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Development and Optimization of Ready to Serve (RTS) Beetroot Drink

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ABSTRACT

Beetroot is rich in various nutrients. Hence the present study was conducted to develop a ready to serve (RTS) drink using beetroot juice. The juice of beetroot was extracted and added with different concentrations of sugar and citric acid to optimize the best-suited combination of ingredients. Standardization of RTS was done using ranking sensory evaluation test. Two variants of ginger and black pepper flavor were also prepared and standardized. The standardized amount of ingredients after sensory analysis for RTS was found to be 17.7% juice content, 7.5% sugar and 0.1% citric acid. The black pepper variant was standardized at 0.4% black pepper in the original product and the ginger variant standardized at 1.5% ginger extract. The beetroot drink and its variants having an optimized amount of ingredients were analysed for their physico-chemical properties. Shelf life analysis for a period of one month was also carried out.

Key words: Beetroot, Black pepper, Ginger, Ready to serve, Shelf Life analysis.

INTRODUCTION

Red Beetroot (*Beta Vulgaris*) is a popular vegetable throughout the world (Latorre *et al*. 2011). Beetroot grown throughout the America, Europe and Asia; is a cultivated form of *Beta Vulgaris* Subsp. Vulgaris (U.S. Department of Agriculture). Beetroot belongs to *Chenopodiaceae* family having four basic varieties Crosby Egyptian, Early Wonder, Detroit Dark Red and Crimson Globe (Chawla *et al*., 2016). Beets are composed of 87.57% water, 9.56% carbohydrates (29.3% fiber and 70.7% sugar), 1.61% protein and 0.17% lipids in addition to being a source of potassium, choline, vitamin C and niacin (Varner 2014). Beetroot juice contains minerals such as magnesium, calcium, iron, phosphorous, sodium and zinc, vitamins like biotin, folic acid, niacin, vitamin B6 (Wootton-Beard and Ryan, 2011). Small amounts of hydroxycinnamic acids such as gallic, syringic, caffeic acids and flavonoids have been identified in beetroot (Kazimierczak *et al*. 2014). Sucrose is the main sugar found in beetroot along with small amounts of glucose and fructose (Bavec *et al*. 2010). Beetroot also contains raffinose (Mahn *et al*. 2001). High concentrations of betalains, a group of phenolic secondary plant metabolites and a water-soluble pigment is responsible for the intense red color of beetroot (Georgiev *et al*. 2010).Betalain is divided into betaxanthins giving yellow-orange color and betacyanins giving purple color to beetroot.

Recently, beetroot has gained attention as a health-promoting functional food product (Clifford *et al*., 2015). Carotenoids, betalains, polyphenols, flavonoids and saponins are the active compounds found in red beetroot (Figiel, 2010). The quantity of these active compounds is influenced by species, variety, cultivation area and ripening period and storage. It shows antibacterial and antiviral activity due to strong antioxidant potential (Kowalski and Szadzińska, 2014) and thus can be considered as a factor preventing cancer (Figiel, 2010). It also shows anti-inflammatory and hepatoprotective activities (Khan *et al*. 2011). Beetroot also helps in increasing resistance to oxidation of low-density lipoproteins (Tesoriere *et al*. 2003).

Beetroot is used in tomato paste, jam and jellies, ice cream, sweets, sauces, etc as a red food colorant to improve the color (Gokhale and Lele 2011). Beetroot commercial products such as juices and powders act as performance-enhancing legal nutrition supplements for athletes, especially those in endurance sports due to the presence of inorganic nitrate (NO_3-), bacteria reduces it to nitric oxides (NO) which shows positive effects on muscle efficiency and fatigue resistance also causing reduction in resting blood pressure (Bailey *et al*. 2009). Thus it is suggested that it can prevent and treat hypertension and cardiovascular diseases (Lundberg *et al*. 2010).

