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Changes in microbial quality of fruit juices, syrups, and ready-to-serve carbonated drinks produced with different processing parameters and stored in different conditions within six months

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*This research article is from my master thesis who I completed in the MPKV, Rahuri Agricultural University, Maharashtra State, India

ABSTRACT

The research was carried out during 2015-2017, aimed to consider the microbial quality of juice, syrup, and ready-to-serve (RTS) carbonated beverage prepared from mandarin fruit (Nagpur cultivar). Fully ripened, mature, fresh fruits were washed and peeled, then the juice was extracted using a screw-type pulper. The syrup and ready-to-serve carbonated beverage prepared from the extracted juice. Microbial analysis of juice was carried out by using Potato Dextrose Agar (PDA) culture medium. The results revealed no microbial growth in the ready-to-serve carbonated beverage up to 60 d of storage, after that from 90 to 180 d of storage was negligible. In the syrup, up to 90 d was no detection of microbes; after that, up to 180 d of storage was negligible. In the juice samples under cold storage (S2), all the treatments were within acceptable levels for 180 d. But under room temperature (S1), eight treatments (T1, T2, T3, T4, T5, T6, T8, T9) showed microbial colonies more than acceptable level, and only one treatment (T7-S1P3B1= juice sample in the room storage (SI) which added 350 ppm sodium benzoate as chemical preservative (P3) and packed in the glass bottle (B1) was remained safe for consumption during 180 d of storage. The microbial quality, viz. yeast and mold count were increased during 180 d of storage in the ready-to-serve carbonated beverage, syrup and juice. The microbial growth was observed within the acceptable level in all treatment combinations of ready-to-serve carbonated beverage, syrup; and juice in cold storage and T7 of juice under ambient conditions.

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1. Introduction

The mandarin fruits are juicy, and the fresh mandarin juice has refreshing flavor characteristics, pleasing aroma, and thirst-quenching properties. Besides that, it is the main source of important phytochemical nutrients, antioxidants, vitamins, minerals, soluble and insoluble dietary fibers that help reduce the risk for cancers as well as many chronic diseases, for example arthritis, obesity, and coronary heart diseases (Kamaljeet, 2002). A single mandarin is said to have about 170 phytonutrients and over 60 flavonoids with anti-tumor, anti-inflammatory, and blood clot inhibiting properties (Aslin, 2014; Etebu et al., 2014; Faizi and Mahen, 2020). Kumar (2009) revealed that for juice processing, it is essential to use cultivars with high juice content, good Brix-acidity balance, and having juice with attractive color. Vadakkan et al. (2010) mentioned that fruit juice has a low shelf life and needs preservation via various methods. Carbonation is the best one with few changes in quality parameters. Adding sufficient CO_2 into beverages was reported to enhance the appearance, flavor, taste and overall acceptability due to increased acidity, taste, sparkle and unique taste of CO_2 gas. Sandhan (2003) observed an increasing trend in the microbial count during the storage of carbonated beverages. Ahire (2007) reported that juice stored in cold storage (5.0 °C) condition in glass bottles could be stored satisfactorily for up to 3 months compared to ambient storage. Glevitzky et al. (2009) noticed that the enzymatic and microbiological reactions take place faster, leading to faster degradation and increased microbial potential in the beverages at room temperature storage. Beuchat (1982) studied the thermal inactivation of five yeasts (*Candida krusei, Hansenula anomala, Saccharomyces bailii, S. cerevisiae* and *Torulopsis magnolia*) suspended in five fruit juices (apple, apricot, grape, orange, and pineapple) with potassium sorbate, sodium benzoate, and sucrose. Yeasts were most sensitive to heat when suspended in orange juice. Both preservatives, at a concentration as low as 100 ppm, enhanced the inactivation rate in juices containing no added sucrose. Grandall et al. (1982) noted that preventing microbial spoilage is also an important element of storage stability. Covadonga et al. (2002) revealed that economic losses due to juice spoilage are minimized by good sanitation procedures before and

