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Study on physiochemical properties on carbonated guava drink

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Abstract
Present investigation was undertaken to study the physiochemical properties of carbonated beverage from guava fruit. The guava (*Psidium guajava*) is selected for the preparation of carbonated fruit drink because of its short life span when its compared with other fruits. The extracted fruit juice subjected to carbonation. The physico-chemical parameter of fruit drink is very critical as it determines the quality and stability of the carbonated fruit drink. The best treatment has been selected based on response surface methodology using design of experiments for the development of best formulation and process condition. The best treatment for carbonated fruit drink process temperature is 0^0C in the pressure range of 100 psi with the fruit juice level is 12.5%. The optimized carbonated guava drink process treatment was adopted for the development of carbonated guava drink with increased level of juice concentration varies from 10%, 20% and 30% and TSS from 10^0Bx, 12^0Bx and 15^0Bx respectively. The observed result of physiochemical parameters guava juice level at 30% with 10^0Bx had received highest vitamin C 28.8 mg/100g, constant specific gravity 1.03, high color value ΔE 4.60 and highest of volume of CO_2. The sensory analysis is done to find the best combination of juice concentration and TSS. Further, the developed carbonated guava drink is compared with commercial carbonated fruit drink and for further research shelf life studies is done.

Keywords: Guava, carbonated beverage, storage, shelf life, physiochemical, properties

1. Introduction
Beverages are consumed by people for various reasons such as to quench the taste, as a social drink, as well as medicinal drink to enhance health consciousness among people (Malik *et al.*, 2006) [19]. In India, cold drinks are in demand so there is a need for carbonated drinks (Abid *et al.*, 2013) [1] with increased nutritional value. Carbonation is the process in which impregnation of a liquid with carbon dioxide gas takes place (Calix *et al.*, 2008) [7]. This process has gained its popularity for its enjoyable taste and is an important ingredient of sparkling drinks (Bertos *et al.*, 2004) [6] which also enhances the flavour as well as provides refreshing sensation to carbonation (Leksrisompong *et al.*, 2012) [17]. The concept of carbonated fruit based beverage is to provide nutritional elements of the fruit along with natural pigments and flavour in addition to carbonation effect. Gas flushing is one of the viable alternate method to extend the shelf life of fruit flavour. The country helping farmers towards "Yellow Revolution" by the promotion of carbonated fruit beverages.
The research outcome gain on carbonated beverages is growing 30 percent annually (Khurdiya D. S. and Verma, 1996) [15]. Fruit juice is a drink that is made from fruit material either with or without any additional ingredient. It is a refreshing liquid and the raw material usually contains minor ingredients, particularly vitamins and minerals (Jain and Borkar, 1966) [12]. An alternative to the carbonated soft drink is the fruit juice which contains water, sweetening agent and flavours. The most popular beverage in the world is tea and the next position is occupied by bottled water and soft drinks (Ahmad *et al.*, 2015) [2]. This trend has been set by major soft drink producing companies which adjusted its products with consumer lifestyles, high marketing budgets and sophisticated distribution systems.
Guava is considered as the fourth most important fruit followed by mango, banana and citrus. World production of guava is 500,000 MT (Rueda, 2005) [22] and the major producers of the guava globally are Mexico, Pakistan, Columbia, Egypt and Brazil followed by India. In India, major cultivation of guava is Uttar Pradesh, Bihar, Madhya Pradesh, Maharashtra. Guava (*Psidium guajava* L.) the "Poor men's Apple" is a tropical fruit originated from America belong to the family "myrtaceae" known for its exotic flavour and potent aroma. It is rich in

lycopene and ascorbic acid, with an ascorbic acid content of which is higher than orange juice. In addition it is also contains dietary fiber, vitamin-A, potassium, magnesium and antioxidant pigments such as carotenoids and polyphenols (Singh, S. P, 2010) [27]. Guava is consumed as fresh fruit which is highly perishable in nature tend to be not stored for longer use (Sahota *et al.*, 2010) [23]. Therefore, necessary steps have to be taken to develop advanced technologies for processing and preservation of such enormous produce. Carbonated guava juice becomes economically important in the market (Harnack *et al.*, 1999) [11] due to its natural flavour, nutritional values the consumption of guava juice has been increasing currently and used as an alternative to other beverages such as tea or coffee. The formulation of carbonated fruit beverage offers variety of flavours, nutrients, long shelf life and other physiological benefits with a greater margin of safety in drink with a lower inherent cost in comparison with the fruit juice. In the present study, carbonation was done to improve the nutritional value of the aerated drink and improve the economic value of guava fruit as well as improve the economic status of farmers and processors (Sandhan, 2003) [24]. The objective of the study is focused to develop carbonated guava drink and to evaluate its physiochemical parameters.

