The Pharma Innovation Journal 2023; 12(9): 1925-1930

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(9): 1925-1930

www.thepharmajournal.com
Received: 12-07-2023
Accepted: 17-08-2023

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Standardization of procedure for swallow root based RTS blends with banana fruit pulp and their storage behaviour

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Abstract
A study was conducted to prepare and standardize the recipe of blended RTS based on different proportion of swallow root extract, banana juice (100:00, 00:100, 20:80 and 40:60) and also with pomegranate juice (00:50:50, 20:40:40 and 40:30:30). Physico and bio-chemical properties these different blends are studied at 0, 30, 60 and 90 days after storage and showed the statistically significant variation among all the blends. The maximum desirable results were obtained from RTS blend of T_6 (swallow root extract, banana juice and pomegranate juice 20:40:40) showed TSS (15.23 ̊brix), titratable acidity (0.64%), ascorbic acid (9.11 mg/100 ml), anthocyanins (4.37 mg/100 ml), bacterial count (1.00×10^6 cfu/ ml) and overall acceptability (9.03) which was followed by the RTS blend of T_7 (swallow root extract, banana juice and pomegranate juice 40:30:30). From 0 to 90 days after storage, TSS and microbial count increased gradually where as titrable acidity, ascorbic acid content, anthocyanins and overall acceptability decreased gradually in all the RTS blends.

Keywords: Swallow root, bananas, pomegranate, TSS, titrable acidity, ascorbic acid, anthocyanins

Introduction
Decalepis hamiltonii Wight & Arn familiarly known as swallow root, is a medicinal herb that has been used for centuries by tribals who inhabit western and Eastern Ghats of India. This plant is a monotypic, glabrous, climbing shrub belonging to the order Gentianales and family Asclepiadaceae. *Decalepis* comprises five species of which four are endemic to Southern India, *viz. D. Hamiltonii, D. Arayalpathra, D. Salicifolia* and *D. Nervosa* (Pradeep *et al.*, 2016) [10]. The nutritional composition of edible root tubers of *D. hamiltonii* was evaluated and found that the tubers contain 1,650 KJ of energy, 84.05% of moisture, 1.24 g lipids, 2.39 g of carbohydrates, 2.37 g proteins and 10.51 g of fiber for 100 g of roots (Samydurai *et al.*, 2012) [16].

It is used as a culinary spice due to its high priced aromatic roots. The roots are characterized by a sarasaparilla like taste accompanied by a tingling sensation on the tongue as described in 'Wealth of India'. The roots of this plant are used in food flavour, Ayurvedic medicines, and in making pickles and health drinks. It is also used as a blood purifier, preservative, and as a source of bio insecticide for storing food grains (Rathi *et al.*, 2017) [14]. A number of phytochemical compounds have been isolated, 2-hydroxy-4-methoxy benzaldehyde (HMB) is an abundant aromatic bioactive compound with greater biological significance (Reddy *et al.*, 2013) [15].

Banana (Musaceae) is an herbaceous flowering plant. It is also consumed both as ripe fruit and also as cooked vegetable. Banana is known to be rich not only in carbohydrates, dietary fibres, certain vitamins and minerals, but is also rich in many health-promoting bioactive phytochemicals (Jiwan *et al.*, 2018) [4]. In many places, there is significant losses of the food value of banana due to improper postharvest management practices that cause huge economic loss. Post production losses of banana can be reduced by adopting various postharvest management to prolong its shelf life (Mohapatra *et al.*, 2010) [7].

Fruits have very limited shelf life due to its perishable nature. In order to preserve, they are processed to Ready to Serve (RTS) beverages which are non-fermented beverage prepared from fruits and vegetables of different concentrations with addition of sugar, water and additives (Rathinasamy *et al.*, 2021) [13].

Hence keeping in view of the natural preservative property and appealing flavor of the swallow root and short shelf-life of banana fruit, the present investigation is planned to impart the flavor and prolong shelf life of banana by standardizing RTS blends.

