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Development and quality analysis of watermelon juice blends with bitter gourd: ginger juice during storage

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Abstract
Watermelon (*Citrulluslanatus*), Bitter Gourd (*Memordica charantia*) and ginger juice (*Zingiber officinale*) were formulated to a blend in different proportion (v/v) as T1 (90:05:05), T2 (80:10:10), T3 (80:15:05) and T0 (100:00:00) respectively. Sensory analysis and Physico-chemical analysis were evaluated. Marginal changes in pH, Total Soluble Solids (TSS), acidity and vitamin C were observed. The results of the analysis of Vitamin C analysis content gave 74.91 mg/ 100 ml in standard watermelon juice, 58.11 mg/ 100 ml in T1, 53.72 mg/ 100 ml in T2, 40.62 mg/ 100 ml in T3 respectively. Titrable acidity of all blends increased (0.21-0.47) and pH of the juice decreased progressively during the storage period. This may be due to the fermentation process which generally took place during storage period and presence of lactic acid reducing micro-organism in the juices. The mean overall acceptability scores up to 7 for T2 sample were observed. In microbial analysis, total plate count and total yeast and mould count, was analyzed up to 30 days with every 10 days interval counting and showed that microbial activity was very low on first day but it was gradually increased till 30 days of storage period to 27 x 10^2. These values were within the safe limit for juices, as they have not exceeded the standard values of 10^3cfu/ml. This indicated that the pasteurization (80ºC for 3 min.) of the samples was efficient and the product was safe for consumption by consumer. However the shelf life of prepared beverage was established within 30 days.

Keywords: watermelon, bitter gourd, physico-chemical, TSS, titrable acidity and total bacterial count

Introduction
Now day's healthy foods have many aims: satisfying hunger and providing the necessary nutrients for humans, in improving health, preventing and/or reducing nutrition-related diseases. In recent years, consumers' awareness towards the correlation between food and health has led to an explosion of interest in "healthy foods"; this phenomenon could be partly attributed to the increasing cost of healthcare, the steady increase in life expectancy, and the desire of older people for an improved quality of their later years [1].The beverages play a very important role in the prevention of various diseases and good health. It was observed that beverages can reduce increasing burden on health care system with the help of a continuous preventive mechanism [2]. Beverages are very excellent medium for the supplementation of nutraceutical components for enrichment [3] such as soluble fiber or herbal extract [4]. The new formulations of beverages are rapidly changing.

Most fruits and vegetables are rich in functional compounds like minerals, vitamins, dietary fiber, antioxidants, and do not contain any dairy allergens that might prevent usage by certain segments of the population. Fruit juices are alcohol free liquid products with a variety of degree of clarity and viscosity. They are obtained through mechanical pressing or by breaking up the fruits with or without adding sugar or carbon dioxide. Watermelon juice is a very rich source of carotenoid, which is called lycopene. The lycopene content in watermelon is higher than many other fruits and vegetables. Lycopene present in watermelon is a very potent antioxidant; it means it can fight with free radicals in the body and can protect from damage of cells. Additionally, benefits of watermelon also include presence of vitamin A (beta carotene) and vitamin C, both known for having antioxidant and anti-aging properties that can prevent diseases. Excessive damage related with free radical can cause various serious diseases, including cancer, heart disease, Alzheimer's disease, arthritis etc.

Bitter gourd (*Memordica charantia*) is the vegetable which is also known Kerela in India. It belongs to the family Cucurbitaceae. It is a most common vegetable which is cultivated throughout India during warm season. It is native to India or China, the fast growing vine is grown throughout Asia and is becoming popular worldwide. The bitter gourd is a good source of vitamin-C and fair source of protein, minerals but is the weak source of sugar [5]. Bitter gourd has various other health benefits like weight loss etc. This vegetable is a most popular vegetable in some Asian countries, where the health benefits of the bitter gourd are well known particularly, it has the capability to lower the blood glucose level in diabetics. Bitter gourd has been used to cure diabetes as conventional medicine. It is now commercially available in local market as Tea, which is prepared from fruits and leaves of bitter gourd. In recent years many scientists globally have started to focus the anti diabetic properties of bitter gourd. The main aim is to provide safe and clear production and dosage recommendations. So that consumers will appreciate the large health benefit from consuming fresh bitter gourd fruit or bitter gourd products. Bitter gourd can control the channels proteins which participate in transport of glucose, which also reduces transportation of glucose into the blood. This effect of bitter gourd is very important for both Type I and Type II diabetic patients and it also helps to protect from high blood glucose levels after taking meals.