during citrus processing. Pasteurization, concentration, or low-temperature storage protocols help reduce the number of micro-organisms in the final product. Citrus juices are acidic beverages with high sugar content. Under these conditions, acidolactic bacteria, molds, and yeasts comprise the typical microbiota present in citrus juices. Lactic acid bacteria are the primary spoilage bacteria in fruit drinks; however, their numbers are greatly reduced after pasteurization, concentration, and refrigeration. Molds and yeasts tolerate high-osmotic and low-pH conditions and grow at refrigeration temperatures and can therefore cause spoilage in the processed product. Polydera et al. (2003) determined that yeasts, mold, and lactic acid bacteria are the micro-organisms responsible for the spoilage of orange juice during storage. Himani (2003) studied microbiological evaluation of kinnow squash, RTS, and concentrates and found that yeast and mold counts were found to be much below the permissible limits and were detected higher than permissible limits only in the long storage period. Ashurst (2010) mentioned that all beverages are at risk of suffering deterioration due to microbial action. Almost all fruit drinks are acidic in nature; the principle risk is microbial spoilage rather than contamination by a pathogenic organism. The main effects of microbial contamination are likely to be the development of off-flavors and changes in physical appearance. Bhardwaj (2011) studied microbiological analysis of stored juice samples of Kinnow mandarin for six months and observed that all the samples were contaminated with a large variety of bacterial, fungal, and mold species but within the acceptable limit. Kumar et al. (2011) noted that there was no microbial growth in the RTS beverages prepared with sweet lime juice; the increase in microbial load after 45 d of storage was negligible and safe for consumption. Chukwumalume (2012) carried out studies on microbiological assessment of preservative methods for African star apple juice. The juice sample was pasteurized and preserved with sodium benzoate 0.1% at ambient and refrigeration temperature for six weeks. The results showed that the combination of pasteurization, use of sodium benzoate, and storage at refrigeration temperature gave the best storage stability with a minimum microbial load. Oranusi et al. (2012) mentioned that most fruit juices are acidic enough and have sufficient sugar to favor yeast growth. Molds are generally considered the least important group of micro-organisms causing spoilage in fruit juice because of their limitation and inability to grow in the absence of air (anaerobic conditions) except for a few molds such as *Penicillium* and spore-forming *Aspergillus*. The presence of microbial contaminants in all the products could reflect the quality of the raw materials, processing equipments, environment, packaging materials and the personnel in the production process. Bhardwaj (2013) found that the untreated fruit juices and pulp were highly contaminated with bacteria, yeast, and mold. The minimum increase in bacteria, yeast, and mold population was observed when juice was processed at 85°C temperature for 15 min with the addition of preservative chemicals. Verma et al. (2014) conducted an experiment on the utilization of aonla (Indian gooseberry) and lime for the development of fruit-based carbonated soft drinks, studied the microbial evaluation of the carbonated fruit drinks, and found a non-significant presence of microbes in the blends. The yeasts-mold count ranged from 0.30×101 to 0.35×101 cfu mL^{-1}. Carbonation removes air, creating anaerobic conditions, and hence controls mold and yeast. Ogodo et al. (2016) observed *Aspergillus* species, *Rhizopus* species and *Penicillium* species in commercially packed fruit juices. *Penicillium* sp. and *Aspergillus* sp. could produce mycotoxins, which could lead to health hazards for the consumers. The research was done to consider the microbial quality (yeast and mold) of juice, syrup, and ready-to-serve (RTS) carbonated beverages of Nagpur mandarin fruit at different processing parameters which stored in the two storage conditions within 180 days of storage in 2015-2017.

