

Development of Functional Herbal RTS Beverage

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Abstract

Herbal plants like *Tinospora cordifolia* and *Ocimum* (basil) possess phytochemicals such as polyphenols, flavonoids and tannins which attribute to a strong free radical scavenging activity and these plant derived antioxidant nutraceuticals modulate many oxidative stress related degenerative diseases. Nutraceutical rich extracts of above herbs can be added in fresh juice of sweet orange for the preparation of refreshing, thirst quenching and energizing ready to serve drink that not only improves the health but also fulfills the nutritional requirements. The mosambi juice was analyzed for its physico-chemical composition, herbal extracts for % radical scavenging activity and different formulations of beverage were prepared by different proportions of orange juice, *tinospora* and basil extract. The prepared beverages were packed in glass bottle with cork cap and stored at room temperature for a period of 2 months. The products were analyzed for its keeping quality and acceptability at intervals of 10 days. The prepared beverages were evaluated by a taste-testing panel for sensory attributes. Herb mixed beverage having formulation 6% basil and 1.5% *T.cordifolia* was found to be optimum among the other formulation. The above optimized beverage can be stored effectively for two months. It is concluded that extracts of 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.

Keywords: Nutraceutical; Ready to serve; Herbal extract; Orange juice; Sensory evaluation; Antioxidants

Introduction

Natural antioxidants like phenolic compounds, are flavonoids which are secondary plant metabolites present in food products of plant origin [1,2] can trap the free radicals directly or scavenge them through a series of coupled reactions with antioxidant enzymes [3]. They also exhibit a wide range of biological effects, including antiageing, antimutagenicity, and protective effects on oxidative stress [4-7]. *Tinospora cordifolia* an indigenous plant used in Ayurvedic medicine is commonly known as (*Gulantha*) belonging to the family *Menispermaceae* has chemopreventive [8], anti osteoporotic [9], hepatoprotective [10], immuno modulatory [11], Antihyperglycaemic [12], antitumor [13] and antiallergic [14] properties. They were effective in scavenging superoxide anion radical and inhibited deoxyribose degradation by scavenging hydroxyl radical directly. *Tinospora cordifolia* contains phytochemicals such as polyphenols, flavonoids and tannins which has a strong free radical scavenging activity. In methanol, ethanol and water extracts *Tinospora cordifolia* exhibited an excellent antioxidant activity [15]. *Ocimum* belonging to family Lamiaceae is commonly used to treat different diseases [16] and its antioxidant effectiveness was even more than that of BHA (butylhydroxyanisole) or BHT (butylhydroxytoluene) [17]. 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 [18]. The sweet orange fruit is processed commercially in to various forms mainly juice, frozen concentrates, squash, RTS drinks, etc. providing 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 [19]. Hence, in the light of above research facts, the present investigation was undertaken with objective to incorporate antioxidant rich herbal extracts of *tinospora* stem and basil leaves for 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

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 material 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 12.5mmHg, 60°C, ground in a laboratory mixer grinder for 30 sec. Powder sample of 10 gm was mixed with milli Q water (1:10 ratio) and was shaken in orbital shaker at 350 rpm and

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40°C for 4 hours 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.

Titrateable acidity, pH and Total Sugar: The juice was analyzed for its Total Soluble Solid (TSS), acidity (as citric acid), pH, and total sugar were determined according to Ranganna [20].

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 [21].

Total soluble solids: Total Soluble Solids (TSS) of fruit juice was 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 [22]. 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 were judged by a trained panel of 15- member using a 9 point Hedonic rating (9-like extremely and 1-dis like extremely) [23] 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%, TSS- 15 °B, Acidity- 0.3%. The procedure for the preparation on 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 the T. cordifolia were 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 T5, were prepared using different combinations of T. cordifolia ranging from 0.5%-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 constant at 15.24°B. 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. 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” [24].

Storage studies: RTS beverages were subjected to storage studies at room temperature for a period of 3 months by drawing samples at bimonthly intervals to evaluate changes in chemical and organoleptic parameters. The products were also evaluated for sensory qualities viz., 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’ [25]. Sensory scores were analysed 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 and 1.5% T.cordifolia. The changes in the optimized formulation (T3) upon storage of 60 days were summarized as below.

Physico-chemical parameters of processed mixed juices

The Physico-chemical compositions of studied mosambi juice were presented in table 1 which reveals that the total soluble solid was 14.1, acidity 1.8%, total sugar 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 the 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 to 15.02 °Brix, which does not show any significant difference during storage and similar result was reported

