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Process Optimization for Dragon Fruit Juice based Herbal Beverage Fortified with Stevia (*Stevia rebaudiana*) and Safed Musli (*Chlorophytum borivilianum*)

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ABSTRACT

The aim of this investigation was to optimise the process of formulating a herbal beverage made from the juice of dragon fruit (*Hylocereus* spp.), enhanced with the extracts of *Stevia rebaudiana* leaves and *Chlorophytum borivilianum* (safed musli) roots. Dragon fruit juice, the foundational component of the fortified beverage, was extracted using a mechanised fruit juicer. Through preliminary trials, various amounts of stevia extract (10%, 12%, 14% and 16%, v/v) and dragon fruit juice (80%, 85% and 90%, v/v) were assessed. 2% safed musli extract was added consistently to all samples. The physico-chemical characteristics of the beverage, microbiological load and sensory aspects were evaluated during a 60-day storage period. Based on the sensory test, the formulation with 90% dragon fruit juice, 10% stevia extract and 2% safed musli extract have showed the maximum degree of acceptance. After 60 days of storage under ambient circumstances, microbial analysis revealed that samples with the highest concentration of stevia extract (16%) and the lowest content of dragon fruit juice (80%) had the lowest microbial count (1.033×10^5 cfu ml^{-1}). Furthermore, whereas total sugars and sensory scores decreased with storage, notable increases in total soluble solids (TSS), acidity and total plate count (TPC) were noted. These results lay the groundwork for creating a herbal beverage made from dragon fruit that is sweetened with stevia and has better shelf life and acceptability among consumers.

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INTRODUCTION

Fruits often possess a limited lifespan due to their very perishable character. They are frequently manufactured into Ready-to-Serve (RTS) beverages, which are non-fermented drinks created with different fruit and vegetable concentrations, water, sugar and other additions, to increase their shelf life. Due to their natural bitterness or astringency, the excellent nutritional profiles of various fruits, which include high sugar, vitamin and mineral content, are typically underutilised, which limits their commercial potential. We can minimise this problem and enhance these fruits' flavour, nutritional value and shelf life by mixing them with other appropriate fruits and vegetables.

Particularly valued for their high nutritional content, reviving attributes, delicious flavour and possible medical benefits are herbal beverages. In light of this, fruit-herb blending is considered an efficient method for preserving and using underutilised fruits in beverage production. In addition to extending the shelf life of fruits, RTS herbal beverages may have other health advantages, such as lowering the chance of contracting specific diseases and acting as a natural hunger suppressant (Rathinasamy *et al.*, 2022).

Dragon fruit, a non-climacteric fruit is produced and used both as for fresh consumption and processing. The fruits are the richest source of vitamins, minerals, anti-oxidants that are crucial for preserving the best possible state of human health and immunity. Compared to other fruits, dragon fruit is higher in fibre, potassium, iron, salt, calcium and other health-promoting nutrients (Suryono, 2006). Dragon fruits have received consideration throughout few previous years among the people in society, mainly in Asian nations, due to its colour, nutritive content and many supplementary aspects (Harivaindaran *et al.*, 2008). It is a large supply of antioxidants which is a value-added quality to any agriculture produce (Rebecca *et al.*, 2010).

The fruit pulp is full of minerals like calcium, iron, potassium, salt and carotene, in addition to antioxidants, vitamin C and polyunsaturated (healthy) fatty acids. Moreover, fruit pulp can hold up to 6000 mg of vitamin C per 100 g, according to Rahmawati and Mahajoeno (2009). Seeds of Dragon fruit are a precious source of Omega-3 and Omega-6 polyunsaturated fatty acids. Dragon fruit may help the body maintain regular functions including removing heavy, poisonous substances from the body. The red pigment found in dragon fruit is known as lycopene and research has demonstrated that lycopene is associated with a lower risk of prostate cancer. Dragon fruit can also be harmful to one's health if too much is eaten. Dragon fruit is regarded as the best fruit for the future and has a lot of potential to create new processed goods with added value as demand for natural, herbal and healthful beverages rises (Pavithra and Mini, 2023).

The high efficacy of medicinal plants in natural and herbal medicines has led to a significant demand for them. Medicinal, flavouring and aromatic values are the main properties for prized the medicinal plants. Presently, people use these plants for a variety of purposes in their daily lives, including medication, cosmetics, foods, drinks and other items. In actuality, medicinal plants are very significant in today's world. The idea is predicated on the belief that the therapeutic and medical applications of the plants as herbal food products have a lot of potential.