2 Material and Methods

2.1 Procurement of raw materials

The ripened guava fruits, beetroot, ginger and sugar were procured from Farmers Producer Organization, Cauvery Delta Mart, Thanjavur and tender guava leaves, basil leaves were collected from the local farm at Thanjavur. PET bottles (11.6mm thickness and 13gwt. of the bottle) were purchased from the Tamilini Plastic Bottle Suppliers, Thanjavur for the further studies. The ascorbic acid was purchased from Madras Scientific Pvt. Ltd., Trichy and all chemicals and reagents used for analysis were either from Sigma Aldrich Chemicals (St. Louis, MO) of analytical reagent (AR) or laboratory reagent (LR) or guaranteed reagent (GR) grade.

2.2 Extraction and formulation of guava juice

The guava fruits were sorted based on maturity and quality before processing. Juice was extracted from guava fruit by heat processing at 90^0C for 10 minutes to break the tissues in the fruit. Guava fruit juice by straining through a muslin cloth. The filtered juice was then heat processed at 85^0C for 15 minutes for clarification. The clarified guava juice was sweetened by adding sugar syrup and fortified with ascorbic acid at the rate of 25 to 50 mg per litre as an acidifying agent. The guava juice was thermally treated at 110^0C for 8 seconds and cooled at room temperature 25 ± 2^0C.The developed formula of the carbonated guava drink is standardized with functional ingredients namely beetroot juice (1% to 3.5%), guava leaves (3.5% to 7.5%) and basil leaves juice (3.5% to 7.5%).For the improvement of nutritional profile with increased per cent of guava fruit juice from 10, 20 and 30 % with lower sugar concentration of 10, 12 and 15 % respectively. The detailed treatment for standardization of carbonated guava drink is presented in the following Table 2.1.

Table 1: Various combination of guava juice and sugar concentrations in carbonated guava drink

Treatments	Guava juice and sugarconcentrations
Gu1	10 % Guava fruit juice + 10^0 B x Total soluble solid (TSS)
Gu2	20 % Guava fruit juice + 10^0 B x Total soluble solid (TSS)
Gu3	30 % Guava fruit juice + 10^0 B x Total soluble solid (TSS)
Gu4	10 % Guava fruit juice + 12^0 B x Total soluble solid (TSS)
Gu5	20 %Guava fruit juice +12^0 B x Total soluble solid (TSS)
Gu6	30 % Guava fruit juice + 12^0 B x Total soluble solid (TSS)
Gu7	10 % Guava fruit juice + 15^0 B x Total soluble solid (TSS)
Gu8	20 % Guava fruit juice + 15^0 B x Total soluble solid (TSS)
Gu9	30 % Guava fruit juice + 15^0 B x Total soluble solid (TSS)

2.3 Carbonation

After cooling, the pasteurized guava drink was subjected to carbonation. The prepared guava drink was filled in 250ml PET bottles in the temperature range of 0^0C,2^0C and 4^0C at the pressure range of 100psi by using carbonation unit at Aaruran Soda Company, Thiruvarur. After carbonation the bottles were sealed tightly by using capping machine and it is stored at cool and ambient temperature for further studies. The carbonated guava drink was prepared as shown in figure 1.

