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Studies on development of kiwi fruit RTS beverage incorporated with lemongrass

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Abstract

The present investigation has been carried out to develop the kiwi fruit RTS beverage incorporated with lemongrass. Sensory evaluation revealed that, RTS with 2 per cent of lemongrass concentrated extract is selected by the panel. For avoiding the stabilization in RTS guar gum was added and also its effect on sensory parameters were analyzed. Sample (B) with 2 percent of lemongrass concentrated extract and 0.2 per cent of guar gum was selected. Chemical composition revealed that TSS, pH, titrable acidity, total sugar, reducing sugar, ascorbic acid and total phenolic content of the RTS beverage was 12.8 Brix, 3.2, 0.384 per cent, 8.2 per cent, 6.4 per cent, 14.6 mg/100ml and 102.9 mg GAE/100ml respectively. Theoretical energy value of RTS beverage was 57.13 Kcal/100g. Microbial analysis revealed that, RTS beverage was acceptable during the storage of 90 days. Refrigeration temperature was better over ambient temperature for storage.

Keywords: Development, kiwi fruit RTS, lemongrass

Introduction

Demand for the natural fruit juice beverages is continuously increasing over the synthetic beverages. Ready to serve beverages are popular among every age group because of their refreshing nature and taste (Chandra *et al.*, 2018) [1]. As a result consumer demand for convenient and easily available product is continuously increasing. Ready to Serve Fruit Beverages means an unfermented product which is prepared from juice or Pulp/ Puree or concentrated juice or pulp of sound mature fruit and shall contain Total Soluble solid not less than 10.0% and fruit juice content not less than 10.0% (FSSR 2011) [2].

Kiwi fruit is introduced into the world in 20th century. In recent years, its production and consumption has increased (Izali *et al.*, 2007) [3]. The kiwifruit is unique because of its high nutritional content, different flavors, vitamins, minerals, antioxidants, phytochemicals and fiber content. In terms of nutrient content, the kiwifruit is amongst the richest fruits: it is also very valuable in terms of health. It is usually consumed fresh but in recent years along with increased production, industrial use is increasing. It is used in the canned food industry, for marmalades, fruit sauces and candies and for fruit juice concentrates, either separately or mixed with strawberries or apples. The fruit is also canned, dried, frozen, and used for the preparation of nectars (Göksel and Atak 2016) [4].

Kiwifruit is considered as antioxidant rich fruit and studied for decreasing oxidative damage and it is observed that human kiwifruit increased the resistance of DNA to oxidative damage induced by H₂O₂ (Collins *et al.*, 2001) [5]. Consumption of kiwi fruit per day reduce the systolic and diastolic blood pressure and also reduces the platelet aggregation (karlsen *et al.*, 2014) [6]. Kiwifruit extracts are analyzed for different biological activities which shows the cytotoxic activity (Noboru and Joseph 2002) [7].

Lemongrass (*Cymbopogon citratus*) is tall and aromatic grass which belongs to family Graminae (poaceae), genus Cymbopogon and species citratus, Cymbopogon has about 55 species. Their leaves are arising from rhizomatous root stock and can grow up to 1.8m in height and 1.2m in width (Shah *et al.*, 2011) [8]. Lemongrass Leaves, stem and root are also abundant source of essential vitamins such as vitamin B₅, vitamin B₆ and vitamin B₁. Fresh herb contains small amounts of anti-oxidant vitamins such as vitamin C and vitamin A (Carlin *et al.*, 1986) [9].

Lemongrass contains several bio-compounds in its decoction, infusion and essential oil extracts. Anti-oxidant, anti-inflammatory, anti-bacterial, anti-obesity, anti-nociceptive, anxiolytic and antihypertensive supports pharmacological claims (Olorunnisola *et al.*, 2012) [10]. Lemongrass folk medicine associated with health claims such as treatment in coughs, constipation, elephantiasis flu, gingivitis, headache leprosy, malaria, ophthalmia, pneumonia, vascular disorders, diarrhoea and stomach ache. It has been claimed to be anti-inflammatory,

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vasorelaxing, diuretic, remedy in treating ringworm infestation, for nervous, gastrointestinal disturbances, fevers and hypertension (Nambiar and Matela 2010) [11].

In view of above facts and related nutraceutical profile of lemongrass and kiwifruit blend of these two is used to formulate the beverage. The present research study was carried out to develop the standardize the recipe and process for preparation for RTS beverage of kiwifruit incorporated with lemongrass with its study of storage study and chemical composition and effect of storage temperature on its physicochemical parameters such as TSS, pH, titrable acidity, reducing sugar, total sugar, ascorbic acid and total phenolic content.

Materials and Methods

Materials

The fresh kiwi fruits were obtained from local market, Parbhani and lemongrass was obtained from college garden. The proposed research was carried out in Department of Food Engineering, College of Food Technology, VNMKV, Parbhani.

