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Storage Stability of Functional Beverage Prepared From Bitter Gourd, Lemon and Amla for Diabetes

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ABSTRACT

Background: Functional beverages become popular in treating non-communicable diseases like diabetes which is influenced by dietary habits and life style of people nowadays. From the standpoint of safe consumption, maintenance of their quality is vital. Therefore, the present study was aimed to evaluate the storage stability of functional beverage prepared from fruits of anti-diabetic potential at ambient temperature.

Methods: Three different formulations of bitter gourd: lemon: amla (8:2:3, 6:4:3 and 5:5:3) along with control formulation were prepared and subjected to storage at ambient temperature (30±2°C) and RH of 75-80% in which physico-chemical, sensory and microbial tests were performed at pre-decided intervals.

Result: Analyses indicated that physico-chemical quality parameters of total soluble solids (5.2-3.7), titrable acidity (5.1- 4.5) and ascorbic acid content (45.81-30.48) were significantly decreased while pH increased from 3.5 to 4.0 among formulations during storage. Furthermore, scores for sensorial attributes got decreased and there was no microbial growth in beverage formulations up to two months of storage. Considering these results, formulation T4 prepared with 6% bitter gourd + 4% lemon + 3% amla juices was found to be more acceptable and safer beverage for consumption with minimal percentage change and better retention of quality characteristics which could be commercialized as healthy diet drink for diabetics.

Key words: Functional beverage, Microbial attributes, Physico-chemical parameters, Sensorial attributes.

INTRODUCTION

Foods that provide additional physiological benefits such as preventing or delaying onset of chronic diseases besides meeting basic thirsty and nutritional requirements are termed as functional foods. Consumption of functional food would deliver health and wellness to consumer (Sharma, 2005) and it has no cytotoxicity and mutagenicity effects (Chen *et al.*, 2009). Of various items of functional foods, functional beverages receive a growing demand in the current world with the stand point of diseases that are enhanced by changing lifestyle pattern. Asli Emine ozen (2012) mentioned that functional beverages satisfactorily demonstrate to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well-being and or reduction of risk of disease.

Diabetes is one of the common diseases which are much influenced by dietary habit of people and literature available in this regard clearly states that people nowadays have tendency to use natural medicinal plants and their parts rather than using general synthetic medicine to treat various symptoms of diabetes. An option in the utilization of medicinal plants is to formulate them into herbal-based functional beverages. These are not intended only to satisfy hunger, taste and thirst but also to provide necessary nutrients to human for prevention of nutrition-related diseases (Menrad *et al.,* 2000). They play an important role in health promotion and disease prevention by means of reducing increasing burden on health care system by a continuous preventive mechanism.

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An initial step was taken to formulate functional beverage using locally available fruits and vegetables those have functional property of anti-diabetic effects with the intention of treating diabetes. From number of studies it was found that bitter gourd has potential to control diabetes. Bitter gourd is anti-diabetic, stimulant, stomachic, laxative, blood purifier and control diabetes (Raman and Lau, 1996). Bitter gourd fruit juice has also been shown to stimulate, significantly, both glycogen storage by the liver (Welihinda *et al*., 1986) and insulin secretion by isolated -cells islets of Langerhans. However, the raw juice is very much bitter in taste and unpalatable for fresh consumption. Therefore, raw bitter gourd juice was blended with other fruit juice extracts

of lemon and amla which also have anti-diabetic values. After formulation, its storage stability must be ensured for longer duration to be used by consumers without any adverse effects on human health as well as environment. With this purpose the present study was undertaken to evaluate the storage stability of formulated beverage at ambient temperature.

MATERIALS AND METHODS

Experimental site and design

The experiment was carried out at Food Science Laboratory of Faculty of Agriculture Eastern University of Sri Lanka in 2014. Experiment consisted of four treatments, replicated three times was laid out in Randomized Complete Block Design (RCBD).

Beverage formulations and storage

Most preferred functional beverage formulations selected through sensory evaluation from previous experiment were developed with varying juice percentages (8:2:3, 6:4:3 and 5:5:3) of bitter gourd: lemon: amla along with control formulation in which bitter gourd juice was not blended with lemon and amla. In all treatments 85% of water was added and remaining 2% was shared in between other minor ingredients of black salt, cinnamon, black pepper and citric acid. Developed beverage formulations were subjected to storage at 30±2°C and RH of 75-80% for a period of two months.