Ginger (*Zingiber officinale*) is a herbaceous aromatic perennial plant which possesses medicinal properties due to its bioactive compounds [6], anti-oxidants [7] and anti-inflammatory activities [8]. Effective anti-oxidants in ginger such as gingerols, zingerone and vitamin C contents, which has been inconsistent and may have capacity to blood thinning and reduce cholesterol levels that may make it useful for treating heart disease [9]. The blended ginger drinks with fruits and vegetables are generally acceptable on the basis of its medicinal and its anti-microbial properties and the production of beverages blended with ginger is highly recommended. The blended juice may help to improve taste, aroma and nutritional value of beverage. Moreover, we could develop a new product through blending in the form of nutritional health drink, which may be used as an appetizer. But the research on mixed fruit with vegetables juice is still deficient. The aim of present study is to develop watermelon blends ready to serve beverage with bitter gourd and ginger and determine their physicochemical properties during preservation.

Materials and methods

Sampling and initial processing of samples

Freshly harvested, fully ripened watermelon and mature bitter Guard and Ginger fruits used for the preparation were collected from a local fruit market in Greater Noida, Uttar Pradesh India and were brought to the Food Process and Technology laboratory, GBU, Greater Noida, India. The fruits were washed thoroughly in running water. The seeds from peeled watermelon were removed manually. The collected fruits were grinded in separate and the extraction of juice was done by using an electric juicer. The extracted juices were filtered through muslin cloth. Bitter Guard and Ginger were peeled with the help of stainless steel knife, they were sliced and grounded with addition of distilled water 1:1 (v/w) and filtered through muslin cloth to have fresh juice and then stored at refrigerated temperature for analysis.

For this study one standard with 100% watermelon and three blends sets of watermelon, Bitter Guard and Ginger juice were prepared having different ratio *e.i.* 90:05:05 (T1), 80:10:10 (T2), and 80:05:15 (T3) respectively. All the samples were pasteurized at 80 ºC for 3 min. [10] and stored at refrigerated temperature for 30 days.

Physicochemical analysis of the Watermelon juice and their blends with Bitter Gourd and Ginger juice

Analysis of Physico-Chemical properties

The Phycio-chemical properties analysis include: pH, Titratable acidity, Total Soluble Solute (TSS) (Brix) and Vitamin C.

Total acidity (as % citric acid) and vitamin C were determined by titrimetric method [11].

Total Soluble Solute (TSS) was analyzed directly with the help of refractometer ATAGO (0-32º Brix).

pH determination was done using pH meter using standard methods [12]. All the experiments were carried out in triplicate at 10 days interval up to 30 days and the mean values of the result were reported.

Sensory analysis

A qualitative descriptive analysis was developed to characterize the sensory quality of a set of 4 samples of watermelon juice blended with bitter guard and ginger juice in four different ratios. A panel of 10 semi-trained members was selected and then carried out the overall acceptance test based on color, taste, aroma, appearance and flavor for the prepared ready to serve beverage (Blended juice) using 9-point Hedonic scale, where 9 is “like extremely” and 1 is “dislike extremely” as described [13].

Analysis of Shelf life

The shelf life of a Ready-to-Serve drink can be defined as the time period within which the drink is safe to consume and/or has an acceptable quality to consumers. The benefits to conduct the self life analysis is to prevent recalls, maintain quality, protect brand/reputation, Improve profitability, avoid expensive litigation and for consumer safety. It is also very useful for product safety, functionality and for food quality/marketability. For self life analysis two different methods are used: Direct Method (Real Time) and indirect method (Accelerated). In the proposed study direct method was used in which juice samples were stored under the selected conditions up to 30 days than the expected shelf life of the beverage was analyzed at regular intervals of 10 days to see the spoilage.