2. Materials and methods

2.1. Materials

The research was conducted at the Postharvest Technology Center of Horticultural Crops and Microbial Quality Analysis in the Laboratory of the Mycology Department of Mahatma Phule Krishi Vidyapeeth (MPKV) Agricultural University during 2015-17. Fully ripened (horticultural maturity = ready to eat), mature (physiological maturity = ready to harvest), fresh, and good mandarin fruits of Nagpur mandarin cultivar from an orchard located in Ahmednagar district were provided. The juice was extracted, the syrup prepared (Faizi et al., 2020) from extracted juice, and ready-to-serve (RTS) carbonated beverage was made from previously prepared syrup by dilution (Faizi, 2022). The packing materials such as pet bottles, glass bottles and standy pouches were obtained from Postharvest Technology Centre, MPKV, Rahuri for packing the juice, syrup, and carbonated RTS drinks. Food grade and analytical grade chemicals obtained from manufacturers M/s. Thermo Fisher Scientific India Pvt. Ltd., Mumbai; M/s. Qualigens Fine Chemicals, Mumbai; M/s. E. Merck (India) Ltd., Mumbai and M/s. S. D. Fine-Chem Ltd., Mumbai, was used. All the glass wares used were obtained from manufacture M/s. Borosil Glass Works Ltd. (BGWL), Ahmedabad, Gujarat. Carbonation cum crown corking machine (Make: M. G. Industries, Coimbatore) was used for carbonation and crown corking of the ready-to-serve beverage. The screw-type pulper machine (Make: M.G. Industries, Coimbatore) was used to extract juice from fruits.

2.2. Methods

Mandarins were washed with cold tap water and peeled (Figure 1A). Then the juice was extracted using a screw-type pulper. The juice and the pomace were collected separately in two outlets. The juice was filtered through a clean muslin cloth. The extracted juice was pasteurized at 65°C for 15 min by adding sodium benzoate as the preservative. Then, at that temperature juice was filled in the pre-sterilized 200 mL glass bottles, 200 mL pet bottles, and 200 mL stand pouches (Figure 1B) and sealed with a crown cork and pouch sealer. All packed juice samples were sterilized (were kept in the boiling water for 10-20 min) as well after closing. Sugar syrup was prepared by adding water to sugar to boiling at a temperature of 90°C. Then the sugar syrup temperature decreased to 60°C, and the juice was mixed well. The syrup was bottled in the presterilized 200 mL transparent glass bottles and pet bottles (Figure 1C) and then sealed. After bottling, all syrup samples were sterilized.

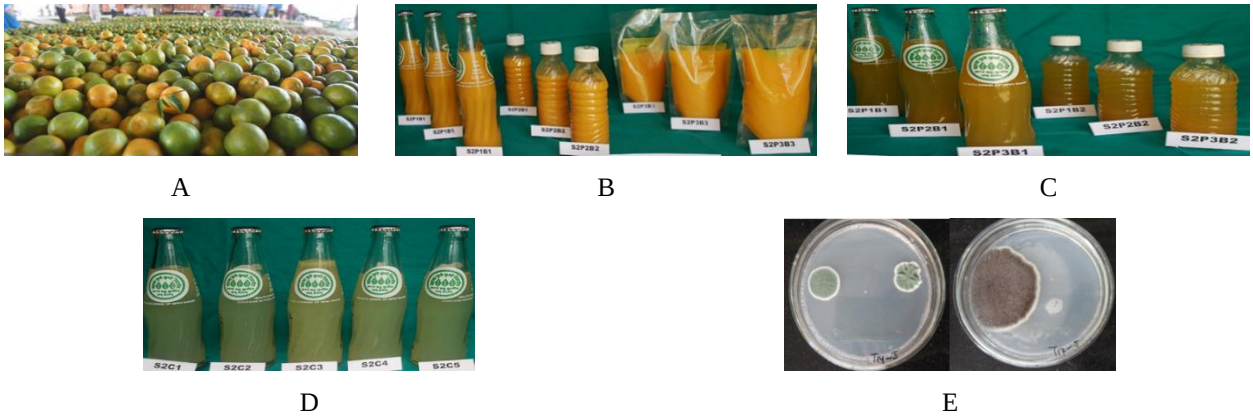

Figure 1. Fresh mandarin cultivar Nagpur which used for juice extraction (A). Prepared juice for microbial analysis in glass bottles, pet bottles, and standy pouch (B). Prepared syrup for microbial analysis in glass bottles and pet bottles (C). Prepared ready-to-serve (RTS) carbonated beverage for microbial analysis in glass and pet bottles (D). Mold colony forming of the spoiled juice samples in the PDA medium (E).