Physico-chemical analysis

Beverage formulations were analyzed at biweekly interval for the following nutritional and physico-chemical quality characteristics such as Total Soluble Solids (TSS), Citric acid, pH and Vitamin C according to AOAC methods (2006).

Sensory evaluation

Organoleptic evaluation for sensory attributes such as colour, taste, aroma, appearance and overall acceptability was conducted at 0, 60 days using 9-point hedonic scale where 1 and 9 denotes dislike extremely and like extremely respectively through a panel of 25 semi-trained judges to evaluate acceptance.

Microbial analysis

It was carried out at monthly interval by total plate count method to find the safe consumption period.

Statistical analysis

Normal distribution of data was tested prior to the analysis. Followed by, the data obtained in the experiment were analyzed statistically using analysis of variance technique (ANOVA) using computer aided SAS statistical analysis package to evaluate the significance at $P<0.05$. Standard errors were calculated using MINITAB 14 statistical package. Mean separation for chemical tests were done by Duncan Multiple Range Test (DMRT) and means of sensory evaluation were compared by Tukey's Studentized Range Test (TSRT).

RESULTS AND DISCUSSION

Effect of storage on TSS of functional beverage

Total soluble solid is the amounts of solids dissolved within a substance. It is referred to as the degrees Brix which is equivalent to percentage of sugar (sucrose) in the solution. Initially formulations had higher TSS content than the control due to the incorporation of lemon and amla juices which have comparatively higher carbohydrate contents than bitter gourd. Mean values of TSS of functional beverage formulations depicted in Fig 1 explains that TSS values were significantly decreased (5.2-3.7) during storage. Formulation T4 was found to be superior in terms of TSS (sucrose) where minimum percentage decrease (11.1) was observed.

The decrease in TSS may be due to chemical interactions taking place among organic constituents of the beverages (Ghorai and Khurdiya, 1998). Since vitamin C is soluble in water and oxidation sensitive, it gradually decreased; this might be the main reason for lowering the value of acidity and TSS (Simsek, 2011). Addition of cinnamon powder also could be attributed to decrease in TSS. Because it inhibits the enzymes α-glucosidase and invertase which are responsible for hydrolysis polysaccharide and disaccharides (Adisakwattana *et al*. 2011; Shihabudeen *et al*. 2011). Thus, leads to the significant decrease in TSS with the increase in storage period. The findings of the present study are in concordance with the findings of Mishra *et al*. (2012) in vitamin C rich beverage from amla and grapes and Puranik *et al*. (2013) in optimized herbal functional RTS with 6% basil and 1.5% *Tinospora cordifolia*.

Effect of storage on titrable acidity of functional beverage

Titrable acidity (TA) of beverage formulations throughout the storage period is shown in Table 1. The functional beverage formulations showed a significant decrease (5.1-4.5) in titrable acidity with the advancement of storage period. Maximum mean value was recorded in T5, followed by T4 which has 4% BGJ (Bitter Gourd Juice) +6% LJ (Lemon Juice) +3% AJ (Amla Juice). T4 was found to be statistically superior to other treatment because of minimum percentage decrease (6.37) in titrable acidity during storage.

The decrease in titrable acidity could be attributed to chemical interaction between organic constituents of beverage induced by temperature and enzymes (Satkar *et al*. 2013). Kumar *et al*. (1992) also stated that decrease in TA might be due to conversion of acids into salts and sugars by enzymes particularly invertase. However due to inhibitory effect of cinnamon on invertase enzyme, rate of decrease in titrable acidy was minor throughout the storage. These results are in conformity with the findings of Sheela and Sruthi (2014) in bitter gourd: mosambi and bitter gourd: lemon RTS beverage. Similar results were also reported by Satkar *et al*. (2013) during the storage of bitter gourd RTS beverage at ambient (27±0.5°C) and refrigerated temperature (5±1°C). Also decreasing trend in titrable acidy