Material and Methods

Materials

Procurement of raw material

The current research program was conducted in the Department of Postharvest Management Laboratory at the College of Horticulture, Anantharajupeta, Annamayya district of Andhra Pradesh.

Fresh swallow root rhizomes were procured from farmers' fields in the B. Kammapalli village, which is at the distance of 6 km close to Anantharajupeta. Matured and ripened bananas, Pomegranate fruits which are fully matured and red coloured and ingredients like sugar and ghee are procured from the local market near Rly. Kodur which is at the distance of 3km from Anantharajupeta.

Methods

Preparation of swallow root extract

Fresh swallow root rhizomes were soaked in water, then scrubbed clean under running water to get rid of any dirt or soil. Rhizomes were dried by spreading them on a clean surface after cleaning. With a stainless steel knife, cleaned rhizomes were divided into equal pieces that measured 2 cm in length. Using a cabinet tray dryer set at 60℃, the cut pieces were dried until they were completely dry, crisp, and the desired weight was reached. A container with the 100 g of dried roots are dissolved in 1 litre of water, left to sit for around 12 hours. Place the container on a stove with a lighted fire for 1 hour, or until the water turns a wine-red colour. Water is filtered via a sieve to remove the silt of dried roots. This liquid concentration is now cooled to room temperature and filtered once more through a small screen to get rid of the minute root or other dirt particles. The liquid concentration is packaged and kept for use.

Extraction of fruit juices

Banana fruits were peeled, cut into pieces, and blanched for two to three minutes to stop browning. By using a tabletop grinder, the fine pulp is produced. Using a Hurrom H-100 juicer, the pomegranate fruits were peeled, the arils were separated, and then clear juice was extracted free of the seeds.

Preparation of RTS blended juices

Juices from banana and pomegranate fruits were extracted and blended at various concentrations with and without swallow root extract, including a ratio of 100 percent swallow root extract, 100 percent banana pulp, 20:80 and 40:60 of swallow root extract and banana pulp, 50:50 of banana pulp and pomegranate juice, and 20:40:40 and 40:30:30 of swallow root extract, banana pulp, and pomegranate juice. A fruit beverage that is ready to serve (RTS) has at least 10% fruit juice, 10% total soluble solids, and only around 0.3% citric acid. Since it is not diluted before serving, it is referred to as a ready-to-serve beverage.

Treatment details

T_1 – Swallow root extract: Banana juice (100:00).
T_2 – Swallow root extract: Banana juice (00:100).
T_3 – Swallow root extract: Banana juice (20:80).
T_4 – Swallow root extract: Banana juice (40:60).
T_5 – Swallow root extract: Banana juice: Pomegranate juice (00:50:50).
T_6 – Swallow root extract: Banana juice: Pomegranate juice (20:40:40).
T_7 – Swallow root extract: Banana juice: Pomegranate juice (40:30:30).

Observations recorded

Physico-chemical analysis of swallow root based RTS blends with banana fruit pulp were analyzed for TSS, Titratable acidity, ascorbic acid, anthocyanins, microbial count and sensory evaluation during storage at 30 days intervals for 90 days at refrigerated conditions.

Physico-chemical and sensory analysis

RTS blends was stored at low temperature (4-70 C) in refrigerated conditions for 3 months depicted in fig 1. The physico-chemical, microbiological and sensory quality characteristics of the blends were carried out at 0, 30, 60 and 90 days of storage

TSS

The total soluble solids are determined as stated by Ranganna (1986) [12] and are expressed in ° Brix. At room temperature, a reading was obtained straight from the scale and recorded in ° Brix.