Table 1. Treatment details of juice, syrup and ready to serve (RTS) carbonated beverages

Tre. No.		Treatment Combinations
		Juice
TI	S1P1B1	Room Storage (19.80 - 27.60°C and 43.00 - 70.60% RH) + 150 ppm Sodium Benzoate + Glass Bottle.
T2	S1P1B2	Room Storage + 150 ppm Sodium Benzoate + Pet Bottle.
T3	S1P1B3	Room Storage + 150 ppm Sodium Benzoate + Stand Pouch.
T4	S1P2B1	Room Storage + 250 ppm Sodium Benzoate + Glass Bottle
T5	S1P2B2	Room Storage + 250 ppm Sodium Benzoate + Pet Bottle
T6	S1P2B3	Room Storage + 250 ppm Sodium Benzoate + Stand Pouch.
T7	S1P3B1	Room Storage + 350 ppm Sodium Benzoate + Glass Bottle.
T8	S1P3B2	Room Storage + 350 ppm Sodium Benzoate + Pet Bottle.
T9	S1P3B3	Room Storage + 350 ppm Sodium Benzoate + Stand Pouch.
T10	S2P1B1	Cold Storage (5.0±2.0°C and 92-95% RH) + 150 ppm Sodium Benzoate + Glass Bottle.
T11	S2P1B2	Cold Storage + 150 ppm Sodium Benzoate + Pet Bottle.
T12	S2P1B3	Cold Storage + 150 ppm Sodium Benzoate + Stand Pouch.
T13	S2P2B1	Cold Storage + 250 ppm Sodium Benzoate + Glass Bottle
T14	S2P2B2	Cold Storage + 250 ppm Sodium Benzoate + Pet Bottle
T15	S2P2B3	Cold Storage + 250 ppm Sodium Benzoate + Stand Pouch.
T16	S2P3B1	Cold Storage + 350 ppm Sodium Benzoate + Glass Bottle.
T17	S2P3B2	Cold Storage + 350 ppm Sodium Benzoate + Pet Bottle.
T18	S2P3B3	Cold Storage + 350 ppm Sodium Benzoate + Stand Pouch.
		Syrup
TI	S1P1B1	Room Storage (19.80 - 27.60°C and 43.00 - 70.60% RH) + 150ppm Sodium Benzoate + Glass Bottle.
T2	S1P1B2	Room Storage + 150 ppm Sodium Benzoate + Pet Bottle.
T3	S1P2B1	Room Storage + 250 ppm Sodium Benzoate + Glass Bottle
T4	S1P2B2	Room Storage + 250 ppm Sodium Benzoate + Pet Bottle
T5	S1P3B1	Room Storage + 350 ppm Sodium Benzoate + Glass Bottle.
T6	S1P3B2	Room Storage + 350 ppm Sodium Benzoate + Pet Bottle.
T7	S2P1B1	Cold Storage (5.0±2.0°C and 92-95% RH) + 150 ppm Sodium Benzoate + Glass Bottle.
T8	S2P1B2	Cold Storage + 150 ppm Sodium Benzoate + Pet Bottle.
T9	S2P2B1	Cold Storage + 250 ppm Sodium Benzoate + Glass Bottle
T10	S2P2B2	Cold Storage + 250 ppm Sodium Benzoate + Pet Bottle
T11	S2P3B1	Cold Storage + 350 ppm Sodium Benzoate + Glass Bottle.
T12	S2P3B2	Cold Storage + 350 ppm Sodium Benzoate + Pet Bottle.
		Ready to Serve (RTS) Carbonated Beverage
TI	S1C1	Room Storage (19.80 - 27.60°C and 43.00 - 70.60% RH) + Carbonation Pressure (C)= 70 psi (Pound Per Square Inch).
T2	S1C2	Room Storage + Carbonation Pressure (C)= 80 psi (Pound Per Square Inch).
T3	S1C3	Room Storage + Carbonation Pressure (C)= 90 psi (Pound Per Square Inch).
T4	S1C4	Room Storage + Carbonation Pressure (C)= 100 psi (Pound Per Square Inch).
T5	S1C5	Room Storage + Carbonation Pressure (C)= 110 psi (Pound Per Square Inch).
T6	S2C1	Cold Storage (5.0±2.0°C and 92-95% RH) + Carbonation Pressure (C)= 70 psi (Pound Per Square Inch).
T7	S2C2	Cold Storage + Carbonation Pressure (C)= 80 psi (Pound Per Square Inch).
T8	S2C3	Cold Storage + Carbonation Pressure (C)= 90 psi (Pound Per Square Inch).
T9	S2C4	Cold Storage + Carbonation Pressure (C)= 100 psi (Pound Per Square Inch).
T10	S2C5	Cold Storage + Carbonation Pressure (C)= 110 psi (Pound Per Square Inch).