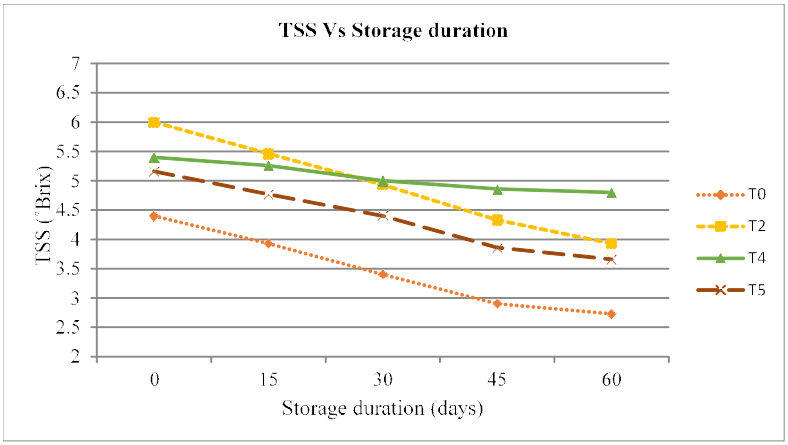

Fig 1: Effect of storage duration on TSS of beverage formulations.
(T0 -13% bitter gourd juice only (BGJ), T2 -8% BGJ+2% lemon juice (LJ) +3% amla juice (AJ), T4-6% BGJ+4% LJ+3% AJ, T5-5% BGJ+5% LJ+3% AJ).

Table 1: Titrable acidity values of beverage formulations during storage.

Treatments	0 days	15 days	30 days	45 days	60 days	Treatment mean
T0	4.56±0.03^{d}	4.40±0.05^{d}	4.17±0.03^{d}	4.01±0.04^{d}	3.87±0.02^{c}	4.23±0.03^{d}
T2	4.93±0.08^{c}	4.73±0.06^{c}	4.65±0.04^{c}	4.46±0.02^{c}	4.25±0.02^{b}	4.60±0.04^{c}
T4	5.33±0.06^{b}	5.13±0.03^{b}	5.11±0.02^{b}	5.05±0.02^{b}	4.99±0.01^{a}	5.13±0.02^{b}
T5	5.66±0.02^{a}	5.45±0.04^{a}	5.33±0.04^{a}	5.18±0.04^{a}	5.07±0.06^{a}	5.33±0.03^{a}
Storage mean	5.1±0.00^{a}	4.9±0.00^{b}	4.8±0.00^{c}	4.6±0.00^{d}	4.5±0.00^{e}	

Values are means of 25 replicates ± standard deviation.
Values followed by different superscripts are significantly different at $p<0.05$.
(T0 -13% bitter gourd juice only (BGJ), T2 -8% BGJ+2% lemon juice (LJ) +3% amla juice (AJ), T4-6% BGJ+4% LJ+3% AJ, T5-5% BGJ+5% LJ+3% AJ).

throughout the storage was recorded in kinnow juice blended with pomogrenate, aonla and ginger juices by Bhardwaj and Mukherjee (2011).

Effect of storage on pH of functional beverage

pH is a key determinant in safe shelf life of beverages and acidity and pH are inversely proportional to each other (Jan and Masih, 2012). On day of preparation pH of formulations was within the range of 3.68-3.33 in which control formulation had higher pH values than the other formulations. pH of functional dietetic drinks was started to increase (3.5-4.0) gradually as the storage period proceeded (Table 2). Formulation T4 was found to be superior in terms of pH than other treatments with its minimum percentage decrease (11.69).

The increase in pH might be due to decrease in citric acid and degradation of ascorbic acid during storage. The pH of all formulations of functional beverage were in the acceptable range of 3.58-3.96 (below 4) after 2 months of storage and this is supported by Cole *et al*. (2000) that pH of most soft drinks and juices is less than 4. The findings are in line with the work reported by Sheela and Sruthi (2014) who observed the increasing trend of pH during storage from 3.2-4.5 and 2.9-4.0 respectively in bitter gourd: mosambi and bitter gourd: lemon RTS beverage. Trends of decreasing acidity and increasing pH found in this study are well supported by previous researchers (Balaswamy *et al*. 2011; Bhuyian *et al*. 2012).

Effect of storage on ascorbic acid content of functional beverage

Vitamin C is one of the vitamins present in considerably higher amounts in selected fruit and vegetables and subjected to losses thus helps to determine the shelf life of beverages. As lemon and amla juices have higher vitamin C contents formulations were recorded for higher vitamin C values than control. Fig 2 shows that there was significant decline (45.81-30.48) in mean values of vitamin C with gradual passage of storage. Among formulations, T4 was found to be superior statistically too than the other formulations because of its minimum percentage decrease (16.94) in vitamin C throughout the storage.

