

## Quality Evaluation of Herbal Juice Developed from Traditional Indian Medicinal Plants Using *Citrus limetta* as Base

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### Abstract

Lime juice, basil extract, ginger and sugar syrup were optimized and blended to form a herbal juice which was pasteurized at 90°C for 25 sec cooled and stored at refrigerated temperature 5°C for 20 days. Physico-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 lime juice greatly increased the antioxidant potential of the juice and also Vitamin C. Vitamin C and pH and subsequently decreased as the storage time increased. Ascorbic acid content was reported to decrease by 10-12 mg/100 gm in all the juice blends. The antioxidant potential gradually decreased during the storage period from 50.5 in 10% to 56.1 in 25% juice blends. The mean overall acceptability scores of more than 8 for juice blends samples up to 20% basil extract incorporation indicated the commercial scope for manufacturing good and nutritious juice blends, 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 extract were more effective for inactivating the microbial flora. However the shelf life of the juice blends 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.

**Keywords:** Lime juice; Basil extract; Ginger; Herbal juice; Physicochemical evaluation; Sensory evaluation; Antioxidants

### 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 [1]. 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 [2]. One small study showed it to reduce blood glucose levels in type 2 diabetics when combined with hypoglycemic drugs [3]. It has also shown beneficial for reducing the cholesterol levels and blood glucose levels [4], for radiation radiation poisoning [5] and cataracts [6] due to its high antioxidant content. It is also very useful for respiratory disorders [7]. Ginger (*Zingiber officinale*) 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* [8]. Ginger is also used in Chinese and Japanese medicines for cholesterol and blood glucose lowering effects [9-11].

Fresh lime juice (*Citrus limetta*) possesses medicinal property which is well known from ancient ages in India. It is also called as sacred fruit in the Vedas. Vitamin C as a primary component of the lime juice increases the resistance of individuals to several diseases, helps in wound healing and increases the health of eyes. It improves the maintenance of good dentition of bones and bleeding of the gums [12].

Citrus fruits are rich in flavonoids and limonoids which are known to possess antitumor and anti-inflammatory activities. Lime is vital in the treatment of gastric disorders like indigestion, constipation and peptic ulcer. It stops the occurrence of indigestion, burning in the

chest due to high acidity in the stomach, abrupt bilious vomiting and excessive accumulation of saliva in the mouth. In chronic constipation by promoting biliary secretion from the liver, it improves intestinal motility. Acidic juice of the fruit acts as curative for tonsillitis. Oral ingestion of lime juice mixed with salt in water provides relief from burning sensation and also stops bleeding in cystitis (inflammation of urinary bladder) (Figure 1).

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 and beverages of Ayurveda made or utilized for the health benefits of an individual [13] (Figure 2). Moreover, one could think of a new product development through blending in the form of a natural health drink, which may also be served as an appetizer.

### Materials and Methods

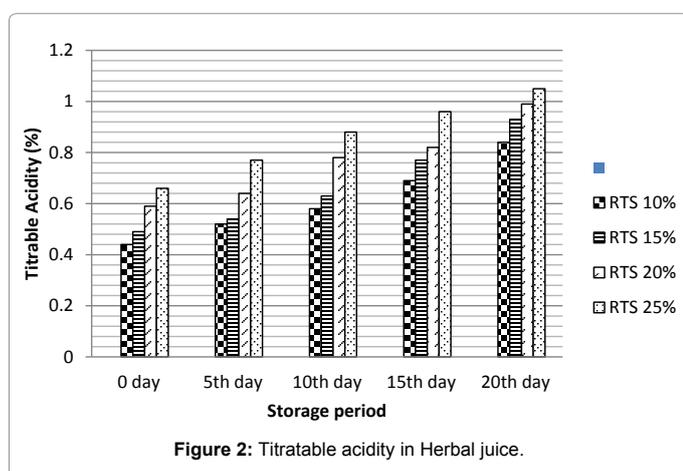
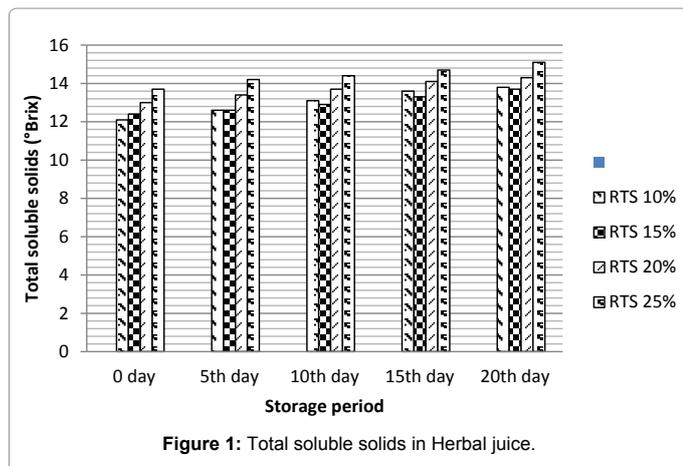
Fully matured and fresh fruits and the required raw materials were procured from local market of Jalandhar, Punjab due to its easy proximity and were brought to Lovely Professional University, Phagwara. Juice blends were developed in different ratios as mentioned in Table 1 (Figure 3).