Safed musli or *Chlorophytum borivilianum* is a member of the family Liliaceae and Kritikar *et al.* (1935) have classified this *C. borivilianum* as a medicinal plant due to its inherent attributes. It is commonly known as Doli roots. Natural growing areas for it include the foothills of the Himalayas, Uttarakhand, Chhattishgarh, the southern region of Rajasthan, the western regions of Madhya Pradesh, Uttar Pradesh and Gujarat. Safed musli roots are utilised in therapeutic preparations to prevent leucorrhea. Nursing mothers and postpartum ladies often use it as a tonic due to its aphrodisiac properties. According to recent reports, due to its sexual properties, it is utilised as a tonic for nursing mothers and recently delivered women (Widyaningsih and Kristiningrum, 2018). Additionally, commercial sterioidal hormone preparation uses root. It is typically applied to boost

vitality. In addition, it is a common ingredient in Ayurvedic remedies that are recommended for diabetes, diarrhoea, joint discomfort and blood purification.

Plate 1: Fresh dragon fruit

Plate 3: Safed musli roots

Plate 5: Stevia leaves

Plate 2: Dragon fruit juice

Plate 4: Safed musli extract

Plate 6: Stevia extract

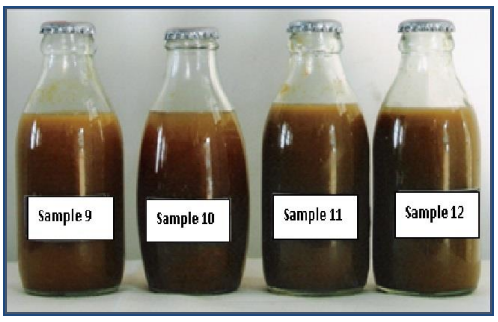

Plate 7: Blended herbal beverage of dragon fruit juice and stevia extract

Stevia possesses the scientific name as *Stevia rebaudiana* Bertoni of family Compositae. It was introduced to Thailand since 1977. The leaf of stevia plant, also referred to as sweet honey leaf, is an herbaceous perennial. One of the sugary compounds that were extracted from the leaves is called stevioside (Kohda *et al.*, 1976). Stevioside is the chemical name for the diterpene glycoside that has an aglycone component known as steviol. While the taste of stevia is comparable to that of sugar (sucrose), stevia is 300 times more sweet (Wongrattanasathit, 1990; Jain *et al.*, 2007). According to the studies, stevia has been utilized for a variety of purposes since ancient times, such as a medicinal and a sweetener (Goyal *et al.*, 2010). The refined goods that are currently made from the dried stevia leaves are widely utilised in Japan. In the Thai market, leaf powders combined with specific herbs are available. In Japan, people are using stevia to sweeten food and beverages as the country banned artificial sweeteners over 40 years ago. In general, weight reduction and diabetic individuals can benefit from it. After conducting more than 40,000 clinical trials, the Japanese concluded that stevia was safe. In the interest of health care, the manufacture of herbal products is more cost-effective and profitable (Goyal and Samsher, 2015).

Natural herbal fruit beverages and drinks are becoming more and more popular these days across many societal segments. Attempt were therefore made to standardize a process to develop dragon fruit based herbal beverage fortified with stevia and safed musli extract in order to replace sugar. Studies were also conducted to evaluated quality of the prepared beverage.

MATERIALS AND METHODS

Study Area

Raw materials, namely Safed Musli (*Chlorophytum borivilianum* Santapau & Fernandes) roots and Stevia (*Stevia rebaudiana*) leaves, were collected

from the Herbal Garden of BHU, Varanasi (25.2677° N, 82.9913° E) and RGSC, Mirzapur. Fully matured and sound dragon fruits were procured from a dragon fruit grower of Chunar, Mirzapur for the experiments of this study. All the experiments were conducted as per the scientific methods suggested by the researchers.

Development of Herbal Beverage

Dragon fruits juice was extracted with the help of fruit juicer (Usha Food Processor) (Plate 1 and 2). The safed musli (Plate 3 and 4) and stevia extract (Plate 5 and 6) were prepared as per process chart given in figure 1 and 2. Herbal beverage was prepared (Plate 7) by blending various quantities 80, 85 and 90% of dragon fruit juice, 10, 12, 14, 16% of stevia extract fortified with safed musli extract 10 ml litre^{-1} of dragon juice. Preservative (KMS) was added @ 0.5% litre^{-1} (w/v) into blend and the blend was put into 200 ml sterile glass bottles for processing at 80 °C temperature for 20 minutes. The bottles were sealed with the help of crown corking machine and cooled. After labeling, the bottles were stores at room temperature to evaluate the sensory quality and microbial attributes, including their physico-chemical properties. The process flow chart of herbal beverage preparation is given in figure 3.

Physico-Chemical Analysis

TSS was determined with a hand refractometer (Tokya, Japan) and express as ^{o}Brix. By titrating against 0.1 N NaOH, the total acidity of the beverage was determined using the AOAC (1995) technique, expressed in units of citric acid. The Lane and Eyan method was used to estimate both total and reducing sugars, followed by Ranganna (1986). Ascorbic acid content was recorded by 2,6-dichlorophenol indophenols titrimetric method (Cunniff, 1995). The growth of micro-organisms (Total Plate Count) in the herbal beverage samples was ascertained by the protocol outlined by Harrigan and McCance (1966). TPC is expressed in terms of log cfu ml^{-1} of herbal beverage. For sensory evaluation, the 9-point hedonic rating test approach was employed, which is advised by Ranganna (1986).