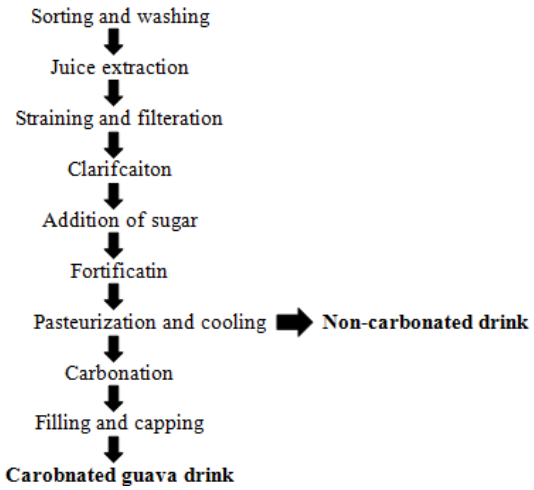

Fig 1: Process flow diagram for carbonated guava drink

2.4 Quality analysis

The physiochemical, nutritional and sensory properties of carbonated guava drink was analysed. Viscosity was measured using Brookfield viscometer -S 62 spindle at 100 rpm (Macdonald *et al.*, 2000) [18]. TSS reading was noted using ATAGO digital refractometer in degree Brix (Abid *et al.*, 2013) [3]. pH value was measured using digital pH meter (Lee *et al.*, 2005) [16]. The specific gravity was measured using pycnometer (Slaughter *et al.*, 2003) [28]. Hunter lab colour flex meter (Hunter Associates Laboratory, Inc. Reston, Virgina, USA) was used for the measurement of colour of guava drink (Ganjloo *et al.*, 2011) [10]. The Aqua Lab water activity meter 4TE was used for the measurement of $a_w$of guava juice (Jawaheer *et al.*, 2003) [13].

For the analysis of titratable acidity, 10ml sample of guava juice was diluted with water at ratio 1:10. From that 25 ml of solution was taken in beaker and 2 to 3 drops of phenolphthalein indicator was added and titrated against 0.1N NaOH till colour changed to pink. Then the values were noted and acidity was determined by the following formula (Sangrame *et al.*, 2000) [25].

$$\text{Titratable acidity (\%)} = \frac{Titrate value \times acid factor \times 100}{volume of aliquot}$$

The total sugar, reducing and non-reducing sugar was determined by Ranganna, (1986) [21]. Ascorbic acid was detected by using the procedure followed by Wall, (2006) [32]. The total phenol content present in guava was determined using Folin-Ciocalteu method described by Cao *et al*. (2011). Antioxidant activity measured was expressed as the percentage of DPPH Scavenged (%DPPH) (Shekhar and Anju, 2014) [26]. Proximate value of the sample and total microbial count was conducted according to the AOAC method number 966.23 (Verma *et al.*, 2014) [31] was determined and CO_2 analysis was done using CO_2 analyser (Chevaux *et al.*, 2001) [8]. Where CO_2 analyzer consist of 2 probes where probe 1 is used for measuring CO_2 and Temperature measurement probe 2 is used to measure the Humidity and Dew point where the samples stored in PET bottles were exposed to the probe the digital meter in the CO_2 analyzer automatically detects the % of dissolved Co_2 in the head space. Sensory evaluation was performed by 9-point hedonic scale in terms of color, flavour, taste, and appearance the overall acceptance for the juice samples (Thongrote *et al.*, 2016) [29].

Statistical analysis is the best insight into a system to assist experiments. Data obtained from the experiments were analyzed using the statistical software Design-Expert.

The probability level and Fishers F-test was used as the basis for determining the statistical significance of the coefficient for the different factors. The three dimensional graphical representation and their respective contour plots were determined by the interaction of independent variables and the dependent variables (Bashir and Abu-Goukh, 2003) [5]

Analysis of Variance was done for statistical analysis after the sensory evaluation. Using one way ANOVA the statistical analysis is done for all the samples. It was done using a SPSS statistics base (SPSS Inc., Chicago, USA). Using the Duncan's Multiple Range test, the differences between the means of the treatments were determined with a statistical significance between the sample treatments at $p<0.05$.The relationship between variables were examined using coefficient of determination (R^2).The data represented in a mean of three replicates. The calculations and graphics were performed using electronic worksheets from Microsoft

3. Results and Discussion

The carbonate guava drink was processed at 0^0C temperature in the pressure range of 100psi with increased level of juice concentration varies from 10%, 20% and 30% and TSS ranged from 10^0Bx,12^0Bx and 15^0Bx respectively. The physicochemical properties and acceptability of the carbonated guava drink with various combinations of fruit juice and total soluble solid *via* Gu1, Gu2, Gu3, Gu4, Gu5, Gu6, Gu7, Gu8 and Gu9 respectively were studied.

3.1 Effect of fruit juice concentration and its TSS in carbonated fruit drink on physiochemical parameters

The physicochemical parameters have been evaluated between the treatment from Gu1, Gu2, Gu3, Gu4, Gu5, Gu6, Gu7, Gu8 and Gu9 respectively and the results were presented in the Figure 1 and Table 1. The result was observed that among the treatment the TSS was high in combination of guava juice at 30% with TSS at 12^0Bx (Gu6) and pH was low in in combination of guava juice at 10% with TSS at 10^0Bx (Gu1). Titrable acidity was low for combination of guava juice at 10% with TSS at 15^0Bx (Gu7) and high for combination of guava juice at 30% with TSS at 15^0Bx (Gu9). The water activity was constant throughout the treatment. The viscosity was high in case of combination of guava juice at 30% with TSS at 10^0Bx (Gu3) sample and low for combination of guava juice at 10% with TSS at 10^0Bx (Gu1) and combination of guava juice at 10% with TSS at 12^0Bx (Gu4) sample. The specific gravity was constant for most of the samples ranged from 1.03 and 1.04. The color value was high for combination of guava juice at 30% with TSS at 10^0Bx (Gu3) samples and low for combination of guava juice at 10% with TSS at 10^0Bx (Gu1) samples.