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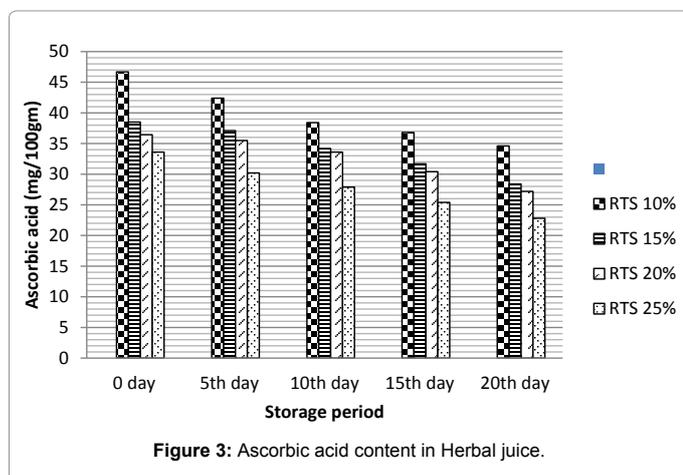
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| Samples | Lime juice | Basil extract | Ginger extract | Sugar syrup | Total volume |
|---------|------------|---------------|----------------|-------------|--------------|
| RTS 10% | 40ml       | 10ml          | 5ml            | 45ml        | 100ml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

Table 1: Blending ratios of Herbal juice.



## Herbal juice 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 herbal juice. Ginger was first cut into small pieces and then blended very finely in a pestle mortar. Juice was extracted using distilled water. Lime juice was extracted using a household mixer and then used along with the pulp. Sugar syrup was made using 100 gms of sugar and 50 gms of water heated to 102°C. Lime 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 juice blends 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 25 sec cooled and stored at refrigerated temperature 5°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 (Figure 4).

## Titrate acidity and pH

10 gm well mixed juice was diluted to 250 ml with boiled water. Titration was done with 0.1 N NaOH, 0.3 ml phenolphthalein for each 100 ml of the solution to pink. End point was taken for 30 seconds [14]. pH was taken by using pH meter.

## Ascorbic acid

Sample solution equivalent to 0.2 mg ascorbic acid mL<sup>-1</sup> 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<sup>-1</sup> concentration until the pink color developed completely. The same process was repeated with blank [14] (Figure 5).

## Antioxidant activity

Free radical scavenging activity of extracts was measured by: the evaluation of the free radical - scavenging effect on the 1,1-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 mix and kept in the dark for 30 minutes. The absorbance was measured later, at 515 nm, against a blank of methanol without DPPH [14].

## Microbiological studies

Prepared juice blends were studied 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 juice blends 9-point Hedonic scale, where 9 is "like extremely" and 1 is "dislike extremely" as described by Amerine et al. [15].

## Statistical analyses

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

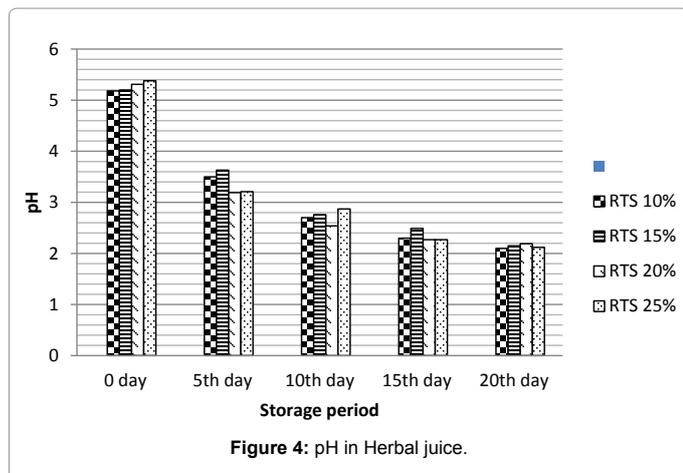


Figure 4: pH in Herbal juice.