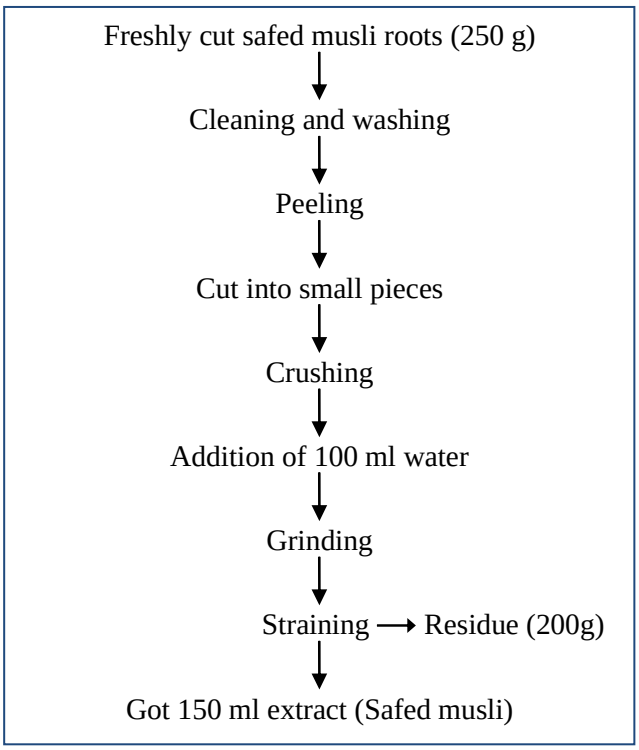

Figure 1: Method followed for safed musli extract preparation

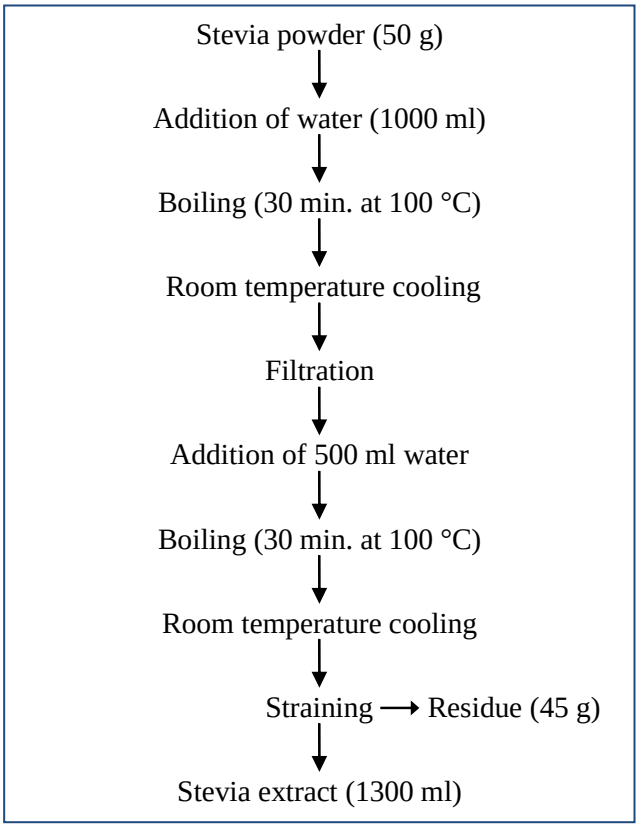

Figure 2: Method followed for stevia extract preparation

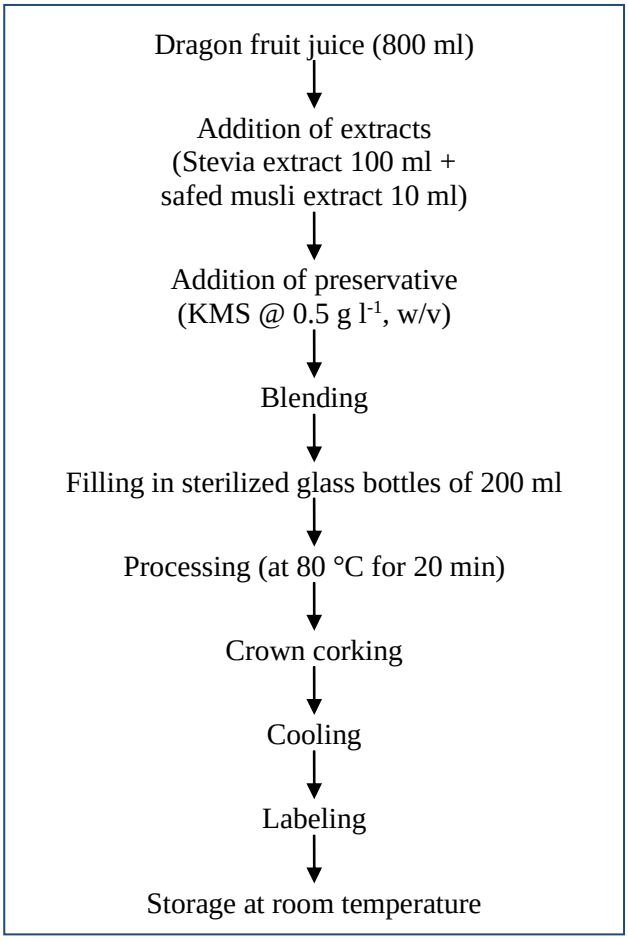

Figure 3: Method followed for fortified herbal beverage preparation

Statistical Assessment

All the experiment was carried out in three replications and observations were made both when the food was fresh and once a month while it was being stored for up to 60 days. Using Randomised Block Design (RBD), as defined by Panse and Sukhatne (1985), OPSTAT (Statistical analysis software) was adopted to carry out the statistical assessment of the data.

RESULTS AND DISCUSSION

Effect of Composition and Storage Periods on Physicochemical Parameters

Total Soluble Solids (TSS)

TSS of blended herbal beverage decreased with increment in the level of stevia extract at a specific level of dragon fruit juice, *e.g.*, S_1: 10.61, S_2: 10.45, S_3: 10.34 and S_4: 11.16 °Brix (Table 1). However, TSS of beverage increase with increased in the level of dragon fruit juice at a specific level of stevia extract (*e.g.*, S_1: 10.61, S_5: 11.90 and S_9: 13.08 °Brix). Study revealed that TSS increased significantly during storage (S_1 10.33 to 10.98 °Brix from fresh to 60 days) as given in table 1. Polysaccharide hydrolysis into simple sugars or inversion may be the cause of the variations in TSS content. The conversion rate was higher at room temperature than it was at chilled temperature, possibly as a result of temperature effects. Shagiwal and Deen (2022) also reported similar results, showing that TSS of RTS increased consistently from 13.00% to 13.52% and from 13.00% to 13.40%, respectively, under both ambient (22.8-34.0 °C) and refrigerated (4-6 °C) conditions. The current results are consistent with the considerations of previous researchers such as: Harendra and Deen (2021) on blended RTS based on ginger, aloe vera, kagzi lime and mango; Gill *et al.