This decrease might be due to the factors such as storage temperature, oxidative enzymes, processing techniques, metal contamination and the presence of atmospheric oxygen in the head space (Din *et al*. 2011). The results of this study are in close agreement with the works of Din *et al*. (2011) on functional and dietetic beverages prepared from different ratios of bitter gourd and Baljeet *et al*. (2013) in whey-based pine apple and bottle gourd beverage and indicated that there was a significant decrease from 1.43 to 1.2 after the storage of 20 days.

Table 2: pH of functional beverage formulations during storage.

Treatments	0 days	15 days	30 days	45 days	60 days	Treatment mean
T0	3.68±0.005^{a}	3.79±0.02a	3.96±0.01^{a}	4.15±0.01^{a}	4.21±0.02^{a}	3.96±0.008^{a}
T2	3.53±0.005^{c}	3.68±0.01b	3.85±0.04^{a}	4.06±0.04^{a}	4.19±0.03^{a}	3.86±0.02^{a}
T4	3.42±0.01^{e}	3.58±0.02c	3.63±0.02^{b}	3.80±0.10^{b}	3.82±0.10^{b}	3.65±0.03^{b}
T5	3.33±0.01^{f}	3.49±0.01d	3.57±0.04^{b}	3.64±0.06^{b}	3.86±0.13^{ab}	3.58±0.04^{b}
Storage mean	3.50±0.00^{e}	3.60±0.00d	3.7±0.00^{c}	3.90±0.00^{b}	4.00±0.00^{a}	

Values are means of 25 replicates ± standard deviation.

Values followed by different superscripts are significantly different at p<0.05.

(T0 -13% bitter gourd juice only (BGJ), T2 -8% BGJ+2% lemon juice (LJ) +3% amla juice (AJ), T4-6% BGJ+4% LJ+3% AJ, T5-5% BGJ+5% LJ+3% AJ).

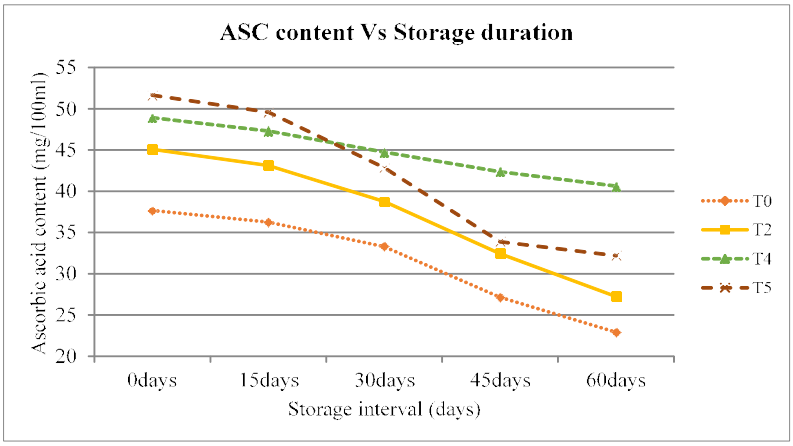

Fig 2: Effect of storage duration on ASC of different beverage samples.
(T0 -13% bitter gourd juice only (BGJ), T2 -8% BGJ+2% lemon juice (LJ) +3% amla juice (AJ), T4-6% BGJ+4% LJ+3% AJ, T5-5% BGJ+5% LJ+3% AJ).

Table 3: Scores of beverage formulations for sensory parameters at 0 and 60 days of storage.

Formulations	Storage days	Sensorial parameters				
		Colour	Taste	Aroma	Appearance	Overall acceptability
T0	0 days	2.9±1.29^{c}	2.0±1.05^{d}	2.2±1.03^{c}	2.4±0.97^{c}	2.2±1.14^{b}
T2		7.3±1.42^{a}	6.4±0.84^{b}	5.3±1.16^{b}	5.3±1.25^{b}	7.0±1.56^{a}
T4		5.2±0.92^{abc}	8.3±1.06^{a}	7.2±1.48^{a}	7.6±1.35^{a}	8.1±0.88^{a}
T5		5.1±1.66^{abc}	7.3±1.34^{ab}	7.7±1.16^{a}	5.9±1.52^{b}	6.5±1.27^{a}
T0	60 days	1.4±0.52^{c}	1.4±0.84^{c}	1.4±0.52^{c}	1.3±0.48^{c}	1.2±0.63^{c}
T2		3.7±1.42^{ab}	3.6±0.84^{b}	2.7±1.16^{c}	2.9±1.20^{b}	4.4±2.12^{b}
T4		4.4±0.84^{a}	7.9±1.37^{a}	6.5±1.58^{a}	7.0±1.33^{a}	7.6±1.27^{a}
T5		2.7±1.25^{bc}	5.0±1.76^{b}	4.9±1.37^{b}	3.1±1.37^{b}	4.9±1.97^{b}