Titratable acidity (%)

10 ml of the sample was taken into a 100 ml volumetric flask, which was then filled with distilled water to made up the volume to 100 ml. Then 10 ml of the homogenised sample was taken into a conical flask and 1-2 drops of phenolphthalein indicator was added and titrated against 0.1 N sodium hydroxide solution, added drop by drop from the burette till the appearance of a pink hue lasts for 30 seconds (Ranganna, 1986) [12]. The final burette reading was recorded, and the formula below was used to compute the percent acidity.

$$\text{Titrable acidity (\%)} = \frac{\text{Titre value} \times 64 \times \text{Normality of NaOH} \times 100}{\text{Volume of sample taken} \times \text{Volume of aliquot taken} \times 1000}$$

Ascorbic acid (mg/100 ml)

According to Ranganna (1986) [12], the ascorbic acid content was measured using the 2-6- dichlorophenol indophenol titration technique. A sample volume of 10 ml was taken, and 3 percent metaphosphoric acid was added to bring the volume to 100 ml. By titrating 10 ml of filtrate against the dye 2-6-dichlorophenol indophenol, ascorbic acid concentration was calculated. The emergence of a pale pink colour lasting for 15 seconds was the end result. The amount of ascorbic acid was calculated using the formula provided and reported as mg/100 ml.

Anthocyanin content was determined by using spectrophotometric method (Ranganna, 1986) [12]. Acidified Ethyl alcohol i.e., ethanolic HCL mixture was prepared by

mixing 85 ml of 95% ethanol and 15 ml of 15 N HCL. Then 10 gms of juice was diluted with 90 ml of ethanolic HCL. By using spectrophotometer the absorbance was recorded at 535 nm.

$$\text{Ascorbic acid (mg/100ml)} = \frac{\text{Titre value x Dye factor x Volume made up x 100}}{\text{Aliquot taken x Wt. of Sample taken}}$$

Microbial count

According to Agarwal and Hasija (1986), a serial dilution and plate count procedure was utilized for the assessment. In a conical flask, 10 ml of the sample was added with 90 ml of sterile water and agitated for 10 minutes. To determine the amount of bacteria in the juice, the serial dilution technique was used. To prepare a 10^{-2} dilution, 1 ml of this solution was transferred to a test tube with 9 ml of distilled water. 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} dilutions were also prepared in the same way. Utilising potato dextrose agar media for fungus and nutritional agar media for bacteria, the total number of microorganisms was counted. For bacteria, a 10^{-6} dilution and for fungus. In duplicate, 1 ml of the appropriate dilution was transferred to petriplates, and sterilised lukewarm in molten PDA media was poured onto the corresponding plates using the pour plate method. After the plates had solidified, they were incubated at 28±1˚C for 3–5 days, during which time colony counts were noted, totaled, and represented as CFU/ ml of sample.

Overall acceptability

The koronye and Ngoddy (1985) described the usage of a 9-point Hedonic scale. The products were coded and served rando mly to the panellists for organoleptic evaluation for overall acceptability

Results and Discussion

TSS (°brix): The maximum TSS of 15.23, 15.66, 16.17 and 16.46 °brix was recorded in the T_6 (swallow root extract: Banana Juice: Pomegranate Juice 20:40:40) followed by T_7 (swallow root extract: Banana Juice: Pomegranate Juice 40:30:30) which was recorded as 14.16, 14.59, 14.77 and 15.15° brix where as the minimum TSS content was recorded in T_1, swallow root extract: Banana Juice (100:00) as 11.85, 12.30, 12.78 and 13.24 °brix at 0, 30, 60 and 90 days after storage respectively represented in table 1.

Over the course of the 90-day storage period, there was a consistent upward trend observed in all treatments. This increase in total soluble solids can likely be attributed to the conversion of insoluble polysaccharides and organic acids into sugars. The presence of sugar syrup in the RTS blend creates an osmotic gradient, causing water from the juices to move into the syrup. This influx of water concentrates the sugars in the solution, increasing the TSS. These findings align with a previous study conducted by Rao *et al*. (2020) [11] which reported similar results in blended juice made from cashew apples, including nannari.