Microbiological analysis

In this study for microbiological analysis, all the prepared juice samples were serially diluted. Serial dilution was done in saline solution; for serial dilution 1.0mL of juice sample was diluted with 9 mL of prepared saline solution and then sample was filtered through sterile Whatman No.1 filter paper to remove solid particles. 1.0 mL of filtrate was used for inoculation. For the isolation and counting of total bacteria and moulds two selective media such as nutrient agar and potato dextrose agar were used. Isolation was done by pour plate method and serial dilution technique. For bacterial enumeration colony forming units (CFU) was used. For computation, average number per plate was divided by juice sample volume used in inoculation and it is expressed as CFU/mL. Media was poured into the samples aseptically using the pour plate method. The mixtures were allowed to

solidify the plate was inverted and incubated at 37°C for 48 h. Colonies was counted and recorded as colony units 1 ml [14].

Statistical analysis

All the data were statistically analyzed by using one way analysis of variance (ANOVA) and analysis were carried using Microsoft Excel data analysis.

Results and Discussion

Sensory Analysis

The sensory evaluation of prepared blends from watermelon, bitter gourd and ginger juice are shown in following figure 1 that shows the mean sensory score and the significant difference among quality attributes of the blended juice. The sensory evaluations of all juice samples including blended samples were done for color, taste, aroma, flavour and overall acceptability. After sensory evaluation the best result were obtained for the T2 sample.

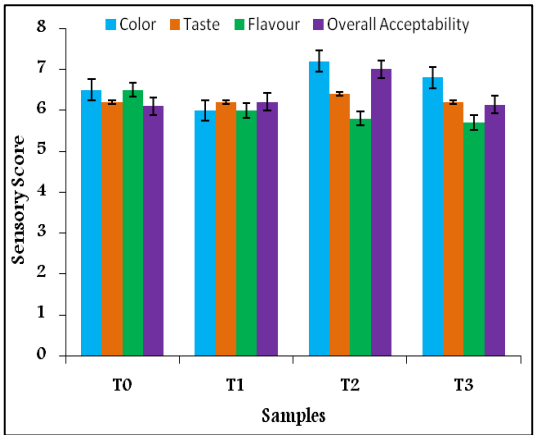

Fig 1: The sensory analysis of blended watermelon juice, bitter gourd and ginger juice

Titrable acidity

The total titrable acidity measures the ionic strength of a juice solution; this determines the rate of chemical reaction. Titrable acidity refers to the total concentration of free protons and un-dissociated acids in a solution that can react with a strong base and be neutralized. It is the measure of the amount of acid present in a solution. The addition of bitter gourd juice and ginger juice to watermelon juice increased the titrable acidity while pH value decreased. Acidity values for all the samples increases linearly during the storage period of 30 days, as shown in Table 4.2. It was observed that maximum acidity (0.49%) was recorded in T3 sample. The minimum increase (0.47%) in acidity was showed in T1 sample. There were also analyzed the similar results which supports the present study for increase in acidity during storage [15]. This increment in the acidity is recognized due to the production of CO_2 that CO_2 forms weak acid on dissolution.

Table 1: Changes in titrable acidity during storage day

Treatment	0 days	10 days	20 days	30 days
T0	0.28	0.31	0.33	0.45
T1	0.17	0.32	0.42	0.47
T2	0.19	0.30	0.43	0.48
T3	0.20	0.35	0.45	0.49
Overall mean	0.21	0.32	0.40	0.47
The *f*-ratio value is 34.96338. The *p*-value is < .00001. The result is significant at $p < .05$.				
S. Ed. (±)	0.0241	0.0108	0.0265	0.0085
Std. Dev.	0.0483	0.0216	0.0171	0.1074

Total soluble solids

It measures the sugar content of sugar solutions (juice, Honey, Syrup) in which the sugar is the major component by using the instrument refractometer. It is referred to as the degrees Brix.TSS analysis is mostly used to determine the concentration of sugar in the food products of vegetables and fruits. In TSS analysis the sugar concentration is expressed in degrees of Brix ($40^{o\ Brix}$ is equivalent to a sugar content of 40%). The Total sugar (Brix) is the sugar content of a juice or an aqueous solution. The mean of one degree brix is 1 g of sucrose present in 100 g of solution and represents the strength of the solution as percentage by weight [16]. The TSS concentration was gradually increased during the storage time of 30 days, which might be possible due to hydrolysis process of polysaccharides into oligosaccharides and monosaccharides. An increasing development in total soluble solids during storage at ambient and low temperature in lime-aonla and mango-pineapple spiced RTS beverages was found [17].