In the food industry convenience is considered as a marketing tool (Drewnowski and Darmon 2005). Food manufacturers thus are looking for convenient and healthy food in order to improve public health (Wootton-Beard and Ryan 2011). Beetroot juice as compared to other vegetable

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juices, has a pleasant taste due to relatively high sugar content (Thakur and Gupta, 2005). There is a paucity of literature available that confirms the use of beetroot for development of a ready to serve drink. Tapping the potential of the beetroot to be used as a source of various nutrients, the present study was conducted to develop a RTS drink from beetroot. These products contribute positively toward increasing consumption of polyphenols with ease and convenience.

MATERIALS AND METHODS

Raw Material

Fresh beetroot, black pepper powder and ginger rhizomes were purchased from local market of Narela, Delhi. All the additives namely citric acid (saby make), sugar (Madhur make) and gum arabic (Aksharchem) were food grade and were purchased through an online shopping portal. The beetroot had moisture content 83.60± 0.1% and ash content of 0.65± 0.01%. Sugar had a moisture content of 0.05 ± 0.41% and ash content of 0.02±0.03%.

Extraction of beetroot juice

Procured beetroot and ginger were washed thoroughly with clean water followed by peeling and cutting them into small pieces. The juice was extracted from beetroot and ginger with the help of domestic juicer (USHA Juicer Mixer Grinder, JMG 3442), strained separately with muslin cloth and stored in bottles.

Optimization of the beetroot RTS and its variants

Standardization of the RTS was done by stepwise standardisation of beetroot juice, citric acid and sugar (Table 1-3) by using ranking sensory evaluation test. The samples were evaluated by 15 semi-trained panelists (Age group 20-35) consisting of students, faculty members and lab technicians from the National Institute of Food Technology Entrepreneurship and Management (NIFTEM). Since the panelists were not professional sensory analysts, therefore, they were made familiar with the procedure of ranking method. The panelists were asked to rank the product on various parameters and also on overall acceptability (Rank 1 for the most preferred sample, 2 for the moderately preferred and rank 3 for the least preferred sample (Sharif *et al*. 2017).

Ranking test for intensity is a product oriented sensory test. This method of testing can be used to gather preliminary product difference details or to screen panelists who can distinguish between samples with established differences. Ranking test is able to find out noticeable differences between the samples, but does not tell about how much difference exists between the samples (Watts *et al*. 1989).

Ginger extract was added in different amounts to prepare the ginger variant of the beetroot RTS (Table 4). Black pepper was added in different amounts and later strained to prepare the black pepper variant of Beetroot RTS (Table 5). Gum arabic was added to increase the thickness of the RTS. 1.00g was added to the 100ml RTS standardised as per the FSSAI Regulation 5.3.10 (Table 1-5).

Table 1: Amount of beetroot juice varied to select the best flavor intensity.

Sample	Amount	
	Beetroot juice	Water
S1	15 ml	85 ml
S2	17.5 ml	82.5 ml
S3	20 ml	80 ml

Table 2: Amount of citric acid solution varied to select the best fit of sourness, keeping the amount of beetroot juice to be added constant.

Sample	Amount		
	Beetroot Juice	Citric Acid	Water
S4	17.5 ml	0.1 g	82.5 ml
S5	17.5 ml	0.15 g	82.5 ml
S6	17.5 ml	0.2 g	82.5 ml

Table 3: Amount of sugar varied to select the best fit of sweetness, keeping the amount of Beetroot juice andcitric acid to be added constant.

Sample	Amount			
	Beetroot juice	Citric Acid	Sugar	Water
S7	17.5 ml	0.1 g	5g	82.5 ml
S8	17.5 ml	0.1 g	7.5g	82.5 ml
S9	17.5 ml	0.1 g	10g	82.5 ml

Table 4: Amount of ginger extract varied to select the best fit, keeping the amount of beetroot juice, sugar andcitric acid to be added constant for the ginger variant of beetroot RTS.

Sample	Amount				
	Beetroot juice	Citric Acid	Sugar	Water	Ginger Extract
S10	17.5 ml	0.1 g	7.5g	82.5 ml	2ml
S11	17.5 ml	0.1 g	7.5g	82.5 ml	1.5 ml
S12	17.5 ml	0.1 g	7.5g	82.5 ml	1ml

Table 5: Amount of black pepper powder varied to select the best fit, keeping the amount of beetroot juice, sugar and citric acid to be added constant for the blackpepper variant of beetroot RTS.