* (2020) on RTS beverage made from rose juice, aloe vera gel and kagzi lime juice; Munesh *et al.* (2018) on mix fruit (apple, guava, carrot and banana) and revealed that RTS beverage increased with increasing duration of storage and the highest value was found as 17.51 °Brix at room temperature after 90 days; Singh *et al.* (2018) on blend RTS made from aloe vera and mango; Mishra and Sangma (2017) on RTS drinks made from aloe vera, sweet lime and amla; and Singh *et al.* (2017) on ginger juice and aloe vera gel ready-to-serve mixture.

Titrable Acidity (TA)

The titrable acidity of herbal beverage varied with different levels of dragon fruit juice and stevia extract. Acidity of fresh samples raised with the higher level of dragon fruit juice at a specific level of stevia extract as given in table 2 (S_1: 0.414, S_5: 0.430 and S_9: 0.445). It was also observed that with increment in level of stevia extract, the TA of samples decreased at a particular level of dragon fruit juice, *e.g.*, S_5 (0.430), S_6 (0.423), S_7 (0.416) and S_8 (0.412). It was found that the acidity of the beverage increases with the increasing storage period and found highly significant (S_1 at fresh, 30

Table 1: Changes in TSS (°Brix)

Storage Days	S_1	S_2	S_3	S_4	S_5	S_6
Fresh	10.33±0.00	10.61±0.29	10.45±0.00	09.86±0.00	11.90±0.50	11.49±0.29
At 30 days	10.87±0.00	10.78±0.29	10.76±0.29	10.12±0.29	12.42±0.29	12.08±0.87
At 60 days	10.98±0.29	11.06±0.50	10.99±0.00	10.78±0.29	12.88±0.58	12.62±0.50

Table 1 continues …

Storage Days	S_7	S_8	S_9	S_{10}	S_{11}	S_{12}
Fresh	11.21±0.29	11.06±0.50	13.08±0.50	12.71±0.29	12.39±0.50	12.21±0.58
At 30 days	11.43±0.29	11.57±0.29	13.39±0.29	13.10±0.50	12.59±0.29	12.44±0.29
At 60 days	11.77±0.76	11.78±0.76	13.65±0.50	13.87±0.29	12.85±0.58	12.65±0.50

