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Research article

DEVELOPMENT AND QUALITY EVALUATION OF RTSBEVERAGESMADE FROMTRADITIONAL INDIAN MEDICINAL PLANTS

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ABSTRACT: Kinnow juice, Basil extract, ginger and sugar syrup were optimized and blended to form a RTS beverage which was pasteurized at 90°C for 25sec cooled and stored at refrigerated temperature 5°C for 20 days. Physic-chemical and sensory analysis was done. Marginal changes in pH, total soluble solids, acidity, vitamin C and antioxidant content were observed.The TSS increased during the storage period and was reported to be increased by 2.5°Brix. Addition of basil extract and ginger to kinnow juice greatly increased the antioxidant potential of the juice and also Vitamin C. Vitamin C, pH and subsequently acidity decreased as the storage time increased. Ascorbic acid content was reported to decrease by 10-14 mg/100gm in all the RTS (Ready to Serve) beverages. The antioxidant potential gradually decreased during the storage period from 54.2 in 10% to 60.1 in 25% RTS. The mean overall acceptability scores of more than 8 for beverage samples up to 20% basil extract incorporation indicated the commercial scope for manufacturing good and nutritious RTS beverage, which will also be helpful in providing good antioxidant and nutraceutical potential to the consumer. Heat pasteurisation (90°C for 25 sec) and basil and ginger extractwas more effective for inactivating the microbial flora. However the shelf life ofthe RTS was established within 10 days, after this the acceptability decreased. The product is recommended children, youth and elderly persons to be used within 10 days.

Key words: Kinnow juice, basil extract, ginger, RTS beverage, physicochemical evaluation, sensory evaluation, antioxidants

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INTRODUCTION

Medicinal plants are being used from the ancient times as the source of medicine and healing properties. They have nutraceutical potential which makes it effective for use in any infection and disease according to the traditional concept of Ayurveda. Holy Basil (*Ocimum sanctum*)is one of the most widely grown herbs for therapeutic use. (Vani *et al*, 2009) The herb is used as a remedy for a variety of conditions including the common cold, headaches, stomach disorders, heart disease, inflammation, malaria, various forms of poisoning, as well as spiritual and flavoring purposes. Recent studies suggest holy basil may be a (cyclooxygenase-2) COX-2 inhibitor, like many modern painkillers, due to its high concentration of eugenol (Prakash *et al* 2005). One small study showed it to reduce blood glucose levels in type 2 diabetics when combined with hypoglycemic drugs (Rai *et al*, 1997). It has also shown beneficial for reducing the cholesterol levels and blood glucose levels (Sethi et al 2004), for radiation radiation poisoning (Devi *et al* 1999) and cataracts (Sharma *et al* 1998) due to its high antioxidant content. It is also very useful for respiratory disorders (Khogare*et al,* 2011). Ginger (*Zingiberofficinale)* has strong antibacterial and to some extent antifungal properties. In vitro studies have shown that active constituents of ginger inhibit multiplication of colon bacteria. It inhibits the growth of Escherichia coli, Proteus sp, Staphylococci, Streptococci and Salmonella (Ody*et al* 2000). Ginger is also used in Chinese and Japanese medicines for cholesterol and blood glucose lowering effects. (Mowery *et al*, 1982; Kobayashi *et al*, 1988; James *et al*, 1999)

Kinnow *(Kinnowmandarin)* is quite important as it has a great variety of beverage, industrial and medicinal uses due to its attractive colour, distinctive flavour and being rich source of vitamin 'C', vitamin 'B', β-carotene, calcium and phosphorous (Sogi and Singh, 2001). Kinnowjuice turns bitter after extraction due to conversion of limonate-a-ring-lactone (non-bitter) to limonin (bitter compound) during storage (Premi et al., 1994), and makes the processing of this fruit limited. For improving the taste, aroma, palatability, nutritive value and reducing bitterness kinnow juice was blended with some other highly nutritive fruit juices and spice extracts. (Room, 1986).

Thus blending of these juices together will improve the nutritional value of the juices and also the antimicrobial effects. Sethi in 2004 conceptualized that fruit drink based on ayurvedic formulations has great medicinal and therapeutic value for an individual and also evaluate that there are different fruit drinks or herbal drinks according to the seasonal requirement for an individual. There are many fruit drinks or RTS beverages of ayurveda has been made or utilized for the health benefits of an individual (De Carvalho et al., 2007). Moreover, one could think of a newproduct development through blending in the form of a natural health drink, which may also be served as an appetizer.

