International Journal of Biotechnology Research Vol. 1(3), pp. 028-037, April 2013
Available online at http://academeresearchjournals.org/journal/ijbr
ISSN 2328-3505 ©2013 Academe Research Journals

Full Length Research Paper

Development of herbal functional RTS beverage

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Accepted 26 March, 2013

Herbal plants like *Tinospora cordifolia* and *Ocimum* possess phytochemicals such as polyphenols, flavonoids and tannins which attribute to a strong free radical scavenging activity and can modulate many diseases. Nutraceutical rich extracts of selected herbs can be added in juice of sweet orange for the preparation of refreshing, energizing RTS drink that improves the health and fulfills the nutritional requirements. The mosambi juice was analyzed for its physico-chemical composition, herbal extracts for % radical scavenging activity. The developed beverage was kept in glass bottle for the period of two months and was analyzed for its keeping quality and acceptability at intervals of 10 days Herb mixed beverage having formulation 6% basil and 1.5% *T. cordifolia* was found to be optimum among the other formulation. It is concluded that extracts of the above herbs can be used as a valuable ingredient for the production of herbal beverage with phenolics and vitamin C as antioxidants.

Key words: Nutraceutical, ready to serve, herbal extract, orange juice, sensory evaluation, antioxidants.

INTRODUCTION

Natural antioxidants like phenolic compounds and flavonoids which are secondary plant metabolites present in food products of plant origin (Helle and Bertelsen, 1995; Yeh and Yen, 2003) can trap the free radicals directly or scavenge them through a series of coupled reactions with antioxidant enzymes (Rao et al., 1996). They also exhibit a wide range of biological effects, including antiageing, antimutagenicity, and protective effects on oxidative stress (Huang et al., 1992; Cook and Samman, 1996; Caputo et al., 2004; Thatte et al., 2000). *Tinospora cordifolia*, an indigenous plant used in Ayurvedic medicine, is commonly known as “Gulancha” belonging to the family Menispermaceae which has chemopreventive (Chaudhary et al., 2008), anti osteoporotic (Kapur et al., 2008), hepatoprotective (Panchabhai et al., 2008), immuno modulatory (Bishayi et al., 2002), antihiperglycaemic (Umamaheshwari and Mainzen, 2007), antitumor (Jagetia and Rao, 2006) and antiallergic (Badar et al., 2005) properties. They were effective in scavenging superoxide anion radical and inhibited deoxyribose degradation by scavenging hydroxyl radical directly. *T. cordifolia* contains phytochemicals such as polyphenols, flavonoids and tannins which have a strong free radical scavenging activity. In methanol, ethanol and water extracts of *T. cordifolia* exhibited an excellent antioxidant activity (Bhawya and Anilakumar, 2010). *Ocimum* belonging to family Lamiaceae is commonly used to treat different diseases (Leung and Foster, 1996) and its antioxidant effectiveness was even more than that of BHA (butylhydroxyanisol) or BHT (butylhydroxytoluene) (Saito and Asari, 1976). In addition to this, a fresh herb has a smoother flavor than a dried one and many alcoholic beverages with basil have excellent sensory properties. These beverages are rich in amounts of vitamin C, thiamin, and riboflavin and also have a good shelf life too due to their antimicrobial substances (Kolesnikova et al., 1976).

The sweet orange fruit is processed commercially into various forms mainly juice, frozen concentrates, squash and RTS drinks which provide 45 kcal, moderate quantity of vitamin C, potassium, bioflavonoid and folic acid and essential items of breakfast. It is refreshing, thirst quenching and energizing drink that improves health and nutritional requirements (Syed et al., 2011). Hence, in the light of the above research facts, the present investigation was undertaken with the objective to incorporate antioxidant rich herbal extracts of *Tinospora*

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stem and basil leaves in the development of delicious and nutritious RTS beverage that could therapeutically help in improving the health of consumers and to study the physicochemical and microbial qualities in storage period at ambient and refrigerated temperatures.