Sample	Amount				
	Beetroot juice	Citric Acid	Sugar	Water	Black pepper
S13	17.5 ml	0.1 g	7.5g	82.5 ml	0.2g
S14	17.5 ml	0.1 g	7.5g	82.5 ml	0.4g
S15	17.5 ml	0.1 g	7.5g	82.5 ml	0.5g

Sensory analysis of the final standardised product and the variants

Beetroot RTS and its flavroured variants were prepared using the optimized quantities of various ingredients and sensory analysis was carried out by using hedonic scales that is a 9 point scale used as a consumer oriented sensory test. It helps in measuring degree of liking of product by the consumer (Watts *et al*. 1989).

The samples were evaluated by 30 semi-trained panelists (Age group 20-35) consisting of students, faculty members and lab technicians from the National Institute of Food Technology Entrepreneurship and Management (NIFTEM). Since the panelists were not professional sensory analysts, therefore, they were made familiar with the procedure of hedonic scale testing.The members were made familiar with the parameters to be analysed namely taste, aroma, appearance, after taste, overall acceptability. Panelists evaluated the RTS and its variants for the parameters using a 9-point hedonic rating scale (1 = dislike extremely and 9 = like extremely).

Physico- chemical analysis beetroot RTS and its variants

The RTS samples stored at room temperatures were analyzed for various physicochemical parameters as follows:

Water activity

Digital benchtop water activity meter (chilled mirror dew-point)(AQUA LAB, S40002534) was used to measure the water activity. The sample was placed in a small sample cup in the temperature controlled chamber. The instrument was switched on and was allowed to run, till the dew point was reached. Readings were observed from the digital display.

Total Soluble Solids (TSS)

Digital bench top refractometer was used to measure the TSS content (ATAGO, 140404N). The calibration was done using distilled water, by placing it over the prism in the chamber. Then the sample was placed on the prism and the chamber was closed. The instrument was allowed to run and the readings were observed from the digital display.

Titratable acidity

The titratable acidity was determined by titration with 0.1N sodium hydroxide (NaOH) according to(AOAC 1995) with some modifications.

$$\% \text{ Citric Acid} = \frac{N \times V \times M}{S \times 10}$$

Where:

N= Normality of standard NaOH solution used for titration

V= Volume of standard NaOH used for titration in millilitres

M= Molecular weight of citric acid divided by the no. of Hydrogen ions

S= Sample in Millilitres or grams

Colour

Colour of beetroot RTS as per *'L', 'a', 'b'* color scale was measured by Hunter Lab Colorimeter (KONICA MINOLTA, CR-400). The Hunter *L, a, b* color space is organized in cube form. The '*L'* axis runs from top to bottom. The maximum for '*L*' is 100 depicts black shade. Positive and negative *'a'* value indicates red andgreen shade respectively. While positive 'b' shows yellow and negative 'b' value depicts blue color. A small amount of the sample was taken in a transparent cellophane pack. The calibrated colorimeter was used to measure *'L', 'a', 'b'* values. The color was reported as ΔE.

pH

A digital pH (LABINDIA, PN13330213) was used to determine the pH of the samples. The pH meter was standardized and calibrated with pH 4.0, 7.0 and11.0 standard solutions.

Microbiological parameters

Total plate count (TPC) and coliform were analyzed for Beetroot RTS and both the flavor variants. The testing was done by an external NABL accredited lab; Appex lab, Ramesh Nagar, New Delhi. Using IS: 5402 - 2012 and IS: 5401(P-1) 2012 as references methods for TPC and Coiiform.

Shelf Life Study of RTS samples

The Beetroot RTS and its variants were prepared according to the above mentioned procedure. Being a moderate acidity product, the RTS was pasteurized and stored in glass bottles for the shelf life analysis. Glass bottles of 200ml were thoroughly cleaned and boiled in water for 15 minutes to sterilize them. Beetroot RTS and both the variants were pasteurized individually by heating to 90-100°C in a pan for 30 minutes. They were hot filled in different bottles, capped and stored at room temperature for 4 weeks. Physico-chemical analysis (water activity, TSS, Titrable acidity, color, pH) and microbial analysis (total plate count and coliform) were done on weekly basis for 4 weeks.