Carbonated RTS beverage was prepared from the syrup. The 40 mL of syrup were added to 200 mL transparent glass bottles (Figure 1D), and bottles were filled with chilled water and different carbon dioxide levels (carbonation). The bottles were sealed simultaneously by a crown corking machine and sterilized. All the juice, syrup, and carbonated RTS beverage were stored at ambient and cold conditions and evaluated at an interval of 30 d for 180 d for microbial quality. Treatment details of juice, syrup and ready to serve (RTS) carbonated beverages is given in the Table 1.

2.3. Microbial analysis

The microbial analysis (yeast and mold count) of juice was carried out accordingly (Adedeji and Oluwalana, 2013; Michael, 2021). Potato Dextrose Agar (PDA) culture medium was used. Ingredients of the PDA culture medium used for analysis were included Potato infusion from peeled potato 200 g, Dextrose 20 g, agar 20 g, water 1 L, and antibiotic (chlortetracycline or streptomycin or chloramphenicol) 25-40 mg. For preparation of the medium, potatoes peeled, then put in 1000 mL distilled water added on it then boiled for 10-20 min. After that sieving by gauze and refill distilled water to 1000 ml. Then added in dextrose and agar and melted down. After that, the medium sterilized at 121°C for 20 min. In the following, a small amount of ethanol was used to dissolve Chloromycetin and put into the culture medium before pouring it into the flat plates. It is mentionable that antibiotics were used to prevent bacteria growth and allowing easy growth of yeast and molds in medium during incubation. An aseptic micropipette used to pipet 1 mL of sample into a test tube containing 9 mL of sterile distilled water. Then, another 1 mL aseptic pipette was used to repeat dilution for 6 times. One mL of each from appropriate dilution was plated (pour plating) in the required medium (PDA), then incubated at 28±1°C for 5-7 d. Then observed and took records. The yeasts and molds colonies expressed as cfu (colony forming units) per mL. Counted the yeasts and molds respectively according to their appearance in the medium when the molds covered the whole plate the average of 2 plates counted for colony count. Calculated the average value based on two plate counts. If the colony counts from all plates were two or more than two cfu/mL, the sample reported as a spoiled sample; if less than two, it was reported acceptable and saved for consumption.

3. Results

3.1. Microbial quality (yeast and mold) of ready-to-serve (RTS) carbonated beverage

The data presented in Table 2 showed that there was no microbial (yeast and mold) growth in the carbonated RTS beverage of Nagpur mandarin up to 60 d of storage in ambient and cold storage situations prepared under relatively hygienic conditions condition. Increase in microbial load after 60 d from 90 to 180 d of storage was negligible and within the acceptable level (less than 2.00 colony forming units per mL) and safe for consumption.

3.2. Microbial quality (yeast and mold) of syrup

The data presented in Table 3 indicated no detection (ND) of microbes (yeast and mold) up to 90 d of storage. There was no microbial detection in treatments T10, T11, and T12 under cold storage conditions during 120 and 150 d compared to other treatments. From 120 to 180 d of storage, there was microbial detection in all the treatments, both in ambient condition/room temperature (RT) and cold storage (CS). Still, it was negligible, within an acceptable level (less than 2.00 colony forming units per mL), and safe for consumption.

3.3. Microbial quality (yeast and mold) of juice

The data presented in Table 4 indicated that there was no detection (ND) of microbes (yeast and mold) in cold storage and treatment T7 under ambient conditions. There was microbial detection in treatments T1, T2, T3, T4, T5, T6, T8, and T9 at ambient conditions up to 90 d of storage. Still, it was negligible, within the acceptable level (less than 2.00 colony forming units per mL), and safe for consumption. From 90 to 180 d of storage, there was microbial detection in ambient conditions (S1) and cold storage (S2). Under CS (S2), all the treatments were within acceptable level during 180 days of storage, but under RT (S1), eight treatments (T1, T2, T3, T4, T5, T6, T8, T9) showed microbial colony (Figure 1E) more than acceptable level (2 or more than two colony forming unit per mL), and only one treatment (T7-S1P3B1) was remained safe for consumption during 180 d of storage.