Fig 1: Carbonated guava drink with various concentrations of fruit juice and TSS

Table 1: Physicochemical parameters of carbonated guava drink in various concentrations of fruit juice (10, 20 and 30%) and TSS (10, 12 and 15^0Bx)

Juice + TSS Concentration	TSS (^{0}Bx)	pH	Viscos. (cp)	Acidity (%)	Sp. Gr. (ρ)	Color (ΔE)	Water activity (a_w)	Vit-C (mg/100g)	Vol of CO_2 (%)
Control	15.7	4.6	12	0.1	1.06		0.9	26.4	0
Gu1	10.8	3.7	18	0.3	1.04	1.10	0.97	13.8	2.2
Gu2	12.5	3.9	26	0.4	1.03	2.09	0.99	18.4	2.3
Gu3	13.8	4.1	46	0.5	1.03	4.60	0.98	28.8	2.5
Gu4	14.7	3.8	18	0.2	1.03	1.51	0.98	14.6	2.1
Gu5	16.2	4.0	24	0.3	1.03	4.07	0.99	19.6	2.3
Gu6	16.9	4.2	42	0.4	1.04	2.75	0.99	25.6	2.5
Gu7	15.7	3.9	24	0.1	1.03	2.56	0.98	13.4	2.0
Gu8	16.1	4.1	36	0.3	1.03	2.61	0.98	17.8	2.3
Gu9	16.7	4.3	44	0.4	1.03	3.08	0.98	20.8	2.5

Note:
Non-carbonated guava drink: Control
Carbonated guava drink: Gu1 to Gu9
Gu1: guava juice 10% + TSS 10^0Bx, Gu2: guava juice 20% + TSS 10^0Bx, Gu3: guava juice 30% + TSS 10^0Bx,
Gu4: guava juice 10%, TSS 12^0Bx, Gu5: guava juice 20%, TSS 12^0Bx, Gu6: guava juice 30% + TSS 12^0Bx,
Gu7: guava juice 10% + TSS 15^0Bx, Gu8: guava juice 20% + TSS 15^0Bx, Gu9: guava juice 30% + TSS 15^0Bx.

Volume of CO_2 released from the sample combination of guava juice at 30% with TSS at 10^0Bx (Gu3) and combination of guava juice at 30% with TSS at 15^0Bx (Gu9) has highest level of 2.5%. Baranowski and Park, 1984 stated that different concentration of pineapple juice ranges from 10%, 12% and 15% were carbonated at 100psi pressure level and the physicochemical properties such as acidity, TSS, pH and sugar were studied and the best results were obtained for 15% fruit juice concentration.

The observed result of physiochemical parameters were indicated that among the treatments the combination of guava juice level at 30% with 10^0Bx had received highest vitamin C 28.8mg/100g, constant specific gravity 1.03p, high color value ΔE4.60 and highest volume of CO_2 2.5%. Similar study have been done in the carbonated beverage contains 25% guava fruit juice,0.5% acidity and 20% TSS (Vasure *et al*, 2014) [30].

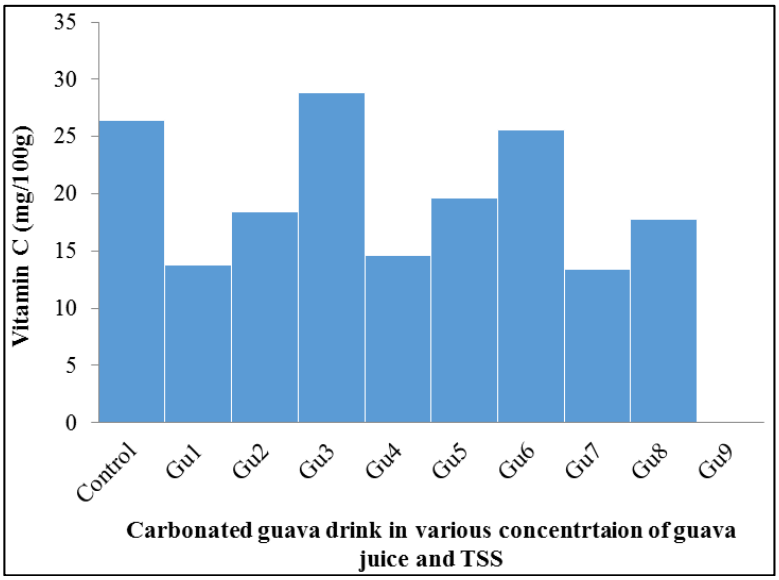

Fig 2: Vitamin-C in carbonated guava drink with various concentrations of fruit juice and Total Soluble Solid