MATERIALS AND METHODS

Materials

The present study was conducted in the year 2011 at Centre of Food Technology, University of Allahabad, Allahabad, U.P., India. The fresh mosambi were procured from local market while holy basil, *T. cordifolia* was collected from the botanical nursery. Other raw materials like sugar, spices were also procured from the local market and citric acid was procured from science corporation, Allahabad. The glass bottle was used for the bottling of juice.

Extraction of mosambi juice

Fresh, fully ripe, sound mosambi were used for extraction of juice. The fruits were cleaned, thoroughly washed, peeled with stainless steel and seeds were removed and blended in a laboratory blender to a pulp and the juice was extracted by filtering through muslin cloth and stored separately for future use.

Preparation of extract

Extraction of Basil juice

The holy basil were washed properly and blended in a laboratory blender to paste with distilled water (1:8) and filtered through muslin cloth to obtain the juice extract.

Aqueous extract of *T. cordifolia*

T. cordifolia stem sample was dried in vacuum oven at 12.5 mmHg, 60°C, ground in a laboratory mixer grinder for 30 s. Powder sample of 10 g was mixed with milli Q water (1:10 ratio) and was shaken in orbital shaker at 350 rpm and 40°C for 4 h and the supernatant was filtered and the process was repeated again with the residue and the supernatant was centrifuged at 2500 rpm, and stored at refrigeration condition for future use.

Titratable acidity, pH and total sugar

The juice was analyzed for its total soluble solid (TSS), acidity (as citric acid), pH, and total sugar which were determined according to Ranganna (1986).

Ascorbic acid

Sample solution equivalent to 0.2 mg ascorbic acid/ml was prepared in water containing 3% (w/v) metaphosphoric acid. It was titrated against standard 2,6 dichlorophenol indophenol (2,6 DCIP) solution of 0.5 mg/ml concentration until the pink color developed completely. The operation was repeated with a blank (Indian Pharmacopoeia, 1996).

Total soluble solids

Total soluble solids (TSS) of fruit juice were analyzed by Digital Refractometer (Rudolph, USA). The fruit pulp was extracted and filtered through muslin cloth. A drop of filtrate was placed on a refractometer prism and the total soluble solids were recorded as °Brix.

Determination of antioxidant activity

Free radical scavenging activity of extracts was measured by the slightly modified method of Alothman et al. (2009). The antioxidant capacity of the extracts was studied through the evaluation of the free radical-scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. An aliquot (100 µl) of fruit extract was mixed with 3.9 ml of 0.1 mM DPPH methanol solution. The mixture was thoroughly vortex-mixed and kept in the dark for 30 min. The absorbance was measured later, at 515 nm, against a blank of methanol without DPPH. Results were expressed as percentage of inhibition of the DPPH radical.

Sensory analysis

To carry out the initial optimization of ingredients, the prepared formulation was judged by a trained panel of 15-member using a 9 point Hedonic rating (9 - like extremely and 1 - dislike extremely) (Murray et al., 2001) for color, flavor, texture and overall acceptability.

Formulations and preparation of RTS

The RTS was prepared with the Food Product Order Specification of Juice (10% of fruit juice; TSS 15 °Brix; Acidity 0.3%). The procedure for the preparation of RTS is given in Figure 1. The prepared mosambi-medicinal herb mixed RTS was divided into 5 batches coded as T1, T2, T3, T4 and T5. Holy basil added to the juice was kept constant at 6% of the RTS while *T. cordifolia* varied from 0.5, 1, 1.5, 2 and 2.5% respectively. The mixtures of herbs were added to the juice on replacement basis of the mosambi juice. The different combinations of RTS were prepared keeping the final volume of the juice constant in the RTS. Five different treatments coded as T1, T2, T3, T4 and T5 were prepared using different combinations of *T. cordifolia* ranging from 0.5 to 2.5% and were kept for the study (Table 1). The juices used for the preparation of RTS were centrifuged for settling the heavy particles. The final TSS of the beverage was kept