Titratable acidity (%): The minimum titrable acidity was recorded in T_6 (swallow root extract: Banana Juice: Pomegranate Juice 20:40:40) as 0.64, 0.53, 0.43 and 0.34% followed by T_7 (swallow root extract: Banana Juice: Pomegranate Juice 40:30:30) recorded as 0.87, 0.64, 0.51 and 0.35% where as the maximum titrable acidity of 1.13, 0.85, 0.65 and 0.52% was recorded in the T_1 (Swallow Root Extract: Banana juice 100:00) at 0, 30, 60 and 90 days after storage respectively depicted in table 1.

A consistent trend of decreasing titratable acidity was observed across all treatments from day 0 to day 90 of storage. This decline can be attributed to natural enzymatic or microbial metabolism, which leads to a reduction in the concentration of organic acids present in the blend. Additionally, depending on the specific ingredients and storage conditions, chemical reactions such as hydrolysis or organic acid degradation may take place, contributing to the decrease in titratable acidity. The reduction in titratable acidity could also be attributed to chemical interactions among the organic components of the juice, driven by temperature and enzyme activity. This phenomenon, which results in decreased titratable acidity, was noted in a study conducted by Palaniswamy and Muthukrishnan (1974) [8]. Similarly, comparable findings were reported in RTS blends of lime and dragon fruit by Deepa and Karetha (2022) [1].

Ascorbic acid (mg/100 ml): The maximum ascorbic acid content of 9.11, 8.76, 8.13 and 6.77 mg/100 ml was recorded in T_6 (Swallow Root Extract: Banana Juice: Pomegranate Juice 20:40:40) followed by T_7 (Swallow Root Extract: Banana Juice: Pomegranate Juice 40: 30:30) which recorded the ascorbic acid content as 8.95, 8.63, 7.86 and 6.55 mg/100 ml where as the minimum ascorbic acid content of 6.46, 5.47, 4.55 and 4.12 mg/100 ml was recorded in T_1 (Swallow Root Extract: Banana Juice 100:00) at 0, 30, 60 and 90 days after storage respectively illustrated in table 2.

Across all treatments, there was a consistent decrease in ascorbic acid levels from day 0 to day 90 of storage. This decline in ascorbic acid content during storage can be attributed to its susceptibility to oxidation, particularly at elevated temperatures. According to Potter and Hotchkiss (1995) [9], this susceptibility to oxidation is a major factor leading to the degradation and loss of ascorbic acid in food preparation and storage. A similar trend was observed in a study involving blends of cashew apple juice, pineapple juice, and nannari, where the ascorbic acid content decreased over the course of 60 days of storage, as reported by Rao *et al*. (2020) [11].

Anthocyanins (mg/100 ml): The maximum anthocyanin content of 4.37, 2.96, 1.72 and 0.92 mg/100 ml was recorded in T_6 (Swallow Root Extract: Banana Juice: Pomegranate Juice 20:40:40) followed by T_7 (Swallow Root Extract: Banana Juice: Pomegranate Juice 40: 30:30) which recorded as 3.84, 2.55, 1.18 and 0.61 mg/100 ml where as the minimum anthocyanin content was recorded in T_2 (Swallow Root Extract: Banana Juice 00:100) as 1.62, 0.97, 0.37 and 0.13 mg/100 ml at 0, 30, 60 and 90 days after storage respectively showed in Table 2.

A consistent decrease in anthocyanin content was observed across all treatments during the 90-day storage period. Alterations in pH levels over time can affect the stability of anthocyanins, potentially leading to their degradation. Anthocyanins are susceptible to chemical reactions, including oxidation and polymerization, which can result in their breakdown and a subsequent reduction in content. Furthermore, this reduction in anthocyanin content could be attributed to the hydrolysis of the protective 3-glucoside bond

during storage, a phenomenon previously documented by Kannan and Thirumanan (2002) [5]. Similar findings were reported by Singh *et al.* (2023) [18], where the total anthocyanin content decreased during the storage of blended guava and colored grape nectar, with consideration for the impact of packaging materials.