Table 2. Effect of storage conditions and carbonation levels on microbial (yeast and mold) quality of carbonated RTS Beverage and their treatment combinations. count (cfu/mL)

Treatment Combinations		Storage Period/ Count Period						
		0 days	30 days	60 days	90 days	120 days	150 days	180 days
TI	S1C1	ND	ND	ND	1.34	1.42	1.48	1.52
T2	S1C2	ND	ND	ND	1.3	1.38	1.44	1.48
T3	S1C3	ND	ND	ND	ND	ND	1.42	1.46
T4	S1C4	ND	ND	ND	ND	1.31	1.37	1.41
T5	S1C5	ND	ND	ND	1.23	1.27	1.33	1.37
T6	S2C1	ND	ND	ND	1.19	1.25	1.31	1.35
T7	S2C2	ND	ND	ND	1.17	1.21	1.27	1.31
T8	S2C3	ND	ND	ND	ND	ND	1.23	1.27
T9	S2C4	ND	ND	ND	1.04	1.12	1.18	1.22
T10	S2C5	ND	ND	ND	1.03	1.11	1.17	1.21

S1= ambient storage/room storage. S2= cold storage. C1= 70 psi. C2=80 psi. C3= 90 psi. C4= 100 psi. C5= 110 psi. ND= not detected. cfu= colony forming unit. C= Carbonation Pressure. Psi= Pound Per Square Inch.

Table 3. Effect of storage conditions, preservative levels, and packing materials on syrup's microbial (yeast and mold) quality and their treatment combinations. count (cfu/mL)

Treatment Combinations		Storage Period/ Count Period						
		0 days	30 days	60 days	90 days	120 days	150 days	180 days
TI	S1P1B1	ND	ND	ND	ND	1.39	1.42	1.46
T2	S1P1B2	ND	ND	ND	ND	1.42	1.45	1.49
T3	S1P2B1	ND	ND	ND	ND	1.28	1.31	1.35
T4	S1P2B2	ND	ND	ND	ND	1.32	1.35	1.39
T5	S1P3B1	ND	ND	ND	ND	1.21	1.24	1.28
T6	S1P3B2	ND	ND	ND	ND	1.26	1.29	1.33
T7	S2P1B1	ND	ND	ND	ND	1.15	1.18	1.22
T8	S2P1B2	ND	ND	ND	ND	1.17	1.2	1.24
T9	S2P2B1	ND	ND	ND	ND	1.07	1.1	1.14
T10	S2P2B2	ND	ND	ND	ND	ND	ND	1.11
T11	S2P3B1	ND	ND	ND	ND	ND	ND	1
T12	S2P3B2	ND	ND	ND	ND	ND	1.02	1.05

S1= ambient storage/room storage. S2= cold storage. P1= 150 ppm sodium benzoate. P2= 250 ppm sodium benzoate. P3= 350 ppm sodium benzoate. B1= glass bottle. B2= pet bottle. ND= not detected. cfu= colony forming unit.

Table 4. Effect of storage conditions, preservative levels, and packing materials on microbial (yeast and mold) quality of juice and their treatment combinations. count (cfu/mL)