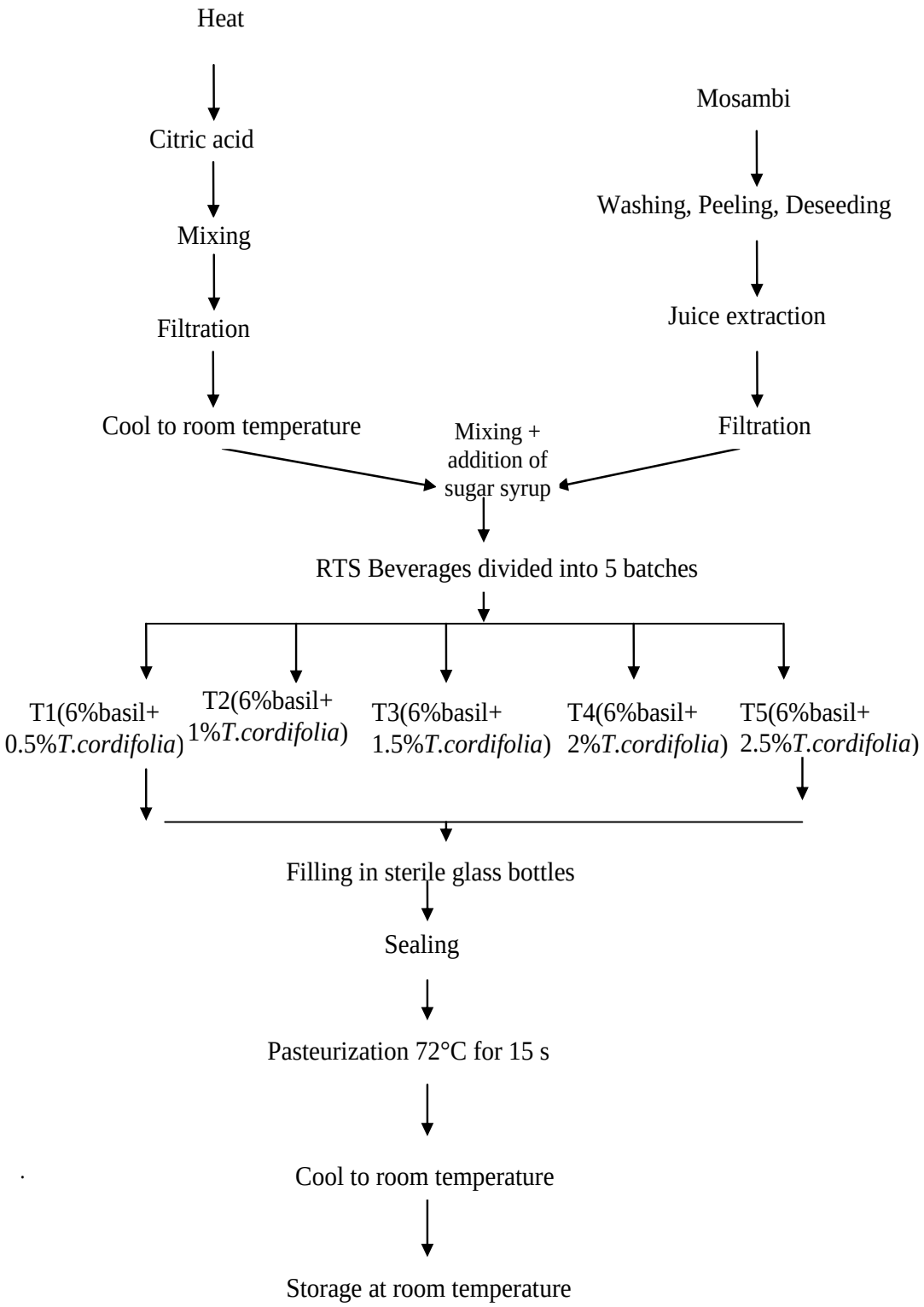

Figure 1. Flowchart for RTS preparation.

constant at 15.24 °Brix. The prepared juice was stored in glass bottles at room temperature for the period of 60 days.