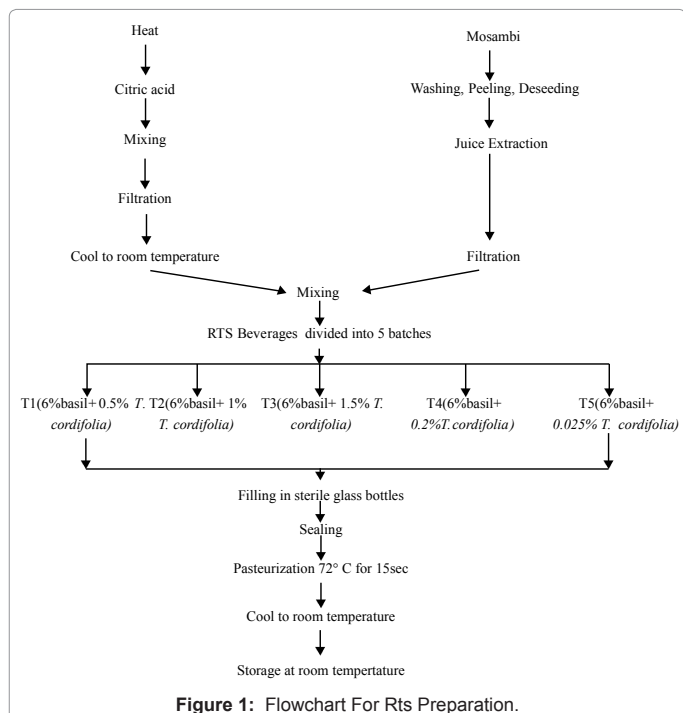


Figure 1: Flowchart For Rts Preparation.

S.No	Particulars	Mousambi juice
1	Color	Greenish
2	O Brix	14.1
3	Acidity%	1.8
4	pH	2.38
5	Total sugar%	9.65

Table 1: Physico-chemical analysis of mousambi juice.

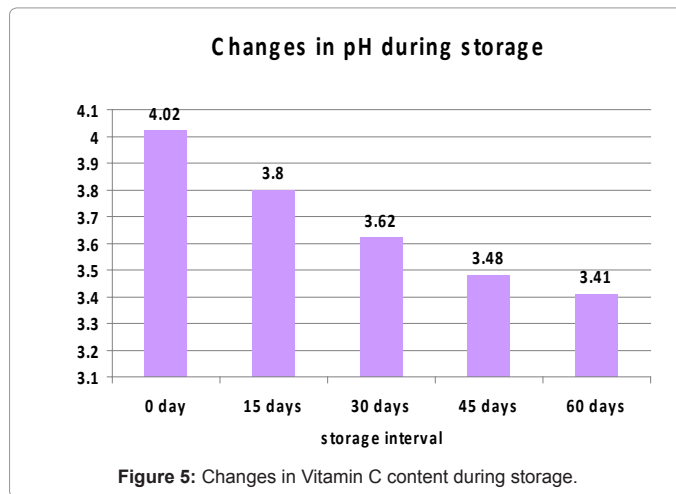
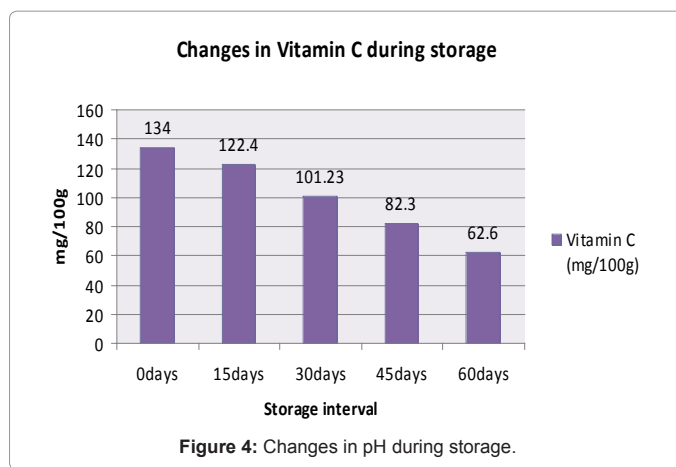
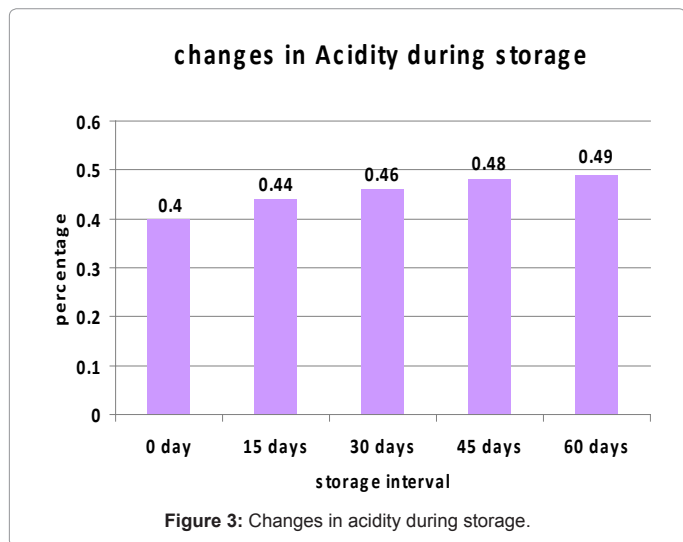
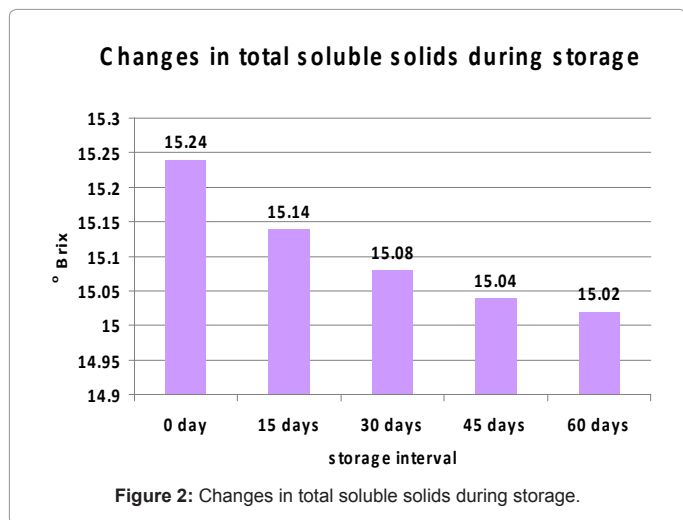
S.No.	Formulations	Flavor	Color	Overall acceptability
1	T1	7.8	7.4	7.6
2	T2	8.0	7.8	8.0
3	T3	8.6	8.1	8.4
4	T4	6.9	7.6	7.2
5	T5	5.5	7.2	6.5