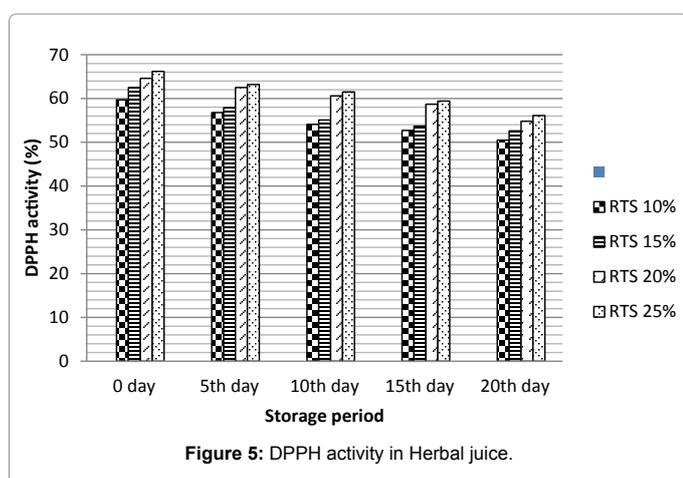


Figure 5: DPPH activity in Herbal juice.

## 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 [16], Deka and Sethi [17] in juice blends and Deka [18] 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).

### Titrateable acidity

There was a significant increase in titrateable acidity content during storage (Table 3); this was due to the increasing amount of lime juice in the respective blends. Titrateable acidity increased from about 0.68 on day 0 in the optimized juice containing 25% of lime to about 1.05 on the 20<sup>th</sup> day.

### Ascorbic acid

The ascorbic acid (vitamin “C”) content of the juices 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 juice 10% at day 0 which gradually decreased till

day 20<sup>th</sup>. Minimum ascorbic acid content was found in juice 25% at the day 20<sup>th</sup> (Table 4).

### pH

There was a significant decrease in pH during storage (Table 5). This might be due to increase in titrateable acidity, as acidity and pH are inversely proportional to each other. Majumdar et al. [19] also reported similar results for a juice blend of bottle guard and basil leaves juice.

### Antioxidant value

The antioxidant capacity (DPPH value) of the juice blend with 25% of holy basil was found maximum and least was found in juice 10% on day 0 (Table 6). The antioxidant potential gradually decreased during the storage period from 59.7 in 10% to 56.1 in 25% juice blends. Similar results were reported by Gao and Rupasinghe [20] on apple carrot juice blends.

### Storage studies

The optimized mixed juice blends were pasteurized at 90°C for 25

| Treatments   | 0 day       | 5 <sup>th</sup> day | 10 <sup>th</sup> day | 15 <sup>th</sup> day | 20 <sup>th</sup> day |
|--------------|-------------|---------------------|----------------------|----------------------|----------------------|
| RTS 10%      | 12.1 ± 0.14 | 12.6 ± 0.05         | 13.1 ± 0.14          | 13.6 ± 0.02          | 13.8 ± 0.18          |
| RTS 15%      | 12.4 ± 0.28 | 12.6 ± 0.17         | 12.9 ± 0.11          | 13.3 ± 0.06          | 13.7 ± 0.11          |
| RTS 20%      | 13 ± 0.19   | 13.4 ± 0.11         | 13.7 ± 0.09          | 14.1 ± 0.11          | 14.3 ± 0.14          |
| RTS 25%      | 13.7 ± 0.14 | 14.2 ± 0.07         | 14.4 ± 0.13          | 14.7 ± 0.08          | 15.1 ± 0.04          |
| F- test      | S           | S                   | S                    | S                    | S                    |
| C.D.(P=0.05) | 0.094       | 0.093               | 0.324                | 0.129                | 0.098                |

Mean ± S.D. (n=3)

Table 2: Total soluble solids in Herbal juice.