Microbiological studies

The prepared beverage was studied for microbial load.

Table 1. Physico-chemical analysis of mosambi juice.

S/N	Particulars	Mosambi juice
1	Color	Greenish
2	°Brix	14.1
3	Acidity %	1.8
4	pH	2.38
5	Total sugar %	9.65

Table 2. Sensory score for the optimized RTS (P > 0.5%).

S/N	Formulations	Flavor	Color	Overall acceptability
1	T1	7.8^{a}	7.4^{a}	7.6^{a}
2	T2	8.0^{b}	7.8^{b}	8.0^{b}
3	T3	8.6^{c}	8.1^{c}	8.4^{c}
4	T4	6.9^{d}	7.6^{d}	7.2^{d}
5	T5	5.5^{e}	7.2^{e}	6.5^{e}

Means with different superscript letters are significantly different at P < 0.05.

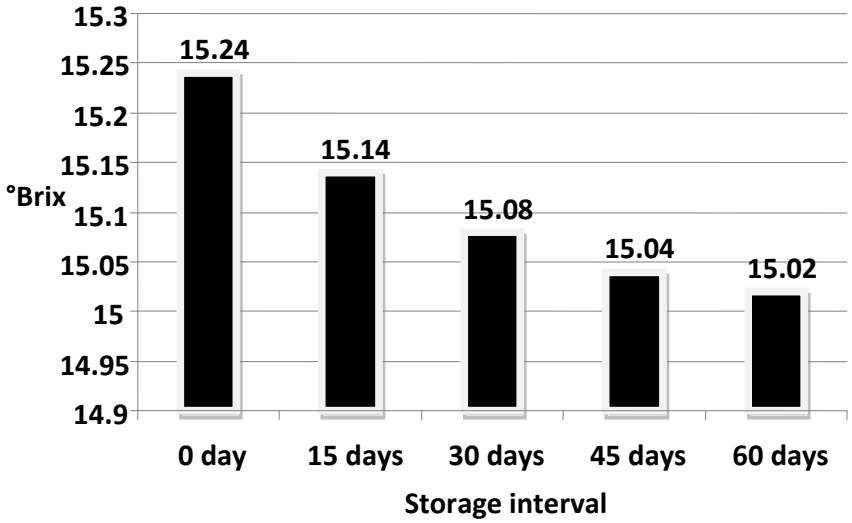

Figure 2. Changes in total soluble solids during storage.

The total microbial load was calculated by standard plate count method. The standard plate count was done according to the method described in “Recommendation method for the microbiological examination of food” (APHA, 1967).

Storage studies

RTS beverages were subjected to storage studies at room temperature for a period of 60 days by drawing samples at bimonthly intervals to evaluate changes in chemical and organoleptic parameters. The products were also evaluated for sensory qualities namely: colour, flavour, taste and overall acceptability by a panel of 10 judges using a 9-point Hedonic scale, where score 1 is for ‘dislike extremely’ and 9 for ‘like extremely’ (Pangborn and Roessler, 1965). Sensory scores were analyzed statistically by ANOVA using SPSS to evaluate the significance at P<0.05.