Statistical analysis

For the statistical analysis of the sensory evaluation, non-parametric test for the samples was carried out using Chi-square test (Goodness to Fit) on the parameter overall acceptability.

RESULTS AND DISCUSSION

Optimisation of RTS Using Ranking Test

Preliminary sensory analysis

The result for the preliminary sensory analysis carried out to determine the best fit of various ingredients by using ranking test is presented in Table 6-11.

Final composition and sensory analysis of beetroot RTS and its variants

Based upon the inferences of the preliminary sensory analysis, the final standardised composition of the RTS and its variants is mentioned in Table 12. The results of the final sensory analysis conducted as a 9 point hedonic scale are presented in Figs 3-5.

Table 6: Standardisation of beetroot juice content.

	Ranking			Inference
	1(Best)	2(Average)	3(Worst)	
S01	5	6	4	Sample 2 was preferred. Out of 15 members, 9 of them ranked sample 2 appropriate wrt Beetroot juice characteristics
S02	9	6	0	
S03	1	3	11	

Table 7: Standardisation of sugar content.

	Ranking			Inference
	1(Best)	2(Average)	3(Worst)	
S04	7	6	2	Sample 05 was preferred. Out of 15 members, 8 of them ranked sample 2 appropriate wrt Sweetness characteristics
S05	8	7	0	
S06	0	2	13	

Table 8: Standardisation of acid content.

	Ranking			Inference
	1(Best)	2(Average)	3(Worst)	
S07	7	8	0	Sample 7 was preferred. Out of 15 members, 7 of them found sample 7 appropriate wrt sourness characteristics
S08	5	6	4	
S09	3	1	11	

Table 9: Standardisation of Ginger juice content.

	Ranking			Inference
	1=Best	2=Average	3=Worst	
S10	4	6	5	Sample 11 was preferred. Out of 15 members, 8 of them found sample 11 appropriate wrt ginger juice characteristics.
S11	8	7	0	
S12	3	2	10	

Table 10: Standardisation of black pepper content.

	Ranking			Inference
	1=Best	2=Average	3=Worst	
S13	4	8	3	Sample 14 was preferred. Out of 15 members, 10 of them found sample 14 appropriate black pepper characteristics
S14	10	5	0	
S15	1	2	12	

*The values depict number of people who ranked a particular sample.

The Figs 3 to 5 shows the number of people and their hedonic scale ratings for the various sensory parameters. Out of 30 panelists, 18 of them ranked the beetroot RTS above 7 in a 9 point hedonic scale ranking for overall acceptability as depicted in Fig 3. The black pepper flavoured Beetroot RTS was ranked above 7 on a 9 point hedonic scale by 18 people for the parameter overall acceptability (Fig 4). 17 panelists ranked the Ginger flavoured beetroot RTS above 7 for the overall acceptability of the product (Fig 5).

Table 11: Sample descriptions.

Sample code	Drink
S01	15% Beetroot juice
S02	17.5% Beetroot juice
S03	20% Beetroot juice
S04	5% Sugar content
S05	7.5% Sugar content
S06	10% Sugar content
S07	0.1% Acid content
S08	0.15% Acid content
S09	0.2% Acid content
S10	2% Ginger juice
S11	1.5% Ginger juice
S12	1% Ginger juice
S13	0.2% Black pepper
S14	0.4% Black pepper
S15	0.5% Black pepper

Physico-chemical characteristics of beetroot RTS and its variants

The optimized beetroot RTS and its variants were analyzed for TSS, acidity, pH, colour and water activity and the results are given in Table 13.

Shelf life study of RTS and its variants

Beetroot RTS and its variants were analyzed for TSS, acidity,

Table 12: Composition of the optimized beetroot RTS and its variants.