Microbial count: On the initial day of storage, no bacterial count was observed among all the treatments. Among the minimum bacterial count was recorded in T_7 (Swallow Root Extract: Banana Juice: Pomegranate Juice 40:30:30) recorded as $0.67x10^6$, $1.00x10^6$ and $1.33x10^6$ cfu/ ml followed by T_6 (Swallow Root Extract: Banana Juice: Pomegranate Juice 20:40:40) recorded as $1.00x10^6$, $1.33x10^6$, $1.67x10^6$ cfu/ ml, where as the maximum bacterial count T_5 (Swallow Root Extract: Banana Juice: Pomegranate Juice 00: 50:50) which recorded the bacterial count as $2.00x10^6$, $2.33x10^6$ and $2.67x10^6$ cfu/ ml the where at 30, 60 and 90 days after storage respectively. Illustrated in Table 3.

From 0 day to 90 days of storage, decreased trend was observed in all the treatments. Based on the environment available to the microorganisms and the storage temperature, an increase in microbial count is seen in all treatments when the storage duration at room temperature is extended. Similar results were also presented by Kumar *et al.* (2010) [6] in aonla-guava blended beverages and Yadav *et al.* (2013) [20] in banana RTS beverages.

Overall acceptability: The maximum overall acceptability score of 9.03, 8.70, 8.07 and 7.60 was recorded in T_6 (Swallow Root Extract: Banana Juice: Pomegranate Juice 20:40:40) followed by T_7 (Swallow Root Extract: Banana Juice: Pomegranate Juice 40:30:30) which recorded the overall acceptability score as 8.68, 8.19, 7.65 and 7.11 where as the minimum overall acceptability score was recorded in T_2 (Swallow Root Extract: Banana Juice 00:100) as 7.67, 7.21, 6.51 and 5.83 at 0, 30, 60 and 90 days after storage respectively represented in Table 3.

The score for all the sensory attributes decreased gradually during storage period. Decrease in overall acceptability scores might be due to the loss in appearance, flavour compounds and uniformity of the product reported by Thakur *et al.* (2013) [19]. Similar findings on decreasing of sensory evaluation of RTS observed by Singh *et al.* (2018) [17] in Aloe Vera fortified mango RTS, Devra *et al.* (2017) [2] in aonla-based blended RTS and Islam *et al.* (2014) [3] in mixed fruit juice made from orange and pineapple.

Table 1: Changes in TSS (Brix) and titratable acidity (%) of swallow root extract based banana juice RTS blends during storage

Treatments	Days after storage							
	TSS (Brix)				Titratable acidity (%)			
	0	30	60	90	0	30	60	90
T_1 – Swallow root extract (100:00)	11.85	12.30	12.78	13.24	1.13	0.85	0.65	0.52
T_2 – Swallow root extract: Banana juice (00:100)	13.85	14.37	14.56	14.78	0.92	0.75	0.63	0.49
T_3 – Swallow root extract: Banana juice (20:80)	13.65	14.88	15.00	15.34	0.88	0.64	0.51	0.35
T_4 – Swallow root extract: Banana juice (40:60)	13.64	14.17	14.48	14.87	0.90	0.72	0.56	0.41
T_5 – Swallow root extract: Banana juice: Pomegranate juice (00:50:50)	12.75	13.32	13.68	13.91	0.91	0.74	0.60	0.43
T_6 – Swallow root extract: Banana juice: Pomegranate juice (20:40:40)	15.23	15.66	16.17	16.46	0.64	0.53	0.43	0.34
T_7 – Swallow root extract: Banana juice: Pomegranate juice (40:30:30)	14.16	14.59	14.77	15.15	0.87	0.65	0.52	0.40
SE(m)±	0.02	0.03	0.03	0.06	0.05	0.03	0.04	0.03
CD at 5%	0.06	0.10	0.10	0.19	0.15	0.10	0.12	0.10

Table 2: Changes in Ascorbic acid (mg/100 ml) and Anthocyanins (mg/100 ml) of swallow root extract based banana juice RTS blends during storage