| Treatments   | 0 day       | 5 <sup>th</sup> day | 10 <sup>th</sup> day | 15 <sup>th</sup> day | 20 <sup>th</sup> day |
|--------------|-------------|---------------------|----------------------|----------------------|----------------------|
| RTS 10%      | 0.44 ± 0.11 | 0.52 ± 0.06         | 0.58 ± 0.12          | 0.69 ± 0.05          | 0.84 ± 0.17          |
| RTS 15%      | 0.49 ± 0.05 | 0.54 ± 0.08         | 0.63 ± 0.17          | 0.77 ± 0.06          | 0.93 ± 0.09          |
| RTS 20%      | 0.59 ± 0.62 | 0.64 ± 0.04         | 0.78 ± 0.16          | 0.82 ± 0.19          | 0.99 ± 0.02          |
| RTS 25%      | 0.66 ± 0.10 | 0.77 ± 0.06         | 0.88 ± 0.08          | 0.96 ± 0.14          | 1.05 ± 0.05          |
| F- test      | S           | S                   | S                    | S                    | S                    |
| C.D.(P=0.05) | 0.764       | 0.858               | 0.784                | 0.145                | 0.916                |

Mean ± S.D. (n=3)

Table 3: Acidity in Herbal juice.

| Treatments   | 0 day        | 5 <sup>th</sup> day | 10 <sup>th</sup> day | 15 <sup>th</sup> day | 20 <sup>th</sup> day |
|--------------|--------------|---------------------|----------------------|----------------------|----------------------|
| RTS 10%      | 46.7 ± 0.12  | 42.4 ± 0.14         | 38.4 ± 0.06          | 36.8 ± 0.12          | 34.6 ± 0.14          |
| RTS 15%      | 38.5 ± 0.09  | 37.1 ± 0.11         | 34.2 ± 0.23          | 31.7 ± 0.17          | 28.4 ± 0.13          |
| RTS 20%      | 36.44 ± 0.06 | 35.5 ± 0.20         | 33.6 ± 0.14          | 30.4 ± 0.18          | 27.2 ± 0.09          |
| RTS 25%      | 33.6 ± 0.10  | 30.2 ± 0.22         | 27.9 ± 0.11          | 25.4 ± 0.13          | 22.8 ± 0.10          |
| F- test      | S            | S                   | S                    | S                    | S                    |
| C.D.(P=0.05) | 0.564        | 0.326               | 0.994                | 0.998                | 1.867                |

Mean ± S.D. (n=3)

Table 4: Ascorbic acid content in Herbal juice.

| Treatments   | 0 day       | 5 <sup>th</sup> day | 10 <sup>th</sup> day | 15 <sup>th</sup> day | 20 <sup>th</sup> day |
|--------------|-------------|---------------------|----------------------|----------------------|----------------------|
| RTS 10%      | 5.18 ± 0.13 | 3.5 ± 0.11          | 2.7 ± 0.06           | 2.3 ± 0.11           | 2.1 ± 0.13           |
| RTS 15%      | 5.2 ± 0.12  | 3.63 ± 0.08         | 2.76 ± 0.04          | 2.49 ± 0.13          | 2.15 ± 0.19          |
| RTS 20%      | 5.31 ± 0.21 | 3.19 ± 0.06         | 2.54 ± 0.05          | 2.27 ± 0.10          | 2.19 ± 0.14          |
| RTS 25%      | 5.38 ± 0.09 | 3.21 ± 0.22         | 2.87 ± 0.12          | 2.27 ± 0.14          | 2.12 ± 0.09          |
| F- test      | S           | S                   | S                    | S                    | S                    |
| C.D.(P=0.05) | 0.628       | 0.684               | 0.866                | 0.892                | 0.984                |

Mean ± S.D. (n=3)

Table 5: pH content in Herbal juice.