Sample	Beetroot Juice (%)	Sugar (%)	Citric Acid (%)	Flavor (%)	Arabic Gum (%)
Beetroot Drink	17.50	7.50	0.10	-	1
Ginger Flavoured Beetroot Drink	17.50	7.50	0.10	1.50	1
Black Pepper Flavoured Beetroot Drink	17.50	7.50	0.10	0.40	1

Table 13: Physico-chemical characteristics of the beetroot RTS andits variants.

Sample	TSS (%)	Acidity (%)	pH	Colour (ΔE)	Water Activity
Beetroot RTS Drink	11.21	0.26	3.95	77.83	0.996
Ginger Flavoured Beetroot Drink	12.70	0.23	4.32	79.02	0.992
Black Pepper Flavoured Beetroot Drink	11.08	0.27	4.03	80.36	0.993

*Each value is average of three determinations.

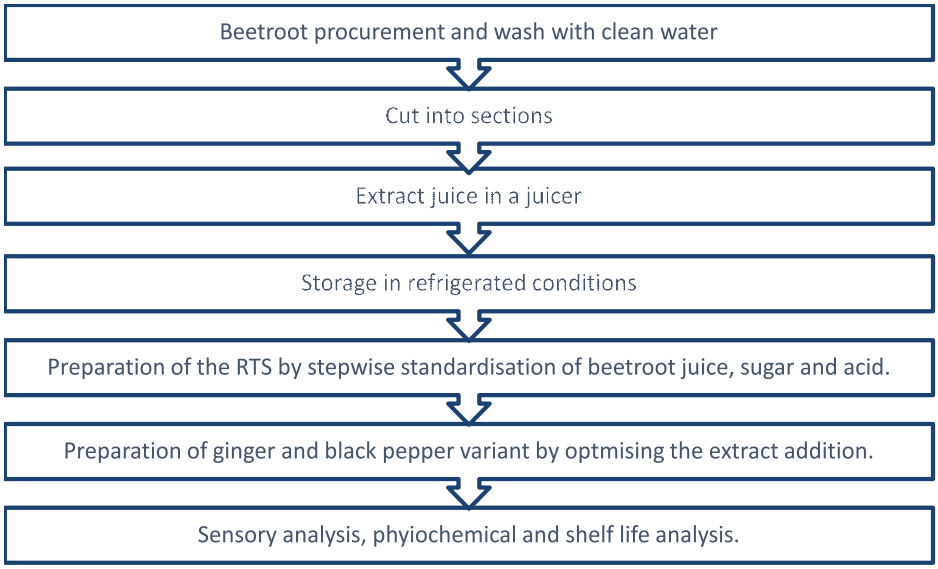

Fig 1: Schematic flow diagram of RTS preparation.

Fig 2: Bottled RTS.

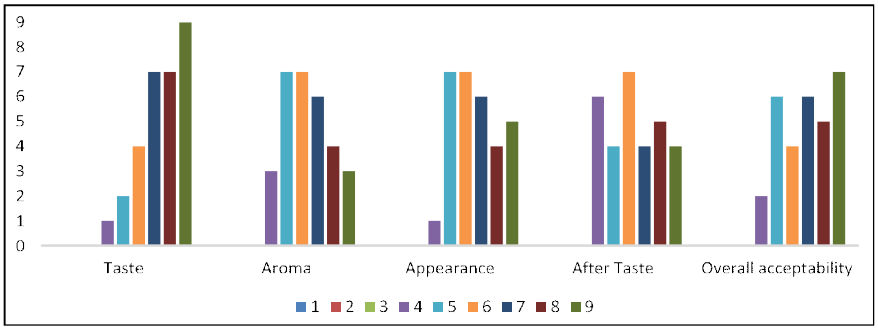

Fig 3: Sensory evaluation of Beetroot RTS; Hedonic scale ranking vs sensory parameters.