Table 1 continues …

	CD ($p<0.05$)	SEm±
Storage	0.2662	0.0967
Composition	0.3474	0.1321
Interaction	NS	0.3298

[NB: S_1: Dragon fruit juice 80% and Stevia extract 10%; S_2: Dragon fruit juice 80% and Stevia extract 12%; S_3: Dragon fruit juice 80% and Stevia extract 14%; S_4: Dragon fruit juice 80% and Stevia extract 16%; S_5: Dragon fruit juice 85% and Stevia extract 10%; S_6: Dragon fruit juice 85% and Stevia extract 12%; S_7: Dragon fruit juice 85% and Stevia extract 14%; S_8: Dragon fruit juice 85% and Stevia extract 16%; S_9: Dragon fruit juice 90% and Stevia extract 10%; S_{10}: Dragon fruit juice 90% and Stevia extract 12%; S_{11}: Dragon fruit juice 90% and Stevia extract 14%; S_{12}: Dragon fruit juice 90% and Stevia extract 16%]

Table 2: Changes in acidity (%)

Storage Days	S_1	S_2	S_3	S_4	S_5	S_6
Fresh	0.414±0.002	0.404±0.001	0.401±0.002	0.394±0.003	0.430±0.004	0.423±0.003
At 30 days	0.416±0.001	0.409±0.002	0.402±0.001	0.408±0.001	0.434±0.002	0.428±0.004
At 60 days	0.420±0.001	0.413±0.003	0.408±0.001	0.405±0.002	0.438±0.001	0.434±0.006

Table 2 continues …

Storage Days	S_7	S_8	S_9	S_{10}	S_{11}	S_{12}
Fresh	0.413±0.001	0.412±0.001	0.445±0.001	0.445±0.012	0.442±0.001	0.439±0.002
At 30 days	0.419±0.002	0.415±0.002	0.457±0.003	0.453±0.001	0.450±0.020	0.444±0.002
At 60 days	0.422±0.001	0.421±0.007	0.463±0.001	0.459±0.001	0.458±0.001	0.452±0.002

Table 2 continues …

	CD ($p<0.05$)	SEm±
Storage	0.0010	0.0029
Composition	0.0009	0.0012
Interaction	0.0029	0.0008

[NB: Abbreviation according to table 1]

and 60 days: 0.414, S_5: 0.416 and S_9: 0.420, respectively). The production of organic acid and the breakdown of pectic compounds may be the cause of an increase in the acidity content. When stored at room temperature rather than in a refrigerator, more citric acid is formed, which could be due to the rapid rate of decomposition of pectic compounds at higher storage temperatures. Shagiwal and Deen (2022)

observed that the acidity content of blended ready-to-serve (RTS) beverages grew continuously under both ambient and refrigerated storage conditions, indicating similar results representing an increase in acidity throughout product storage. It was raised from 0.30% to 0.83% and from 0.30% to 0.62%, respectively. Harendra and Deen (2021) investigated blended ready-to-serve (RTS) beverages prepared from ginger, aloe vera, kagzi lime and mango. Similarly, Gill *et al.* (2020) studied an RTS beverage formulated with rose juice, aloe vera gel and kagzi lime juice. Khalid *et al.* (2019) focused on a ready-to-serve drink combining strawberry and dates, while Selvi *et al.* (2018) examined a guava-lime-ginger RTS beverage. Research conducted by Singh *et al.* (2018) on an RTS blend of aloe vera and mango; and Mehta *et al.* (2018) analyzed a ginger blended RTS, lime and guava. Additionally, Yadav *et al.* (2014) also reported that acidity levels tended to rise with extended storage periods at various temperatures. After 90 days of storage, the sample was stored at refrigerator temperature showed acidity levels of 0.453, 0.533 and 0.573%.

Ascorbic Acid

Table 3 showed that the ascorbic acid of the herbal beverage decreased as the amount of stevia extract increased, at a specific level of dragon fruit juice, *i.e.*, 85% dragon fruit juice with 10, 12, 14 and 16% of stevia extract. The ascorbic acid values of samples S_5, S_6, S_7 and S_8 were 19.910, 19.902, 19.875 and 19.839 mg/ 100 ml, respectively.