MATERIALS AND METHODS

Fully matured and fresh fruits and the required raw materials were procured from local market of Jalandhar, Punjab due to its easy proximity and were brought to Lovely Professional University, Phagwara. Juice blends were developed in different ratios as mentioned in Table 1.

Table 1. Blending ratios of RTS beverages

Samples	Kinnow juice	Basil extract	Ginger extract	Sugar syrup	Total volume
RTS 10%	40ml	10ml	5ml	45ml	100ml
RTS 15%	35ml	15ml	5ml	45ml	100ml
RTS 20%	30ml	20ml	5ml	45ml	100ml
RTS 25%	25ml	25ml	5ml	45ml	100ml

RTS beverage preparation

Fresh holy basil leaves were washed and then blended in pestle mortar .The thick paste was used to extract the juice with the help of distilled water and muslin cloth. Appropriate quantity of that juice was then used in different ratios in the preparation of the RTS drink. Ginger was first cut into small pieces and then blended very finely in a pestle mortar. Juice was extracted using distilled water. Kinnow juice was extracted using a household mixer and then used along with the pulp. Sugar syrup was made using 100gms of sugar and 50gms of water heated to 102 °C. Kinnow juice, holy basil, ginger and sugar syrup were used in the ratios of 40:10:5:45; 35:15:5:45; 30:20:5:45; and 25:25:5:45. Three batches of beverages mixture were prepared. The products were filled in PET bottles which were sterilized at 110°C for 10 minutes, then sealed .After that bottles were pasteurized at 90°C for 25sec cooled and stored at refrigerated temperature5°C for 20 days.

Total soluble solids

Total soluble solids were analysed by using Digital refractometer (Rudolph, USA). Fruit pulp was extracted and passed through muslin cloth, a drop of filtrate was put on a refractometer prism and TSS were recorded as °Brix.

Titrable acidity and pH

10 gm well mixed juice was diluted to 250ml with boiled water. Titration was done with 0.1N NaOH , 0.3ml phenolpthlein for each 100ml of the solution to pink. End point was taken for 30 seconds (Rangana, 2010). pH was taken by using pH meter.

Ascorbic acid

Sample solution equivalent to 0.2mg ascorbic acid $mL^{-1}$was prepared in water containing 3% (w/v) metaphosphoric acid. It was titrated against standard 2,6 dichlorophenol indophenol (2,6 DCIP) solution of 0.5mg mL^{-1}concentration until the pink colordeveloed completely. The same process was repeated with blank (Rangana, 2010)

Antioxidant activity:

Free radical scavenging activity of extracts was measured by: the evaluation of the free radical - scavenging effect on the1,1-diphenyl-2-picrylhydrazyl radical. An aliquot of fruit extract will mixed with 3.9 ml of 0.1 mM DPPH methanol solution . The mixture will thoroughly mixed and kept in the dark for 30 minutes. The absorbance wasl measured later,at 515 nm, against a blank of methanol without DPPH.

Microbiological studies

Prepared RTS were studies for microbial load. The microbial content of all the samples was estimated by using total plate count technique (APHA, 1967)

Sensory analysis

All estimations were carried out in triplicate at 5 days interval and the mean values reported. A panel of 10 semi-trained members carried out the overall acceptance test for the beverage 9-point Hedonic scale, where 9 is "like extremely" and 1 is "dislike extremely" as described byAmerine et al, 1965.

Statistical analyses

The statistical analyses were carried out by Two- way ANOVA classification as described by Snedecor and Cochran 1968.

RESULTS AND DISCUSSION

Physicochemical analysis

Total soluble solids

The TSS increased with gradual passage of storage time, which might be due to hydrolysis of polysaccharides into monosaccharide and oligosaccharides. Similar results were also reported by Jan and Masih (2012), Deka and Sethi (2001) in juice blends and Deka (2000) found an increasing trend in total soluble solids during storage at ambient and low temperature in lime - aonla and mango-pineapple spiced RTS beverages. (Table 2).