3.2 Effect of fruit juice concentration and its TSS in carbonated fruit drink on sensory attributes

The sensory attributes of the all the nine samples were evaluated by the 20 pane list to find out the best combination of guava juice and TSS concentration. The sensory analysis was done using 9-point Hedonic scale for three different concentration of carbonated guava juice using different characteristics such as color, appearance, flavor, taste and overall acceptability and the results were presented in the Table 2. Higher levels of fruit juice concentration in the guava juice significantly increased ($P<0.05$) the sensory attributes of color (8.50± 0.5), appearance (8.15± 1.1), flavor (8.30± 1.3), taste (8.5±0.68).

Table 2: Multiple comparisons using one-way ANOVA

Juice + TSS Concentration	Color	Appearance	Flavour	Taste	OAA
Control	9.00±0.00a	9.00±0.00a	9.00±0.00a	9.00±0.00a	9.00±0.00a
Gu1	6.30± 0.75b	6.05± 1.05bc	5.80± 1.39 d	5.20± 1.67 b	6.35± 1.03 bcd
Gu2	5.95± 0.75b	6.40± 1.18 bc	5.95± 1.50 d	5.10± 1.74 b	6.10± 1.33 bd
Gu3	8.50± 0.5 a	8.15± 1.1 ac	8.30± 1.3 a	8.5±0.68a	8.6±0.59 a

Gu4	7.15± 1.2 c	7.10± 1.1 bc	7.00± 1.1d	7.0± 0.5 c	7.2± 1.0 c
Gu5	6.0± 0.66 b	6.10± 1.29 bc	6.45± 0.94 d	5.80± 1.36 bc	6.2± 1.32 bcd
Gu6	6.65± 0.93b	6.40± 1.09 bc	6.30± 1.30 d	6.10± 1.37 bc	6.7± 1.17 bc
Gu7	6.25± 1.88b	6.65± 1.72 bc	6.05± 1.27 d	6.20±1.88 bc	6.05± 1.14 bd
Gu8	5.85± 1.59b	5.85±1.72 b	5.55± 1.82 c	5.55± 1.87 b	5.45± 1.31 d
Gu9	7.00± 1.33b	7.25± 1.20 dc	7.30± 0.97 ab	7.00± 1.02 c	7.00± 1.16 bc

*Means with different letters within the row are significantly different ($p < 0.05$)

Highest percentage of carbon dioxide in the sample combination of guava juice at 30% with TSS at 10^0Bx (Gu3) contributes better color and overall acceptance. This result was agreed with findings of Chilana *et al.*, 2015 [9]. The carbonated guava drink Gu3 which contains fruit juice concentration 30% with TSS 10% sample has got higher acceptability score (8.6±0.59) which is highly preferred by the panelist among all the treatments. There is no significance difference between carbonated guava drink samples in sensory attributes.

3.3 Comparison of carbonated guava drink with commercial carbonated fruit drink

The developed carbonated guava drink has been analyzed by organoleptic evaluation and panelist has preferred sample Gu3contains 30% of guava juice and 10% of TSS as the best combination when compared with other samples.

The nutritional valueof developed carbonated guava drink is compared with commercial carbonated fruit drink and results are presented in the Table 3. It was observed that the developed carbonated guava drink has protein 0.7g/100g, crude fiber 2.1g/100g, total phenols 346.5mg/100ml, total antioxidants 346.5mg/100ml and ascorbic acid 28.8mg/100g when compared with various commercial carbonated fruit drink. Pawar, (2010) [20] studied nutrient facts of carbonated pineapple juice with the commercial sample and they concluded that carbonation technology for fruit juices can be preferred for commercialization.