On the first day of experiment, presence of TSS content in all juice samples was found 8.5 ^{0}bx in T1 blend sample, 8.1 ^{0}bx in T2 blend sample, 8.0 ^{0}bx in T3 blend sample and the standard sample T0 had 9.7 ^{0}bx. Over the period of 30 days storage the gradual increment of TSS content was found after every ten days interval and at the end of the 30th day of storage time the TSS content was found to be 11.10 ^{0}bx, 9.3 ^{0}bx, 8.7 ^{0}bx and 8.6 ^{0}bx respectively.

Table 2: Changes in Total Soluble Solute (TSS) during storage day

Treatment	0 days	10 days	20 days	30 days
T0	9.7 ^{0}bx	10.0 ^{0}bx	10.4 ^{0}bx	11.10 ^{0}bx
T1	8.5 ^{0}bx	8.8 ^{0}bx	9.0 ^{0}bx	9.3 ^{0}bx
T2	8.1 ^{0}bx	8.4 ^{0}bx	8.6 ^{0}bx	8.7 ^{0}bx
T3	8.0 ^{0}bx	8.2 ^{0}bx	8.3 ^{0}bx	8.6 ^{0}bx
Overall mean	8.575	8.85	9.075	9.425
The *f*-ratio value is 4.5973. The *p*value is .006288. The result is significant at $p < .05$.				
S. Ed. (±)	0.390	0.403	0.464	0.579
Std Dev.	0.78	0.80	0.92	1.15

Vitamin-C

During analysis of Vitamin C for watermelon, bitter gourd and ginger juice blended samples T0, T1, T2 and T3, Vitamin C concentration was observed 74.91, 58.11, 53.72 and 40.62 mg/100ml respectively as show in Table 3. The watermelon was found to contain the highest amount of vitamin C (74.91 mg/100ml of juice), more than three times the bitter gourd and ginger juice. The addition of bitter gourd and ginger juice to watermelon juice reduced the vitamin C content. But during the storage period of 30 days the ascorbic acid concentration of the all juices decreased, which might be probably due to the fact that Vitamin C is sensitive to oxygen, light and heat. It can easily oxidized in presence of oxygen with the help of both enzymatic and non-enzymatic catalyst [18]. The results of present study are also supported by the work done by researchers who also reported a significant loss of vitamin-c concentration (25 to 26%) during storage [19]. This decrease in vitamin-C content might be due to various factors such as storage temperature, oxidative enzymes, processing techniques, metal contamination and the presence of atmospheric oxygen in the head space.

Maximum ascorbic acid (43.14 mg/100 ml juice) was recorded in T1 sample of watermelon juice blended with bitter gourd and ginger juice in ratio 90:05:05(v/v/v). Vitamin C, or ascorbic acid, is a water soluble antioxidant that plays a vital role in protecting the body from infection and disease. It

is not synthesized by the human body and therefore must be acquired from dietary sources primarily fruits and vegetables.

Table 3: Changes in vitamin-C during storage day

Treatment	0 days	10 days	20 days	30 days
T0	74.91	72.62	70.22	68.44
T1	58.11	53.71	49.31	43.14
T2	53.72	49.31	44.03	40.74
T3	40.62	37.22	36.70	33.29
Overall mean	56.84	53.215	50.065	46.40
The *f*-ratio value is 5.3698. The *p*-value is .0077618. The result is significant at $p < .05$.				
S. Ed. (±)	0.707	0.734	0.719	0.763
Std. Dev.	1.41	1.46	1.43	1.52

pH

pH is one of the main quality characteristics that describes the stability of bioactive compounds in fruit juice[20]. From the Table 4 it is cleared that pH of all samples decreased day by day. The table shows that during the storage period of 30 days the pH of the blended beverage samples was gradually decreased this indicated that the acidity of the juice products was increased.

During the storage period of 30 days minimum pH of the juice sample was recorded 4.51 in the sample T3. The pH range of most beverages or juice products is in the ranges between 3.5 and 5.5[21]. This decrease in the pH of beverage was due to the increment in titrable acidity which affects the organoleptic quality of juice and juice product as observed [22]. The results of present study with respect to pH during the storage period of 30 days were also similar with the findings of some scientist [23].