Table 2. Total soluble solids in the RTS beverages

Treatments	0 day	5th day	10th day	15th day	20th day
RTS 10%	12.6 ± 0.15	12.9 ± 0.06	13.4 ± 0.11	14.0 ± 0.03	14.2 ± 0.2
RTS 15%	12.9 ± 0.02	13.5 ± 0.14	13.7 ± 0.13	14.3 ± 0.11	14.5 ± 0.05
RTS 20%	13.2 ± 0.14	14.0 ± 0.16	14.2 ± 0.09	14.6 ± 0.14	14.9 ± 0.12
RTS 25%	13.6 ± 0.12	14.4 ± 0.09	14.7 ± 0.02	14.9 ± 0.07	15.4 ± 0.16
F- test	S	S	S	S	S
C.D.(P=0.05)	0.082	0.093	0.124	0.079	0.091

***Mean ± S.D. (n=3)**

Titratable acidity

There was a significant decrease in titratable acidity content during storage (Table 3), this was due to the decreasing amount of Kinnow juice in the respective blends. Titrable acidity decreased from about 0.61 on day 0 in the optimized RTS to about 0.19 on the 20th day. Significant decrease was assumed to due to the decrease in the Vitamin C content as it is soluble in water and oxidation sensitive (Simsek 2011; Mishra 2011).

Table 3. Acidity in the RTS beverages

Treatments	0 day	5th day	10th day	15th day	20th day
RTS 10%	0.64 ± 0.04	0.57± 0.02	0.39± 0.07	0.22± 0.01	0.19 ± 0.02
RTS 15%	0.61 ± 0.16	0.55± 0.05	0.31± 0.17	0.3± 0.01	0.23 ± 0.12
RTS 20%	0.59 ± 0.09	0.54 ± 0.14	0.44 ± 0.11	0.43 ± 0.07	0.36 ± 0.09
RTS 25%	0.56 ± 0.11	0.52 ± 0.08	0.37 ± 0.06	0.39 ± 0.04	0.31 ± 0.06
F- test	S	S	S	S	S
C.D.(P=0.05)	0.984	0.968	0.884	0.104	0.926

***Mean ± S.D. (n=3)**

Ascorbic acid

The ascorbic acid (vitamin "C□) content of the beverages decreased during storage with the advancement of storage period, which was probably due to the fact that ascorbic acid being sensitive to oxygen, light and heat was easily oxidized in presence of oxygen by both enzymatic and non-enzymatic catalyst. Maximum ascorbic acid content was found in RTS 10% at day 0 which gradually decreased till day 20th. Minimum ascorbic acid content was found in RTS 25% at the day 20th (Table 4).

Table 4. Ascorbic acid content in the RTS beverages

Treatments	0 day	5th day	10th day	15th day	20th day
RTS 10%	52.6 ± 0.52	45.5 ± 0.34	38.4 ± 0.17	36.4 ± 0.16	33.9 ± 0.11
RTS 15%	43.6 ± 0.40	39.6 ± 0.37	31.5 ± 0.22	30.7 ± 0.05	29.7 ± 0.21
RTS 20%	38.0 ± 0.02	31.2 ± 0.09	27.2 ± 0.07	24.4 ± 0.09	25.2 ± 0.03
RTS 25%	29.6 ± 0.40	26.7 ± 0.11	22.9 ± 0.16	21.1 ± 0.12	19.6 ± 0.06
F- test	S	S	S	S	S
C.D.(P=0.05)	0.424	0.187	0.948	1.346	2.187

***Mean ± S.D. (n=3)**

pH

There was a significant decrease in pH during storage (Table 5). This might be due to increase in titrable acidity, as acidity and pH are inversely proportional to each other. Majumdar et al 2011, also reported similar results for a juice blend of bottleguard and basil leaves juice.