Values are means of 25 replicates ± standard deviation.

Values followed by different superscripts are significantly different at p<0.05.

(T0 -13% bitter gourd juice only (BGJ), T2 -8% BGJ+2% lemon juice (LJ) +3% amla juice (AJ), T4-6% BGJ+4% LJ+3% AJ, T5-5% BGJ+5% LJ+3% AJ).

Effect of storage on sensory attributes of functional beverage formulations

The data obtained in organoleptic evaluation given in the Table 3 reveals that the storage duration had significant effect on all sensory parameters and all parameters were gradually decreased during storage. The change in colour parameter might be due to maillard reactions between sugars and amino acids (Gonzalez and Leeson, 2000). Similar results of this study were found by Satkar *et al*. (2013) in bitter gourd RTS beverage and also gradual decrease in colour was reported by Ahmed *et al*. (2008) in mandarin diet RTS drink and Kauser *et al*. (2012) in cucumber-melon functional drink. The change in taste might be due to degradation ascorbic acid (Kauser *et al*. 2012). The taste difference and loss might also be due to time, temperature and duration of storage (Ahmed *et al*. 2008). The decrease in flavor (aroma +taste) during storage could be possibly due to loss of volatile aromatic substances (Thakur and

Barwal, 1998). Decrease in flavor was supported by several researches (Ahmed *et al*. 2008; Kausar *et al*. 2012; Gaikwad *et al*. 2013). Decrease in appearance of beverage formulations might be due to settling of finer particles present in beverages. Din *et al*. (2011) noted that score of 7.5 in freshly made bitter gourd RTS was decreased to 6.1 after 90 days of storage. Similar results were observed by Gaikwad *et al*. (2013) in low calorie herbal aonla-ginger RTS.

Further, significant decreases in overall acceptability of all functional drink formulations at 2 months of storage were observed. Degradation in sensorial attributes especially colour, flavour may in turn results the decrease in overall acceptability. Similar decrease in overall acceptability (7.6-5.7) was reported by Satkar *et al*. (2013) in bitter gourd RTS beverage. Decreasing trend in overall acceptability throughout the storage was reported by Din *et al*. (2011) in functional and dietetic beverages prepared from different ratios of bitter gourd and Gaikwad *et al*. (2013) in herbal aonla-ginger RTS beverage.

Microbial analysis

At 0, 30 days of storage no microbial growth was observed in all beverage formulations and this might be due to low pH, heat treatments during processing (pasteurization and sterilization) and higher acidity of beverage. Higher acidity of RTS beverage from fruit helps to protect the product from the microbial spoilage (Sri and Bobby, 2005). But at 60 days of storage very mild growth of microorganism (bacteria) was observed in T0 and T2 due to their higher pH and no microbial growth was observed in T4 and T5. As the pH increased throughout the storage period considerable number of CFU could be observed after 2 months of storage. And it was concluded that products were safe for consumption up to 2 months of storage in terms of microbial quality but it needs further elaborated study as it impacts the health of consumers. This result is supported by Barwal *et al*. (2005a) who observed full plate growth of micro flora after 60 days of storage at room temperature in RTS beverage developed from bitter gourd fruit.

CONCLUSION

Maintenance of quality parameters during storage is vital important in determination best formulation in order to release the product for commercial production. From the results, it was concluded that functional beverage formulation T4 prepared with 6% bitter gourd juice + 4% lemon juice + 3% amla juice was found to be the most stable as well as acceptable beverage formulation than others during storage considering physico-chemical, sensory and microbial qualities with minimal percentage losses. As this study lacks clinical studies, it is further suggested to check the applicability of this functional beverage formulation in diabetic people *via* clinical studies for several consumers of different ages.

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