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

by [26] 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 [27]. Increasing trends in acidity with increasing storage period have been observed earlier by Kalra and Revathi [28] and Sandhu et al. [29] as well which may be due to formation of various organic acids in the fruits such as sulphurous acid [30]. From the figures 2-5 it was indicated that vitamin C, T.S.S all were gradually decreased during while acidity increased during storage, all these three factor has strong correlation. Since vitamin C is soluble in water and oxidation sensitive which is gradually decreased and this is the main reason for lowering the value of acidity and T.S.S. [31]. 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 is increasing [32] (Figure 4). Trends of decreasing pH and increasing acidity found in these studies are well supported by previous researchers [29,33].

Vitamin-C and DPPH % radical activity

Vitamin-C or ascorbic acid content of optimized beverage was



observed to be high (Figure 5). The figure 5 showed that vitamin-C or ascorbic acid was reduced from 134mg/100g to 62.6 mg/100g in beverage after 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 [34,35]. Antioxidant activity in terms of DPPH % radical scavenging activity was found to be 31.34, 49.63, 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 (28-32°C). The colour, flavour, overall 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 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 does not show significant difference during storage (p < 0.05%) (Figure 7) similar results were obtained by Jain, et al. [36]. In microbiological study, the total no. 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

S.No	No of days	Total plate count cfu/g
1	0 day	2.23
2	15 day	2.58
3	30 day	2.71
4	45 day	3.07
5	60 day	3.45

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

S.No	Particulars	DPPH % scavenging activity
1	Mousambi juice	31.34
2	Basil extract	49.63
3	Tinospora extract	38.34

Table 4: DPPH % scavenging activity analysis of mousambi juice, basil extract and *tinospora* extract.

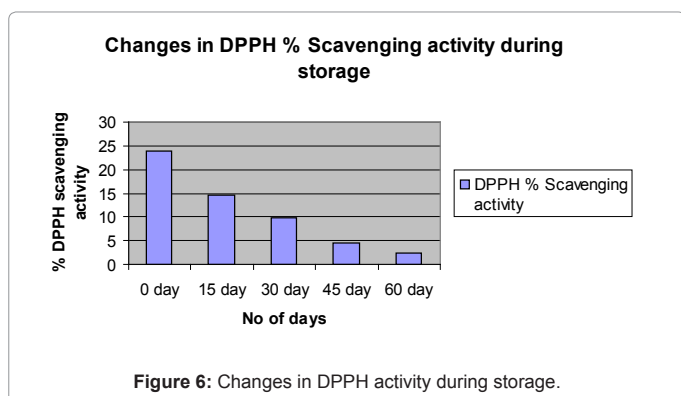


Figure 6: Changes in DPPH activity during storage.

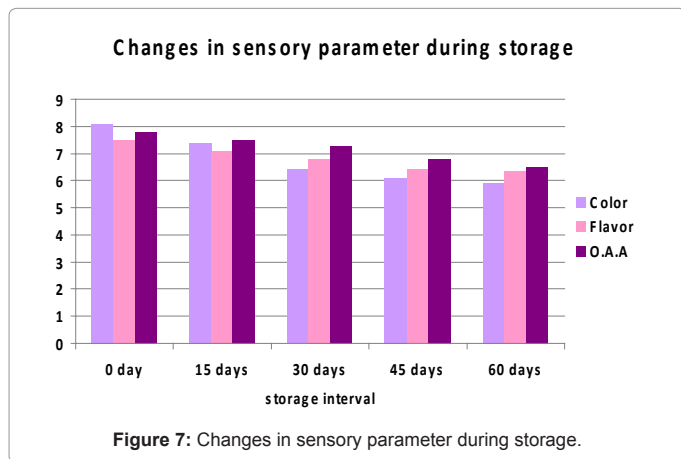


Figure 7: Changes in sensory parameter during storage.

were 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 safety level (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 extracts of 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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