Treatments	Days after storage							
	Ascorbic acid (mg/100 ml)				Anthocyanins (mg/100 ml)			
	0	30	60	90	0	30	60	90
T_1 – Swallow root extract (100:00)	6.46	5.47	4.55	4.12	2.74	1.64	0.86	0.52
T_2 – Swallow root extract: Banana juice (00:100)	7.33	6.97	5.67	5.32	1.62	0.97	0.37	0.13
T_3 – Swallow root extract: Banana juice (20:80)	8.54	8.27	7.63	6.62	2.07	1.47	0.64	0.46
T_4 – Swallow root extract: Banana juice(40:60)	8.35	7.93	7.36	6.14	2.48	1.33	0.58	0.28
T_5 – Swallow root extract : Banana juice: Pomegranate juice (00:50:50)	7.95	7.74	6.97	4.64	3.63	2.14	0.98	0.58
T_6 – Swallow root extract : Banana juice: Pomegranate juice (20:40:40)	9.11	8.76	8.13	6.77	4.37	2.96	1.72	0.92
T_7 – Swallow root extract : Banana juice: Pomegranate juice (40:30:30)	8.95	8.63	7.86	6.55	3.84	2.55	1.18	0.61
SE(m)±	0.01	0.16	0.27	0.17	0.14	0.11	0.08	0.05
CD at 5%	0.03	0.49	0.83	0.53	0.43	0.34	0 0.24	0 0.15

Table 3: Changes in microbial count (bacterial) and overall acceptability (9 point hedonic swallow root extract based banana juice RTS blends during storage

Treatments	Days after storage							
	Bacterial count (CFU/ml)				Overall acceptability			
	0	30	60	90	0	30	60	90
T_1 – Swallow root extract (100:00)	-	-	$0.67x10^6$	$1.00x10^6$	8.37	7.82	7.46	6.59
T_2 – Swallow root extract: Banana juice (00:100)	-	$2.33x10^6$	$3.00x10^6$	$3.67x10^6$	7.67	7.21	6.51	5.83
T_3 – Swallow root extract: Banana juice (20:80)	-	$1.67x10^6$	$1.33x10^6$	$2.33x10^6$	8.24	7.62	7.16	6.57
T_4 – Swallow root extract: Banana juice(40:60)	-	$1.33x10^6$	$1.67x10^6$	$2.00x10^6$	8.33	7.67	7.23	6.72
T_5 – Swallow root extract: Banana juice: Pomegranate juice (00:50:50)	-	$2.00x10^6$	$2.00x10^6$	$2.67x10^6$	8.50	7.88	7.32	6.86
T_6 – Swallow root extract: Banana juice: Pomegranate juice (20:40:40)	-	$1.00x10^6$	$1.33x10^6$	$1.67x10^6$	9.03	8.70	8.07	7.60
T_7 – Swallow root extract: Banana juice: Pomegranate juice (40:30:30)	-	$0.67x10^6$	$1.00x10^6$	$1.33x10^6$	8.68	8.19	7.65	7.11
SE(m)±	-	0.33	0.4	0.36	0.07	0.08	0.06	0.09
CD at 5%	-	1.01	1.2	1.08	0.21	0.24	0.19	0.27