| Treatments   | 0 day       | 5 <sup>th</sup> day | 10 <sup>th</sup> day | 15 <sup>th</sup> day | 20 <sup>th</sup> day |
|--------------|-------------|---------------------|----------------------|----------------------|----------------------|
| RTS 10%      | 59.7 ± 0.21 | 56.8 ± 0.20         | 54.1 ± 0.09          | 52.7 ± 0.12          | 50.5 ± 0.11          |
| RTS 15%      | 62.5 ± 0.06 | 57.9 ± 0.13         | 55.1 ± 0.05          | 53.7 ± 0.09          | 52.6 ± 0.13          |
| RTS 20%      | 64.6 ± 0.08 | 62.5 ± 0.15         | 60.6 ± 0.03          | 58.7 ± 0.14          | 54.8 ± 0.20          |
| RTS 25%      | 66.2 ± 0.10 | 63.2 ± 0.19         | 61.5 ± 0.11          | 59.4 ± 0.08          | 56.1 ± 0.16          |
| F- test      | S           | S                   | S                    | S                    | S                    |
| C.D.(P=0.05) | 0.546       | 0.479               | 0.627                | 0.624                | 0.712                |

Mean ± S.D. (n=3)

Table 6: DPPH activity in Herbal juice.

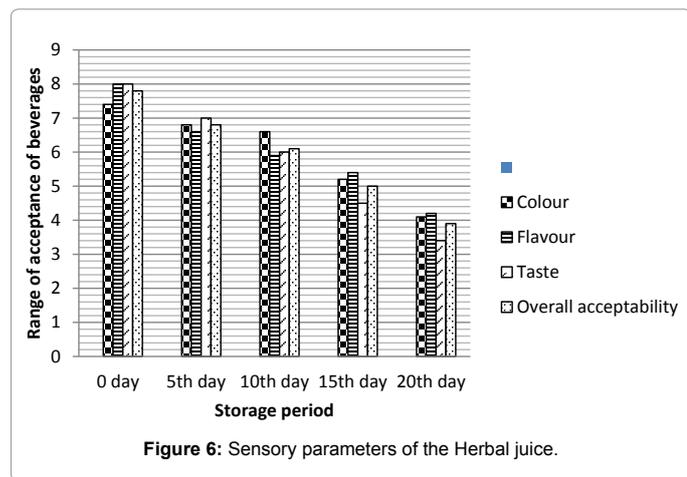


Figure 6: Sensory parameters of the Herbal juice.

| Sensory parameters    | 0 day      | 5 <sup>th</sup> day | 10 <sup>th</sup> day | 15 <sup>th</sup> day | 20 <sup>th</sup> day |
|-----------------------|------------|---------------------|----------------------|----------------------|----------------------|
| Colour                | 7.4 ± 0.15 | 6.8 ± 0.04          | 6.6 ± 0.05           | 5.2 ± 1.04           | 4.1 ± 0.13           |
| Flavour               | 8 ± 0.13   | 6.6 ± 0.06          | 5.9 ± 0.04           | 5.4 ± 0.21           | 4.2 ± 0.14           |
| Taste                 | 8 ± 0.24   | 7 ± 0.08            | 6 ± 0.08             | 4.5 ± 0.25           | 3.4 ± 0.09           |
| Overall acceptability | 7.8 ± 0.11 | 6.8 ± 0.18          | 6.1 ± 0.07           | 5 ± 0.14             | 3.9 ± 0.06           |

Mean ± S.D. (n=10)

Table 7: Sensory acceptance of the Herbal juice 20%.

sec 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 juice blends were studied for a period of 20 days, after every 5 day intervals. The overall acceptability of the juice blends did not show significant difference during storage ( $p > 0.05$ ). In microbiological study, immediately after preparation of juice, the total number of viable count was not uniform. It also showed that the count increased during the storage period. The initial microbial load was found to be 1.74 (log CFU g<sup>-1</sup>) 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 [21] (Figure 6).

### Sensory evaluation

Overall sensory scores obtained by the different juice blends are documented in Table 7. The intention was to incorporate the maximum possible quantity of basil extract in the juice blends 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. juice blends 20%, with the composition of lime juice, holy basil, ginger and sugar syrup in the ratio of 30:20:5:45 [22-24].

### Conclusion

It was concluded from the study that juice blends with incorporation 20% basil extract was acceptable having the ratios 30:20:5:45 of lime juice, holy basil, and ginger and sugar syrup respectively. Physicochemical parameters did not show any significant alteration, however the antioxidant and nutraceuticals potential was increased by addition of holy basil and ginger to the lime juice. Sensory evaluation also revealed that the juice blends are acceptable till 5<sup>th</sup> to 10<sup>th</sup> day of preparation, thereafter the quality deteriorated for consumption. On the basis of above results revealed in the present study it may be concluded that the formulation of mixed blend juice 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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