Table 3: Comparison of nutritional value (g/100g) in carbonated guava drink with commercial carbonated fruit drink

Proximate value	Carbonated guava drink	Commercial Sample 1	Commercial Sample 2	Commercial Sample 3
Energy kcal	10.0	54.0	6.0	6.0
Carbohydrates	1.8	13.6	1.4	1.4
Total sugars	7.1	21.0	1.4	1.4
Crude fiber	2.1	0	0	0
Protein	0.7	0	0	0
Moisture	94.8	~~9~~0	~~9~~0	~~9~~0
Ash	0.6	N.D	N.D	N.D
Total phenols mg/100ml	346.5	N.D	N.D	N.D
Total antioxidants mg/100ml	119.3	N.D	N.D	N.D
Ascorbic acid mg/100g	28.8	N.D	N.D	N.D
Reducing sugar	5.9	N.D	N.D	N.D
Non reducing sugars	1.2	N.D	N.D	N.D

3.4 Storage stability of carbonated guava drink

Developed carbonated guava ready-to-drink has been carried out with quality analysis such as physiochemical and microbial quality. The shelf life study was done for carbonated guava juice for 3 months at two different storage conditions such as ambient and refrigerant temperature. During the storage period it was found that carbonated guava drink has slight modification with acceptable level in quality parameters and it was recorded as increase in TSS and bacterial count and decrease in pH and titrable acidity. The rate of increase in TSS, bacterial count and decrease in pH and titrable acidity was higher in ambient storage temperature than in refrigerant storage temperature. No more changes in color, flavor and taste because of the sparkling nature of fruit drink containing highest volume of CO_2 the flavor remains as it is in the carbonated guava drink and the freshness of the drink was retained and the shelf life of the beverage was also increased upto to 3months.The carbonated guava drink was stored for the period of 3 months with no changes in flavor, taste and color but after three months there is a slight modification in pH, acidity and TSS due to the loss of CO_2 percent in the drink. Similar findings were reported by Khurdiya *et al.*, (1996) [15].

Table 4: TSS, pH, acidity and microbial quality during storageof carbonated guava drink

Parameters	Initial			After 1 month			After 2 month			After 3 month		
	A	R	Mean	A	R	Mean	A	R	Mean	A	R	Mean
TSS	13.8	13.8	13.8	14.1	13.8	13.95	14.5	14.0	14.25	15.0	14.3	14.65
pH	4.1	4.1	4.1	3.9	4.0	3.95	3.7	3.9	3.8	3.5	3.8	3.65
Acidity	0.5	0.5	0.5	0.48	0.49	0.485	0.47	0.48	0.475	0.46	0.47	0.465
Microbial count *×10³Cfu/g)*	0	0	0	1.4	1.32	1.36	2.1	1.8	1.95	3.6	2.5	3.05

Note: A = Ambient storage temperature (± 25^0 C), R = Refrigerant temperature (± 5^0C)

4. Conclusion

Ready-to-drink fruit juice could be prepared by carbonation technique using locally available guava fruit. The changes in physicochemical characteristics were found in respect of different types of fruit juices and concentration of fruit juice. The developed carbonated guava drink has protein 0.7g/100g, fiber 2.1g/100g, total phenols 346.5mg/100ml, total antioxidants 346.5mg/100ml and ascorbic acid 28.8 when compared with various commercial carbonated fruit drink samples Highly acceptable guava based carbonated drink could be prepared by 30% fruit juice, 10^0Bx TSS respectively with a shelf life of 3 months Carbonated fruit drink prepared

with combination of 30% fruit juice, 10^0Bx TSS has got higher acceptability score (8.6±0.5) when compared with other samples. From all the results and comparison obtained in the above study palatable and shelf life drinks can be prepared from guava juice by adjusting the juice concentration and carbonation. The drink made from the guava juice with carbonation technology, yielded eye appealing products and received good scores throughout the storage and sensory period. The guava juice in carbonated drink improves the nutritional value and adds variety to the ready-to-drink. Hence, such product should be evaluated on its own merit, if the concept of fruit base carbonated drink is to survive and becomes acceptable. These developed carbonated guava drink can serve as good vehicle for carrying the added nutrients to target populations for use in combating the nutrient deficiency disorder. Keeping in view of the nutritional benefits, the fruits can be explored for commercial use in preparation of therapeutic drink. The study demonstrated that value added drinks from fruit juices with carbonation technology could be prepared for commercial exploitation this technology can redress the problems of industrialist by minimizing the spoilage losses, avoid fruit glut in the market, and efficiently utilization of astringent, highly nutritive fruits in the form carbonated beverage with retention of nutrients and nutraceutical properties of fruits for a period of three months

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