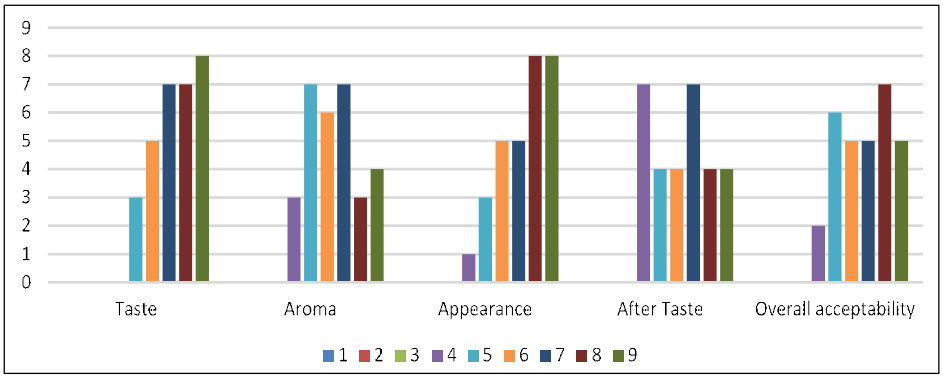

Fig 4: Sensory evaluation of Black pepper flavoured Beetroot RTS; Hedonic scale ranking vs sensory parametrs.

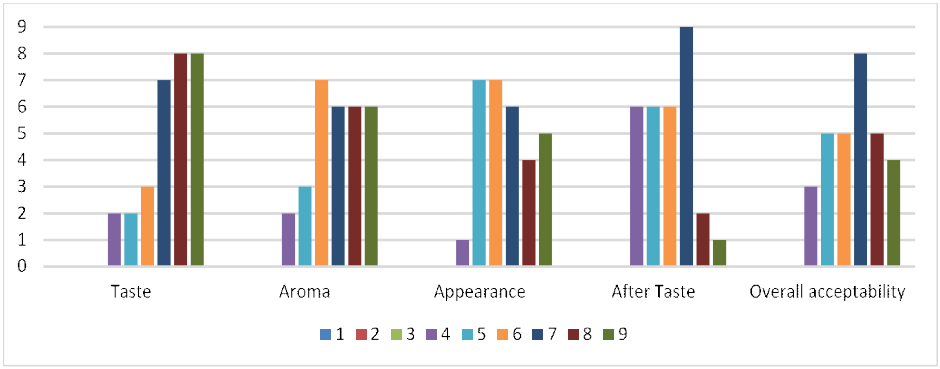

Fig 5: Sensory evaluation of Ginger flavoured Beetroot RTS; Hedonic scale vs sensoryparameters.

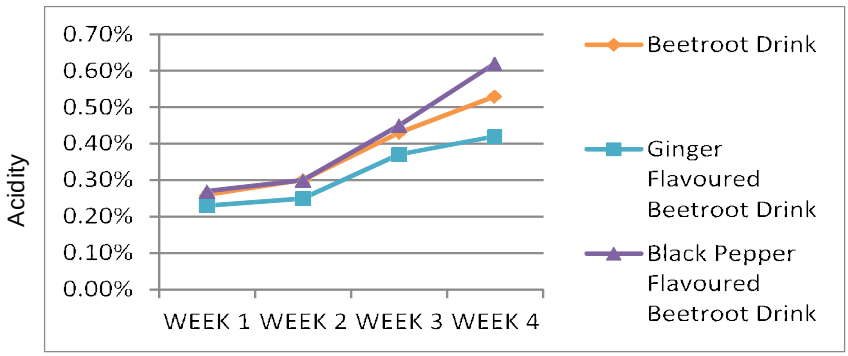

Fig 6: Changes in acidity with time.

pH, color and Total plate count (TPC) on a weekly basis and the results are given in Fig 6 to Fig 10.

Changes in acidity

Fig 6 shows the changes in the acidity of Beetroot RTS and its variants for the storage period of 4 weeks. When stored at room temperature, the acidity for Beetroot RTS increased from 0.26% to 0.53% in the period of 4 weeks. Similarly the acidity for Black pepper variant increased from 0.27% to 0.62% and that of ginger variant increased from 0.23% to 0.42%. A sharp notable increase is seen in all the variants after 2nd week. This may be due to the fermentation by micro-organisms. Similar results were quoted by (Kohli *et al*. 2019) in case of storage of sugarcane juice at refrigerated conditions.