Table 3: Changes in ascorbic acid (mg/ 100 ml)

Storage Days	S_1	S_2	S_3	S_4	S_5	S_6
Fresh	18.625±0.027	18.591±0.043	18.559±0.058	18.522±0.019	19.910±0.011	19.902±0.022
At 30 days	18.436±0.038	18.347±0.048	18.325±0.111	18.319±0.093	19.888±0.038	19.837±0.016
At 60 days	18.410±0.036	18.341±0.022	18.279±0.026	18.237±0.012	19.863±0.010	19.805±0.017

Table 3 continues …

Storage Days	S_7	S_8	S_9	S_{10}	S_{11}	S_{12}
Fresh	19.875±0.019	19.839±0.017	20.495±0.017	20.392±0.006	20.281±0.003	20.190±0.016
At 30 days	19.754±0.025	19.686±0.028	20.366±0.014	20.318±0.015	20.263±0.028	20.155±0.032
At 60 days	19.741±0.023	19.651±0.024	20.252±0.019	20.232±0.010	20.168±0.023	20.092±0.021

Table 3 continues …

	CD ($p<0.05$)	SEm±
Storage	0.03333	0.0123
Composition	0.04050	0.0138
Interaction	0.10822	0.0371

[NB: Abbreviation according to table 1]

It was noticed that higher the level of dragon fruit juice, the ascorbic acid content also increased at a particular level of stevia extract. Ascorbic acid content of the samples prepared with 16% stevia extract and 80, 85 and 95% dragon fruit juice were observed as 18.522, 19.839 and 20.190 mg/ 100 ml, respectively for S_4, S_8 and S_{12}). A decrement in ascorbic acid content was found significant during storage invariably for the entire sample. In general, the ascorbic acid of samples partially decreases with the increasing concentration of stevia extract and drastically decreased with storage period. Ascorbic acid (vitamin C) content may be depleted because oxygen trapped in the product's intramolecular space and containers oxidises ascorbic acid to dehydro-ascorbic acid. The present results concerning variations in the concentration of ascorbic acid (vitamin C) in drinks during storage are consistent with the findings published by Shagiwal and Deen (2022). They observed that during the course of the

storage period, the vitamin C content of ready-to-serve (RTS) beverages created from combinations of ginger, aloe vera and strawberries progressively decreased. The vitamin C content specifically dropped dramatically at both low and ambient temperatures, going from 6.10 mg to 5.80 mg/ 100 ml and 6.10 mg to 5.65 mg/ 100 ml, respectively. Research conducted by Harendra and Deen (2021) focused on blended ready-to-serve (RTS) beverages prepared from ginger, aloe vera, kagzi lime and mango. Khalid *et al.* (2019) investigated a ready-to-serve beverage that included dates and strawberries. Singh *et al.* (2018) investigated a mixture of RTS made from aloe vera and mango; whereas Selvi *et al.* (2018) investigated a ginger-lime-guava RTS beverage. The studies came to the conclusion that the ascorbic acid concentration was declining, indicating that ascorbic acid content was higher in low temperature circumstances, possibly as a result of temperature influencing ascorbic acid oxidation.

Reducing Sugars

Reducing sugars of herbal beverage increased with the increasing level of stevia extract at a specific level of dragon fruit juice (Table 4). Reducing sugars increased with the increasing level of dragon fruit juice at a particular level of stevia extract. It was also observed that reducing sugars increased drastically during storage period irrespective of concentration of stevia extract and dragon fruit juice. Reducing sugars 3.752, 3.982 and 4.699% were noted for the samples prepared with 85% dragon fruit juice and 12% stevia extract at fresh, 30 days and 60 days of interval, respectively. One possible explanation for the rise in reducing sugar content in products is the transformation of non-reducing sugar into reducing sugar (Shagiwal and Deen, 2022). In both ambient and low temperatures, the reducing sugar content of RTS grew consistently until storage period is completed, rising from 0.40% to 1.06% and from 0.40% to 0.83%, respectively. Equivalent findings have been published by earlier researchers, including Harendra and Deen (2021), who studied blended ready-to-serve (RTS) beverages made from ginger, aloe vera, kagzi lime and mango; Gill *et al.* (2020), who investigated RTS beverages prepared from rose juice, aloe vera gel and kagzi lime juice; Khalid *et al.* (2019), who focused on a ready-to-serve drink combining strawberry and dates; Mehta *et al.* (2018), who examined guava-based RTS beverages; Mishra and Sangma (2017), who studied RTS drinks made from amla, sweet lime, ginger and aloe vera; and Singh *et al.* (2017), who researched blended RTS beverages containing ginger juice and aloe vera gel. These discoveries confirm the present research's results.

Table 4: Changes in reducing sugars (%)

Storage Days	S_1	S_2	S_3	S_4	S_5	S_6
Fresh	3.555±0.003	3.581±0.008	3.599±0.005	3.632±0.011	3.737±0.006	3.752±0.016
At 30 days	3.832±0.012	3.853±0.010	3.888±0.006	3.889±0.008	3.971±0.009	3.982±0.011
At 60 days	4.567±0.005	4.675±0.011	4.758±0.010	4.758±0.004	4.786±0.012	4.699±0.004

Table 4 continues …

Storage Days	S_7	S_8	S_9	S_{10}	S_{11}	S_{12}
Fresh	3.788±0.008	3.852±0.009	3.961±0.016	3.998±0.005	4.022±0.009	4.068±0.015
At 30 days	4.111±0.009	4.156±0.011	4.173±0.003	4.198±0.011	4.216±0.007	4.229±0.006
At 60 days	4.875±0.014	4.913±0.015	5.019±0.021	5.022±0.008	5.078±0.013	5.172±0.004

