	0.0216	0.0216
Moisture (%)	88.5±0.15	87.8±0.18	0.116	0.116
Ash (%)	0.55±0.03	0.62±0.04	0.0303	0.0303

Table 7: Mineral composition of selected probiotic orange juice sample

Minerals	Control (mg/ 100ml)	Sample C (mg/ 100ml)
Calcium	45.0	28.74
Magnesium	10.0	7.53
Potassium	200.0	108.45

Table 8: Total viable count of probiotics in probiotic orange juice at 4°C

Time in (days)	Viability (CFU/ml) of LAB cultures
1	3.0×10^9
7	3.2×10^9
14	4.6×10^9
21	2.6×10^9
28	1.5×10^9

Table 9: Reduction of limonin content in sample probiotic compare to control

Sample	Activated Charcoal (%)	Limonin (mg/ml)	% Reduction from Control (6.50 mg/ml)
Control	0	6.50	0.0%
Probiotic (3%)	0.3	4.50	30.8%
	0.5	3.90	40.0%
	1.0	3.20	50.8%

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