Changes in TSS

The TSS decreased from 11.21% to 11.06% in the case of Beetroot RTS over the period of 4 weeks storage at room temperature as shown in Fig 7. The similar result was observed in the case of black pepper variant where the TSS decreased from 11.08% to 11.00% and ginger variant where it decreased from 12.70% to 12.42%. The micro-organisms use sugars present in the RTS as the raw material for fermentation which leads to the decrease in the TSS.Similar results were also seen by Amaravathi *et al*. (2014) in the case of spiced pineapple juice storage and also by (Sri Vidhya and Sri 2018) in the case of stored Beetroot juice.

Changes in pH

The pH decreased from 3.95 to 3.03 for Beetroot RTS over

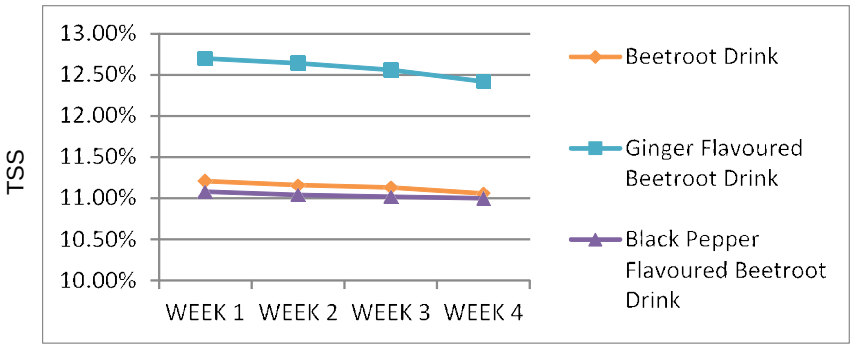

Fig 7: Changes in TSS with time.

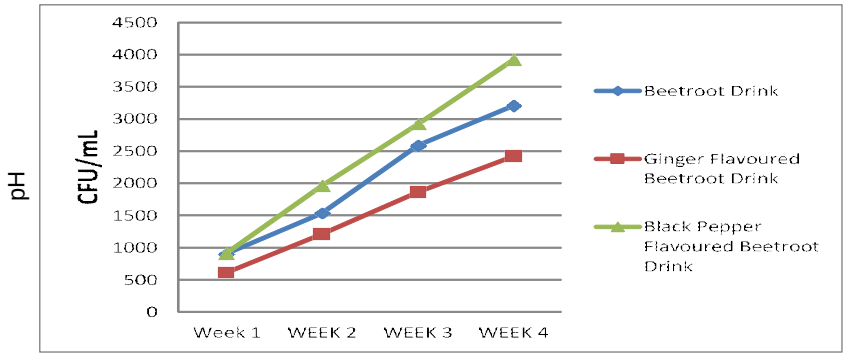

Fig 8: Changes in pH with time.

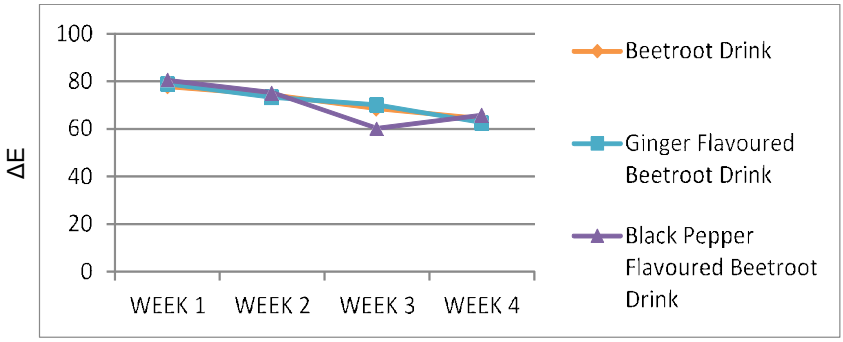

Fig 9: Changes in colour with time.

the period of storage. It decreased from 4.03 to 3.10 in case of ginger flavoured beetroot RTS and 4.32 to 2.64 in case of black pepper flavoured variant which is clear from Fig 8. Sri Vidhya and Sri (2018) observed the similar results in the case of stored beetroot juice. Minimum pH change was observed in the ginger variant which may be due to the antibacterial properties associated with ginger (Nwachukwu and Ezejiaku, 2014).