RESULTS AND DISCUSSION

The formulation optimized on the basis of sensory evaluation (Table 2) was T3 formulation with 6% basil

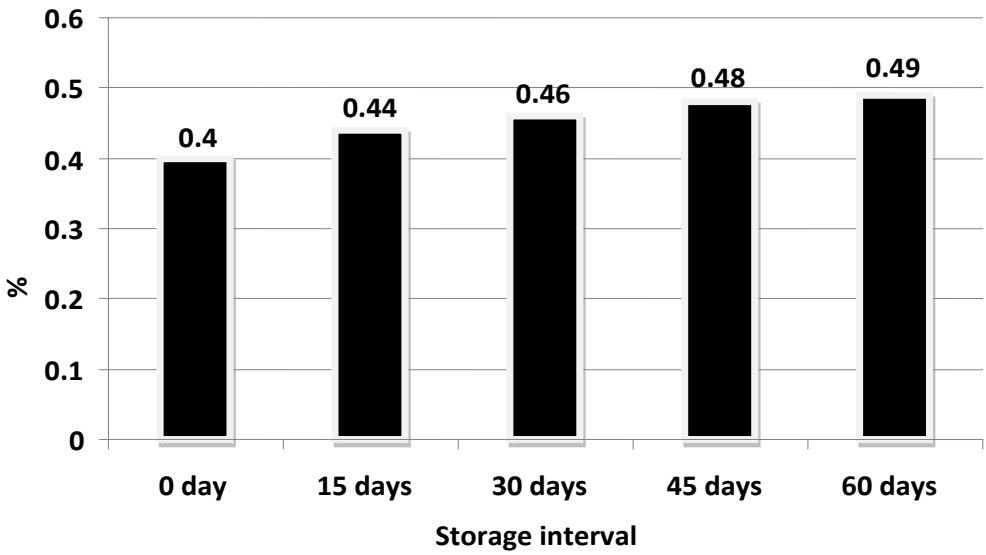

Figure 3. Changes in acidity during storage.

and 1.5% *T. cordifolia*. The changes in the optimized formulation (T3) upon storage of 60 days are summarized as follows:

Physico-chemical parameters of processed mixed juices

The physico-chemical compositions of the studied mosambi juice were presented in Table 1 which reveals that the total soluble solid was 14.1, acidity was 1.8%, and total sugar was 9.65%.

Total soluble solids (TSS), pH and acidity

The total soluble solids initially adjusted in formulations showed a negligible change throughout 2 months of storage period at room temperature (28-32°C). From Figure 2, it was observed that the initial TSS range was found to be 15.24 °Brix, and during storage of two months the TSS range was 15.02 °Brix, which did not show any significant difference during storage. Similar result was reported by Vidhya and Narain (2011) during storage of wood apple bar. Acidity was calculated on the basis of titrable acidity as citric acid. Acidity for the optimized formulation was calculated at an interval of 15 days during storage period of 60 days. The value of acidity observed at the interval of 15 days was given in Figure 3. The initial acidity of optimized mixed fruit beverage was 0.4% which was increased to 0.49% after storage for 60 days. Similar result was reported by Yadav et al. (2010). Increasing trends in acidity with increasing storage period have been observed earlier by Kalra and Revathi (1981) or Sandhu et al. (2001) as well which may be due to formation of various organic acids in the fruits such as sulphurous acid (Baramanary et al., 1995). From Figures 2, 3 and 5, it was indicated that vitamin C, TSS all gradually decreased, while acidity increased during storage; all these three factors have strong correlation. Since vitamin C is soluble in water and oxidation sensitive, it gradually decreased; this is the main reason for lowering the value of acidity and TSS (Simsek, 2011). The pH of the mixed beverage showed decrease in value during storage from 4.02 to 3.41 after 60 days of storage as the acidity of the beverage increased (Majumdar et al., 2011) (Figure 4). Trends of decreasing pH and increasing acidity found in these studies are well supported by previous researchers (Tandon et al., 1983; Sandhu et al., 2001).

Vitamin-C and DPPH % radical activity

Vitamin-C or ascorbic acid content of optimized beverage was observed to be high as shown in Figure 5. The figure showed that vitamin C was reduced from 134 mg/100 g to 62.6 mg/100 g in beverage during 60 days of storage due to the oxidation of vitamin C. This trend of decrease in vitamin C was found in storage studies of developed beverage and pickle too (Mishra et al., 2010; Puranik et al., 2011). Antioxidant activity in terms of DPPH % radical scavenging activity was found to be 31.34, 49.63, and 38.34% for mosambi juice, basil extract and *Tinospora* extract respectively (Table 4). The antioxidant activity of optimized ready to serve beverages was found to be 24.56%, which decreased to 4.32% during 60 days of storage (Figure 6).