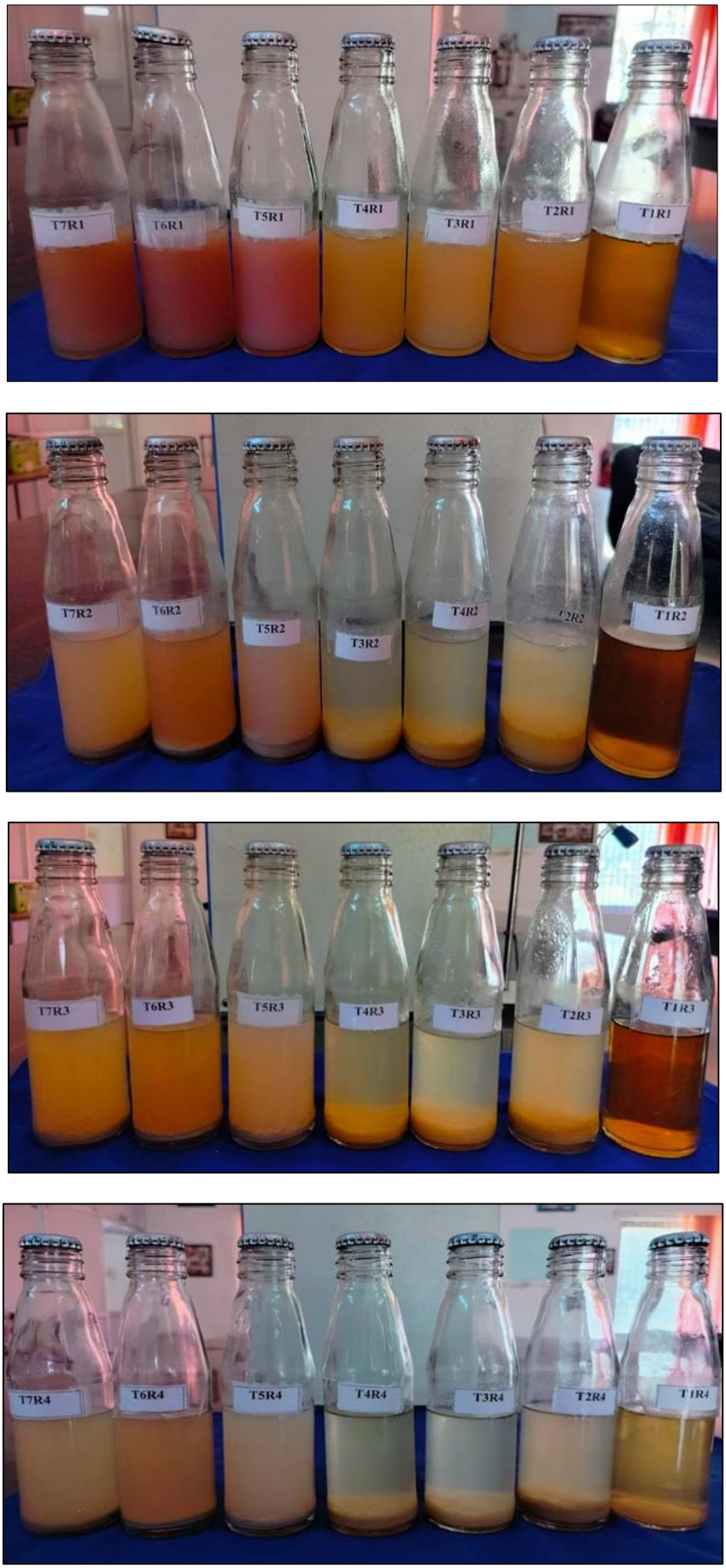

Fig 1: RTS blends at 0 to 90 days of storage period

Conclusion

Juice blending not only improves the quality and nutrition of basic raw material, but also offers to develop the new product. This study demonstrated that the treatment, T_6 (Swallow Root Extract:Banana Juice: Pomegranate Juice 20:40:40) have best results both in physico-chemically and organoleptically as compared to other treatments with regard to most of the bio-chemical characteristics *viz*., TSS, titrable acidity, ascorbic acid, anthocyanins, microbial and overall acceptance throughout the storage period upto 90th days. It was observed that all the treatments recorded gradual increase in TSS and microbial count whereas, titrable acidity, ascorbic acid content, anthocyanin content and overall acceptability decreased from 0 to 90 days after storage. On the basis of results obtained in the study it can be concluded that most of the bio-chemical characteristics of blended RTS beverages were significantly influenced by different treatments and storage period. Further optimization of developed RTS with enhanced shelf life may make it a potential beverage in the near future at a commercial scale.

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