Changes in colour

As shown in Fig 9, the colour of the beetroot RTS expressed as ΔE decreased significantly from 74.83 to 64.27 in 4 weeks. It decreased from 73.26 to 62.66 in case of the ginger variant and 75.23 to 65.71 in the case of black pepper variant. Betalain responsible for giving colour to beetroot to susceptible to degration due to factors like water activity, pH value, atmosphere, light, temperature, metal cations and decolorizing enzymes (Herbach *et al*. 2006). Colour of RTS juice might have degraded mostly due to the factors like light and temperatures as betalain is very heat liable compound and thus shows degradation even at room temperature as observed by (Woo *et al*., 2011).

Microbiological analysis

All the three samples of beetroot drink were analyzed for Total Plate count and Coliform count. TPC was found gradually increasing in all the samples with time. The total plate count was 3210 CFU/ml, 2421 CFU/ml and 3920 CFU/ml at the end of 04 weeks of storage in case of plain beetroot RTS, ginger flavoured drink and black pepper flavoured drink, respectively. Less increase in case of ginger flavoured drink could be due to its antimicrobial properties (Nwachukwu and Ezejiaku 2014). As all the samples were only pasteurized not sterilized, the available fermenting

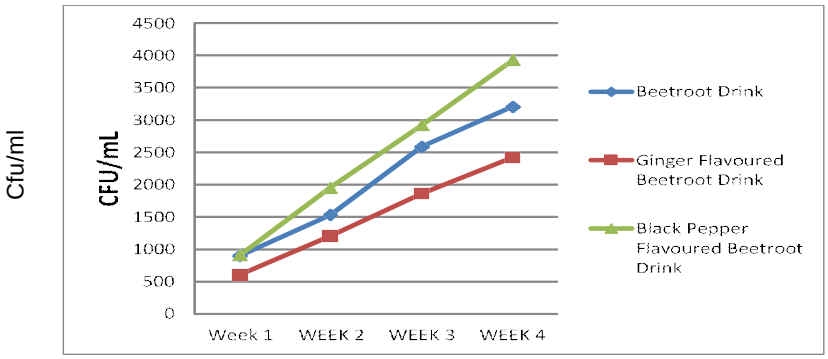

Fig 10: Changes in TPC with time.

Table 14: Chi-Square test (goodness of fit) for the samples.

Sample	Chi-square calculated value	DOF	Tabulated value at 5% significance level	Inference
Beetroot RTS	0.570069354	20	10.85	Null hypothesis may be accepted
Beetroot RTS-Ginger variant	0.255514797	20	10.85	Null hypothesis may be accepted
Beetroot RTS-Black pepper variant	0.373223088	20	10.85	Null hypothesis may be accepted

microbes in the drinks could have multiplied. Increase in acidity of all the samples support the microbial fermentation over the time. The similar results were reported by Kohli *et al*. (2019) in case of stored sugar cane juice. The coliform count of the samples during the storage was <10 CFU/ml.

Statistical analysis

For the statistical analysis of the sensory evaluation, non-parametric test for the samples was carried out using Chi square test (Goodness to Fit) on the parameter overall acceptability for beetroot RTS and its two variants. The results are presented in Table 14. Null hypothesis was assumed that the overall acceptability may be accepted. The results were calculated at p<0.05%. It was seen from the results that the tabulated value was more than the calculated value thus the null hypothesis may be accepted.

CONCLUSION

The beetroot RTS and its flavored variants were developed successfully with a fixed composition and preferred by many consumers. Sensorial evaluations by a semi-trained panel was used at each and every step of product development to ensure the best fit of ingredients to achieve the most desirable product. Physico-chemical analysis showed that the product is of low pH preventing the spoilage by the diversity of organisms. However, the product may still be susceptible to acid tolerant bacteria and wild yeasts. RTS pasteurized and packed in glass bottles didn't show much change in the physicochemical parameters until 2nd week of the storage at the room temperature.

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