Table 4 continues …

	CD ($p<0.05$)	SEm±
Storage	0.0042	0.0012
Composition	0.0035	0.0019
Interaction	0.0110	0.0038

[NB: Abbreviation according to table 1]

Total Sugars

The total sugars of the fresh samples enhanced with the increasing level of stevia extract and dragon fruit juice (Table 5). During the investigation, the values of total sugars were found as S_5 11.485, S_6 11.584, S_7 11.639 and S_8 11.806%, respectively for the herbal beverage samples prepared with 85% fruit juice and 10, 12, 14 and 16% of stevia extract. Statistical analysis revealed that total sugars decreased significantly during storage period. In general, the total sugars of the samples increased within the concentration of stevia extract and dragon fruit juice irrespective of their storage time. However, the total sugars content declined during storage of 60 days (for sample S_1 at fresh, 30 and 60 days of storage, total sugars were recorded as 11.258, 11.247 and 11.223%, respectively). The transformation of non-reducing sugar into reducing sugars may be the cause of an increase in the product's total sugar content. The current data regarding the rise in total sugar content in RTS is thus consistent with findings from other fruit-based drinks (Mehta *et al.*, 2018; Singh *et al.*, 2018; Selvi *et al.*, 2018; Khalid *et al.*, 2019; Gill *et al.*, 2020; Harendra and Deen, 2021).

Table 5: Changes in total sugars (%)

Storage Days	S_1	S_2	S_3	S_4	S_5	S_6
Fresh	11.258±0.004	11.282±0.006	11.339±0.005	11.443±0.026	11.485±0.004	11.584±0.007
At 30 days	11.247±0.009	11.263±0.004	11.334±0.009	11.412±0.0013	11.454±0.011	11.550±0.002
At 60 days	11.223±0.005	11.226±0.007	11.340±0.006	11.395±0.005	11.405±0.012	11.466±0.012

Table 5 continues …

Storage Days	S_7	S_8	S_9	S_{10}	S_{11}	S_{12}
Fresh	11.639±0.011	11.806±0.014	12.225±0.006	12.366±0.006	12.498±0.006	12.626±0.004
At 30 days	11.640±0.005	11.790±0.008	12.216±0.011	12.358±0.009	12.477±0.009	12.614±0.010
At 60 days	11.627±0.004	11.784±0.004	12.207±0.005	12.333±0.007	12.465±0.012	12.612±0.001

Table 5 continues …

	CD ($p<0.05$)	SEm±
Storage	0.0052	0.0019
Composition	0.0066	0.0034
Interaction	0.0211	0.0068

[NB: Abbreviation according to table 1]

Effect of Composition and Storage Periods on Microbial Growth

The findings of the microbial study demonstrated that, when consumed fresh, the quality of the herbal beverages was unaffected by microbial contamination. The microbiological assessment was conducted for fresh and after one month interval till 60 days. No microbes were detected during initial period of storage upto 30 days (Table 6). This may be attributed to incorporation of stevia extract, because stevia had antibacterial and antifungal properties (Tomita *et al.*, 1997); subsequently it acts as a preservative and resulted in reduction of total Plate count in blended herbal beverage sample. After that, the microbial growth was observed and found that TPC increased with increase in storage period. TPC was recorded for the sample prepared with 80% dragon fruit juice and 10% stevia extract 1.071×10^5 cfu ml^{-1} at 60 days of storage. However, in contradiction to this, TPC decreased with increase in stevia extract at a specific level of dragon fruit juice and the values were found as 1.181, 1.110, 1.078 and 1.057×10^5 cfu ml^{-1}, respectively for the samples of S_9, S_{10}, S_{11} and S_{12}. Statistical analysis revealed that the TPC increased significantly during storage.

Table 6: Changes in number of microbes ($\times 10^5$ cfu ml^{-1})

Storage Days	S_1	S_2	S_3	S_4	S_5	S_6
Fresh	ND	ND	ND	ND	ND	ND
At 30 days	ND	ND	ND	ND	ND	ND
At 60 days	1.071±0.013	1.060±0.005	1.052±0.006	1.033±0.004	1.109±0.005	1.088±0.002

Table 6 continues …

Storage Days	S_7	S_8	S_9	S_{10}	S_{11}	S_{12}
Fresh	ND	ND	ND	ND	ND	ND
At 30 days	ND	ND	ND	ND	ND	ND
At 60 days	1.050±0.002	1.042±0.001	1.181±0.005	1.110±0.002	1.078±0.007	1.057±0.006

Table 6 continues …

	CD ($p<0.05$)	SEm±
Storage	0.0138	0.0072
Composition	0.0345	0.0123
Interaction	0.0786	0.0289