Table 4. Changes in pH during storage day

Treatment	0 days	10 days	20 days	30 days
T0	6.17	5.75	5.36	5.18
T1	5.71	5.38	5.26	5.12
T2	5.51	5.36	5.16	4.80
T3	5.40	5.31	4.83	4.51
Overall mean	5.69	5.45	5.15	4.90
The *f*-ratio value is 6.28447. The *p*-value is .008283. The result is significant at $p < .05$.				
S. Ed. (±)	0.170	0.101	0.114	0.155
Std. Dev	0.3401	0.2022	0.23	0.3103

Microbial Analysis

Microbial examinations of microorganisms are generally used to monitor indices of food spoilage. The result of the microbial analysis of blends of watermelon, bitter gourd and ginger juice during storage is as shown in Figure 2 and Figure 3. All the colonies grown in the plate were counted in colony forming unit per milliliter (cfu/ ml).The total bacterial cell count for all the juice samples on the selective media was very low on first day of microbial analysis, this result confirmed that the pasteurization of the juice samples was effective and the juice product was safe for consumption by consumer. This value steadily increased till 30 days of storage up to 15 x 10^2. [24] These values were within the safe limit for juices, as they have not exceeded the standard values of 1x 10 ^{10}.

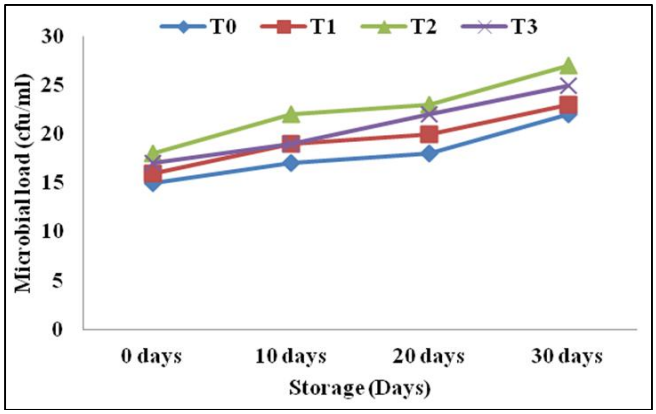

Fig 2: The total bacterial count in blended watermelon, bitter gourd and ginger juice during storage day

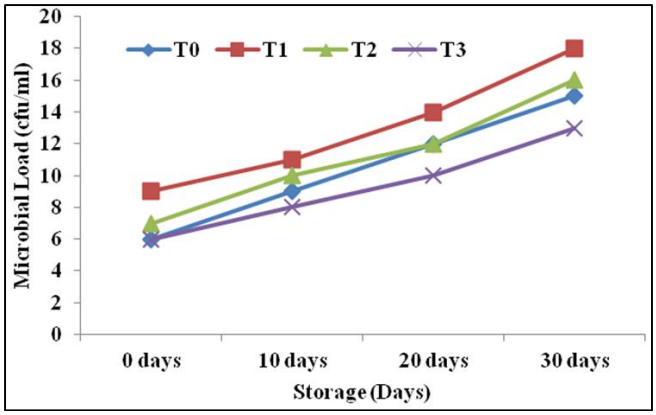

Fig 3: The total yeast & moulds count in blended watermelon, bitter gourd and ginger juice during storage day

Conclusion

Blending of fruit juices and vegetables juices could enhance their nutritional quality and development of new products. It was concluded that the best sensory evaluation score results were obtained for the watermelon, bitter gourd and ginger juice blends of 80:10:10 (V/V.V) proportion with 8.1 ^{0}bx TSS, containing vitamin C (53.72/ 100 ml of juice). Bitter gourd juice is well accepted and can be one possibility to increase the consumption of bitter gourd as anti- diabetic vegetable. Bitter gourd is sometimes called "plant insulin" because it has the efficacy for glucose managing. For a diabetic patient, a food based treatment having bitter gourd can become an easy available and very low cost effective option to insulin and therapeutic drugs. Microbial status of blended juices to ensure food safety for a specific control over public health risk. The average counts for bacteria of the prepared blended beverage samples examined were below the maximum allowable limit in foods to be marketed for consumption (10^3cfu/ml). In microbiological analysis the the microbial values were within the safe limit, as their numbers had not exceeded the standard values of 10^3 cfu/ ml, so the developed juice beverage was microbiologically safe. Thus, blend can be recommended for production at commercial level to make nutritious and healthy RTS.

Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript. This manuscript has not been published and is not going to be considered for publication elsewhere. The authors certify that neither the manuscript nor its main contents have already been published or submitted for publication in another journal.

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