Storage studies

The optimized beverage was stored at ambient temperature (28 to 32°C). The colour, flavour, overall

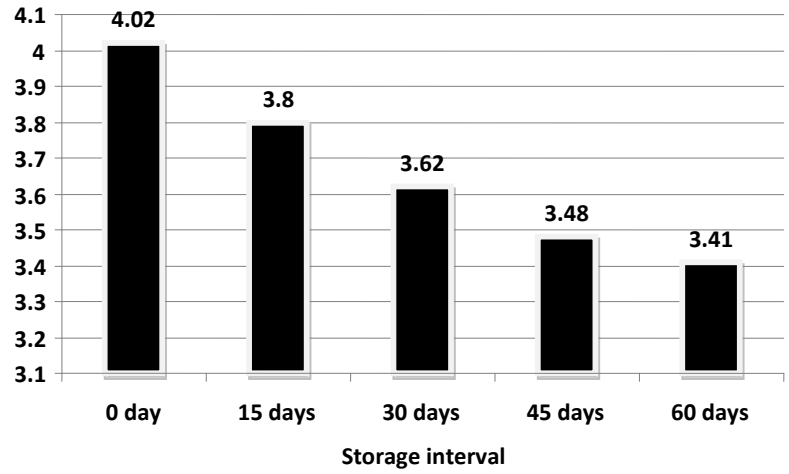

Figure 4. Changes in pH during storage.

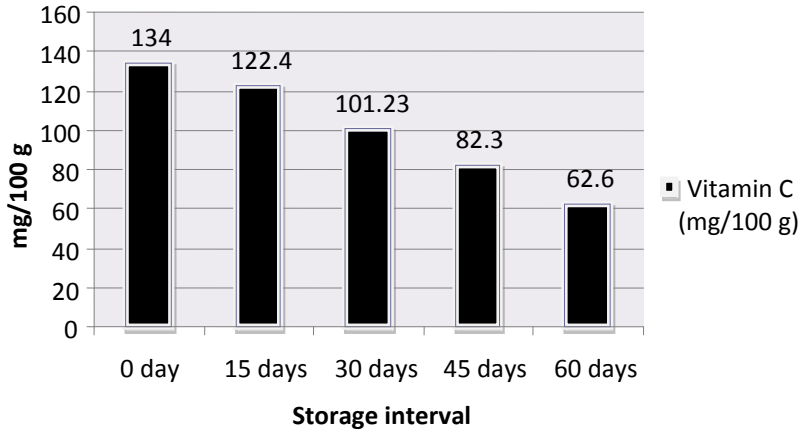

Figure 5. Changes in Vitamin C content during storage.

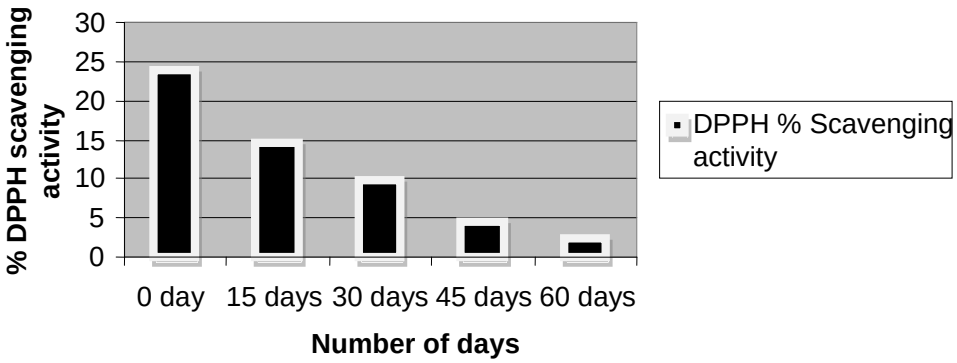

Figure 6. Changes in DPPH activity during storage.