Table 5. pH content in the RTS beverages

Treatments	0 day	5th day	10th day	15th day	20th day
RTS 10%	5.49 ± 0.13	3.19 ± 0.01	2.55 ± 0.05	2.40 ± 0.11	2.22 ± 0.01
RTS 15%	5.43 ± 0.05	3.37 ± 0.04	2.50 ± 0.01	2.39 ± 0.05	2.29 ± 0.06
RTS 20%	5.38 ± 0.23	3.26 ± 0.22	2.45 ± 0.10	2.36 ± 0.07	2.19 ± 0.02
RTS 25%	5.36 ± 0.03	3.21 ± 0.16	2.38 ± 0.11	2.31 ± 0.09	2.16 ± 0.15
F- test	S	S	S	S	S
C.D.(P=0.05)	0.028	0.674	0.736	0.782	0.814

***Mean ± S.D. (n=3)**

Antioxidant value

The antioxidant capacity (DPPH value) of the juice blend with 25% of holy basil was found maximum and least was found in RTS 10% on day 0 (Table 6). The antioxidant potential gradually decreased during the storage period from 54.2 in 10% to 60.1 in 25% RTS. Similar results were reported by Gao and Rupasinghe in 2012 on apple carrot juice blends.

Table 6. DPPH activity in the RTS beverages

Treatments	0 day	5th day	10th day	15th day	20th day
RTS 10%	64.7 ± 1.11	62.3 ± 0.32	61.3 ± 0.05	57.1 ± 0.25	54.2 ± 0.04
RTS 15%	66.5 ± 1.82	63.9 ± 0.04	62.1 ± 0.05	59.5 ± 0.16	56.6 ± 0.75
RTS 20%	68.4 ± 0.11	66.5 ± 0.28	64.9 ± 0.02	61.3 ± 0.13	59.9 ± 0.25
RTS 25%	70.2 ± 0.04	67.2 ± 0.84	64.7 ± 0.14	63.4 ± 0.11	60.1 ± 0.28
F- test	S	S	S	S	S
C.D.(P=0.05)	0.386	0.296	0.286	0.274	0.368

***Mean ± S.D. (n=3)**

Storage studies

The optimized mixed RTS beverageswere pasteurized at 90°C for 25sec cooled and stored at refrigerated temperature 5°C for 20 days. The sensory parameters, TSS, titrable acidity, pH, vitamin C content and antioxidant activity of these RTS were studies for a period of 20 days, after every 5 day intervals. The overall acceptability of the RTS beverages did not show significant difference during storage ($p>0.05$). In microbiological study, immediately after preparation of juice, the total number of viable count were not uniform. It also showed that the count increased during the storage period. The initial microbial load was found to be 1.84 (log CFU g^{-1}) which was not increased significantly during the storage period. The resistance to microbial activity was assumed because of the basil and ginger content as they are potent antimicrobials.

Sensory evaluation

Overall sensory scores obtained by the different beverages are documented in Table 7. The intention was to incorporate the maximum possible quantity of basil extract in the beverage with higher sensory scores and adjustment of acidity to get good taste. It was observed that the highest sensory score of was obtained with maximum incorporation of 20% of basil extract i.e. RTS 20%, with the composition of Kinnow juice, holy basil, ginger and sugar syrup in the ratio of 30:20:5:45.

Table 7. Sensory acceptance of the RTS 20% beverage

Sensory parameters	0 day	5th day	10th day	15th day	20th day
Colour	7.8 ± 0.04	7.9 ± 0.24	7.4 ± 0.06	6.9 ± 0.13	5.2 ± 0.14
Flavour	8.6 ± 0.06	7.2 ± 0.16	7.0 ± 0.09	5.4 ± 0.12	4.2 ± 0.19
Taste	9 ± 0.12	8 ± 0.21	6.8 ± 0.01	4.8 ± 0.16	3.8 ± 0.07
Overall acceptability	8.5 ± 0.08	7.7 ± 0.14	7.06 ± 0.03	5.7 ± 0.07	4.4 ± 0.09

***Mean ± S.D. (n=10)**

CONCLUSION

It was concluded from the study that RTS beverage with incorporation 20% basil extract was acceptable having the ratios 30:20:5:45 of Kinnow juice, holy basil, ginger and sugar syrup respectively. Physicochemical parameters did not show any significant alteration, however the antioxidant and nutraceutical potential was increased by addition of holy basil and ginger to the kinnow juice. On the basis of above results revealed in the present study it may be concluded that the formulation of mixed blend juice beverage is possible to satisfy consumer taste and preferences. Utilization of the medicinal plants in diet or by incorporation and optimizing their use in fruit beverages, an individual will get all the benefits related to health and also reduces the risk of serious diseases like diabetes and other cardiovascular diseases.

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