[NB: Abbreviation according to table 1]

Table 7: Changes in overall acceptability

Storage Days	S_1	S_2	S_3	S_4	S_5	S_6
Fresh	7.023±0.232	6.755±0.212	6.348±0.218	6.129±0.074	7.778±0.222	7.528±0.117
At 30 days	6.723±0.111	6.588±0.114	6.248±0.333	6.041±0.323	7.611±0.111	7.348±0.214
At 60 days	6.612±0.221	6.385±0.214	6.055±0.314	5.948±0.211	7.523±0.324	7.228±0.123

Table 7 continues …

Storage Days	S_7	S_8	S_9	S_{10}	S_{11}	S_{12}
Fresh	7.232±0.111	7.348±0.212	7.932±0.141	7.728±0.321	7.412±0.211	7.078±0.222
At 30 days	7.222±0.128	6.911±0.333	7.748±0.344	7.615±0.212	7.248±0.214	6.922±0.325
At 60 days	6.948±0.214	6.7878±0.125	7.528±0.379	7.328±0.222	7.028±0.125	6.712±0.231

Table 7 continues …

	CD ($p<0.05$)	SEm±
Storage	0.4141	0.1444
Composition	0.5628	0.2121
Interaction	NS	0.5238

[NB: Abbreviation according to table 1]

The results of previous investigations are advocating the findings of present research as the mixed drink made with lemon juice and mature coconut water has a six-month shelf life at 37 °C (ambient, low and high temperatures) (Chauhan *et al.*, 2014). PET bottles containing the functional beverage from aonla and bael could be kept at room temperature for up to ninety days (Sashikumar, 2016). At refrigeration temperature, Dambalkar *et al.* (2015) found that the beetroot-orange blend health drink had a shelf life of more than 30 days. Roy *et al.* (2016) discovered that the cashew blended beverage has a 60-day shelf-life when stored. After being examined under various circumstances, Pandey *et al.*

(2018) discovered that the shelf life of Kadamba fruit beverage was 150 days at room temperature. According to research conducted by Aware and Kotecha (2018) on storage at both room temperature and refrigeration, RTS beverage made from muskmelon juice has a high storage life of 90 days. According to research done by Hemalatha *et al.* (2018), the Cape gooseberry may be stored for a long time at both room temperature and refrigeration. Its microbiological content is found to be within acceptable bounds, making it safe to eat.

Effect of Composition and Storage Periods on Overall Acceptability of Sensory Quality

The score of overall acceptability revealed that the fresh beverage samples prepared with highest level of dragon fruit juice (90%) and lowest level of stevia extract (10%) were highly preferred and got highest acceptability score of 7.932 (Table 7). All the samples were rated between 'like moderately' to 'like very much'. It was also revealed that overall acceptability score decreased significantly during storage. The samples were rated between 'like slightly' to 'like very much' after 60 days of storage. In general, quality of blended herbal beverage decreased during storage period irrespective of composition of dragon fruit juice and stevia extract. The statistical data showed that various compositions and storage times had substantial effects on widespread acceptance.

Findings of the following researchers are confirming the authenticity of present investigation work and it was discovered that the aloe vera blend's sensory qualities diminished after six months of storage (Sasi Kumar *et al.*, 2013). The nutritious functional beverage was found to have excellent sensory acceptance after being accepted by the senses (Sharma *et al.*, 2014). The results of blended beverage obtained by Sashikumar (2016) showed that when the storage duration lengthens, the colour and organoleptic taste scores decline. It is found that the mixed beverage with a litchi base has a higher acceptance and improved sensory score (Jayachandran *et al.*, 2015). Research is done on the cashew blended beverage's sensory quality and Roy *et al.* (2016) found that the mix with mango and pineapple has a high sensory score. According to Mielby *et al.* (2016), sensory examination of the stevia-sweetened fruit beverage revealed that it was confirmed and the inclusion of lime juice neutralises the enticing flavour and odour produced by the stevia. After four months of storage, sensory evaluations of the aonla RTS beverage's shelf life unveiled that it was no longer suitable (Kumar, 2018). The RTS beverage that had been blended with tamarind to beets in a ratio of 80:20 had the premier sensory rating (Kale *et al.*, 2018). The pleasant flavour of orange-date squash reduces with increase in temperature and storage duration (Khan *et al.*, 2018).

CONCLUSION

It is possible to draw the conclusion that the blended herbal beverage made with a blending of 90% dragon fruit juice and 10% stevia extract which fortified with the 2% safed musli extract, obtained the greatest level of acceptability score when compared to those made with other combinations after analysing all the data collected from the study's results. Additionally, it has been discovered that stevia extract has preservation properties for herbal beverages when stored. As a result, the prepared blended herbal beverage can be kept at room temperature for up to 60 days.

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Conflict of Interest

The authors declare no conflict of interest.

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