Treatment Combinations		storage period/ count period						
		0 days	30 days	60 days	90days	120 days	150 days	180 days
TI	S1P1B1	ND	1.78	1.81	1.84	1.87	3.5*	
T2	S1P1B2	ND	1.82	1.85	1.89	2.6*		
T3	S1P1B3	ND	1.89	1.92	1.98	14*		
T4	S1P2B1	ND	ND	1.67	1.7	1.76	1.77	3*
T5	S1P2B2	ND	1.67	1.7	1.73	1.76	3*	
T6	S1P2B3	ND	1.74	1.77	1.8	1.8	2*	
T7	S1P3B1	ND	ND	ND	ND	1.54	1.6	1.63
T8	S1P3B2	ND	ND	ND	1.67	1.73	1.78	4*
T9	S1P3B3	ND	ND	1.74	1.77	1.78	2.3*	
TI0	S2P1B1	ND	ND	ND	ND	1.41	1.47	1.5
T11	S2P1B2	ND	ND	ND	ND	1.46	1.52	1.55
T12	S2P1B3	ND	ND	ND	ND	1.5	1.56	1.59
T13	S2P2B1	ND	ND	ND	ND	1.27	1.33	1.36
T14	S2P2B2	ND	ND	ND	ND	1.31	1.37	1.4
T15	S2P2B3	ND	ND	ND	ND	1.37	1.43	1.46
T16	S2P3B1	ND	ND	ND	ND	1.2	1.26	1.29
T17	S2P3B2	ND	ND	ND	ND	1.24	1.3	1.33
T18	S2P3B3	ND	ND	ND	ND	1.35	1.41	1.44

S1= ambient storage/room storage. S2= cold storage. P1= 150 ppm sodium benzoate. P2= 250 ppm sodium benzoate. P3= 350 ppm sodium benzoate. B1= glass bottle. B2= pet bottle. B3= standy pouch. ND= not detected. *= discarded samples /terminated shelf life. Cfu= colony forming unit.

4. Discussions

The data revealed that the microbial detection was negligible and within the acceptable level (less than 2.00 colony forming units per mL) in all treatment combinations at ambient storage up to 90 d in juice, up to 180 d in syrup, and carbonated RTS. Fruit beverages that are sold commercially are consumed by individuals of various ages all around world as they are nutritious, also the flavonoids of fruit beverages inhibit cancer cell development (Pinto et al., 2022; Faizi, 2022), but if improperly prepared, that could be harmful to people's health due to microbial growth in it, so to guarantee the protection of the public's health, manufacturing procedures need to be substantially stricter (Ahmed et al., 2018). The microbial detection was negligible and within an acceptable level in all treatment combinations at cold storage up to 180 d in juice, syrup, and carbonated RTS. The microbial growth was found to be within an acceptable level in the juice, syrup and carbonated RTS beverage, which might be due to the acid environment, high sugar level, chemical preservative, packaging materials, and CO_2 gas maintaining the beverage at a safe level and has prevented microbial growth. Similar results were reported by Lotha et al. (1994) on Kinnow mandarin juice; Covadonga et al. (2002) on orange juice; Polydera et al. (2003) on orange juice; Himani (2003) on Kinnow mandarin juice; Kumar et al. (2011) on sweet lime juice; Chukwumalume (2012) on African star apple juice; Oranusi et al. (2012) and Ogodo et al. (2016) on different fruit juices grown in Nigeria. In our research, the findings were acceptable that rely upon strictly applied health standards and regulations during processing, the same idea mentioned by Kumar (2009) and Strano et al.

(2022); otherwise, the product will be exposed to contamination by bacteria, mold and yeasts.

5. Conclusion

Microbial quality (yeast and mold) count was increased during 180 d of storage in the ready-to-serve carbonated beverage, syrup, and juice samples. The microbial growth was recorded within the acceptable level and saved for consumption in all treatment combinations of ready-to-serve carbonated beverage, syrup and juice in cold and room storage. But in the room storage, only T7 of juice remained within the acceptable level, and remained all discarded at different periods. Not only storage conditions but also packaging materials and preservative quantities had shown an important stability of the samples as the juice sample in the cold storage (SI) which added highest (350 ppm) level of sodium benzoate as chemical preservative (P3), and packed in the glass bottle (B1) remained save for consumption. So, for production fruit beverages free of mold and yeast specially in case of Nagpur cultivar of mandarin beverages, always glass bottle + cold storage + using ideal level of preservative is a need and should take it serious at industrial production level.

Compliance with Ethical Standards

Conflict of Interest

As the author of article declare that there are no conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' Contributions

All the procedures including research conception and design, draft manuscript controlling and corrections, data collection and analysis of the manuscript as well as finalization of the manuscript was done by **Zaki Ahmad FAIZI**.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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