acceptability, TSS, acidity, pH and microbial load (Standard plate count) in the beverage were observed during the storage period of 2 months. The data of organoleptic quality attributes measured on 9-point

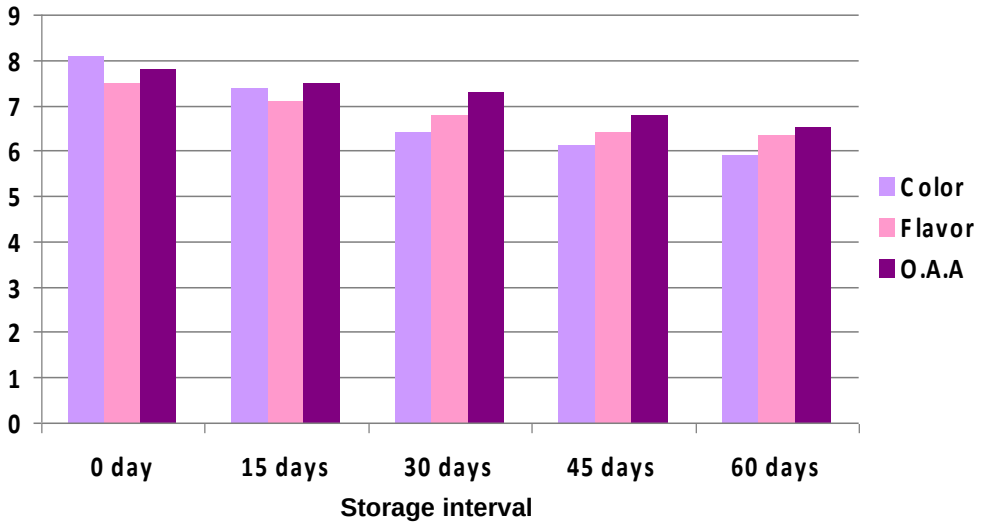

Figure 7. Changes in sensory parameter during storage.

Table 3. Microbiological analysis of optimized RTS (P > 0.5%).

S/N	Number of days	Total plate count (cfu/g)
1	0 day	2.23[a]
2	15 day	2.58[b]
3	30 day	2.71[c]
4	45 day	3.07[d]
5	60 day	3.45[e]

Means with different superscript letters are significantly different at P < 0.05.

Table 4. DPPH % scavenging activity analysis of mosambi juice, basil extract and tinospora extract.

S/N	Particulars	DPPH % scavenging activity
1	Mosambi juice	31.34
2	Basil extract	49.63
3	Tinospora extract	38.34

hedonic scale are presented in Table 2. It was evident from the data that flavour, texture, taste and overall acceptability was higher in formulation T3. It was also observed that with the decrease in storage period, there was decrease in the rating of all organoleptic characters in beverage but the overall acceptability of the RTS beverage did not show significant difference during storage (p<0.05%) (Figure 7). Similar results were obtained by Jain et al. (2011). In the microbiological study, the total number of viable count was not uniform. It also showed that the total colony count increased slightly with the increase of storage period. The initial microbial load of the beverage was found to be 2.53 (log cfu/g) which was not increased significantly after two months of storage. The microbial load was also very low and far below the safely level (Zurowietz, 1996) (Table 3).

Conclusion

Herb mixed beverage having formulation 6% basil and 1.5% *T. cordifolia* was found to be optimum among the other formulations. The above optimized beverage can be stored effectively for two months. It is concluded that the extracts of the above herbs can be used as a valuable ingredient for the production of herbal beverage with all the important properties and medicinal

characteristics of *Tinospora* and basil herbs. This can thus prove to be a good health drink with phenolics and vitamin C as antioxidants.

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