Methods

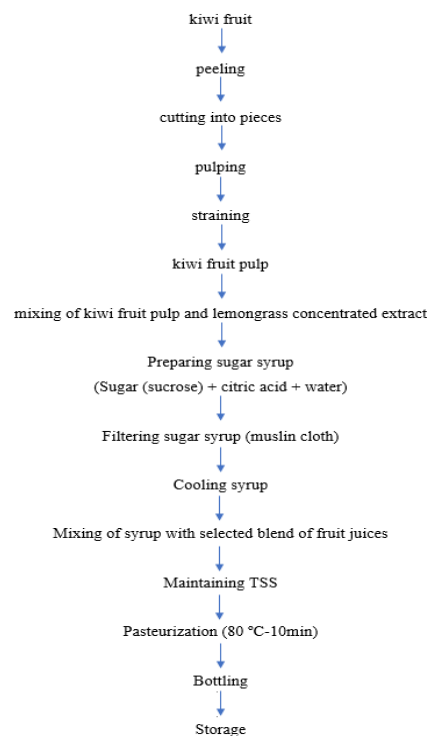
Preparation of kiwi fruit RTS incorporated with lemongrass

Lemongrass leaves aqueous extract is prepared by decoction, obtained extract is clarified, centrifuged and concentrated in rotary vacuum concentrator. Prepared concentrate is incorporated in the kiwi fruit Ready-to-serve beverage as per the FSSAI specifications. The formulation was prepared by blending the kiwi fruit pulp and lemongrass concentrated extract T₁ (10:0), T₁ (10:1), T₂ (10:2) and T₃ (10:3). For avoiding the stabilization in Prepared RTS beverage guar gum at different concentration is added and final sample was selected by sensory evaluation. Efforts were made to study its chemical composition and effect of storage temperature on its physicochemical parameters such as TSS, pH, titrable acidity, reducing sugar, total sugar, ascorbic acid and total phenolic content were analyzed. And also its therotical energy value was determined.

Table 1: Formulation of recipe for kiwi fruit RTS incorporated with lemongrass.

| Sr.no. | Ingredients | Quantity | | | |
|--------|-------------------------------------|----------------|----------------|----------------|----------------|
| | | T ₀ | T ₁ | T ₂ | T ₃ |
| 1. | Kiwi fruit pulp(ml) | 10 | 10 | 10 | 10 |
| 2. | Lemongrass concentrated extract(ml) | - | 1 | 2 | 3 |
| 3. | Sugar(g) | 12 | 12 | 12 | 12 |
| 4. | Citric acid(g) | 0.1 | 0.1 | 0.1 | 0.1 |

a. Flowsheet for preparation of kiwi fruit RTS beverage incorporated with lemongrass.



Determination of Physico-chemical characteristics

- Sensory evaluation of product:** Samples were scored based on a nine point hedonic scale. Judges were asked to rate the product on 9 point Hedonic scale with corresponding descriptive terms ranging from 9 to 1 as 'like extremely' to 'dislike extremely' as recommended by (Ranganna, 2001) [12].
- Total Soluble Solid (TSS):** Total soluble solid (TSS) of juice was determined by digital hand refractometer of range 0-30°Bx. The reading was corrected to 20°C and the mean value was expressed as the per cent °Bx (AOAC, 2000) [13].
- pH:** The pH values were determined with the help of a digital pH meter after calibrating it with buffer solution of pH 4 and 7 (AOAC, 2000).
- Titratable Acidity:** Titratable acidity was estimated by titrating 5 ml aliquot of the sample against standard 0.1N sodium hydroxide solution using phenolphthalein as an indicator. The total titratable acidity was expressed as per cent citric acid present in 100 ml liquid sample (Ranganna, 2011). It was calculated by formula,

$$\text{Acidity (\%)} = \frac{\text{Titre value} \times \text{N of alkali} \times \text{Volume made up} \times \text{Equivalent weight. of acid} \times 100}{\text{Aliquot} \times \text{Volume of sample taken} \times 1000}$$

- Reducing sugar:** The reducing sugar content was determined by the method given by (Ranganna, 1986) using Fehling's A and Fehling's B solution.

Standardization of Fehling's Solution

Equal quantities (20 ml each) of Fehling's solution A and B were mixed in a 250 ml conical flask with 100 ml water. The mixed Fehling's solution was then titrated with standard glucose solution (1%) till blue colour just disappears. Content in the flask was then heated on a hot plate with wire gauge. When liquid begin to boil, 3 drops of methylene blue indicator were added without removing flask from hot plate. Then further titration is continued with glucose solution till the brick red colour is observed and dye colour is decolorized. The volume of glucose solution required to reduce the Fehling's solution was noted as titre value.

$$\text{Fehling's factor} = \frac{\text{Titre value of std. glucose solution} \times 2.5}{1000}$$

Preparation of sample 25 g of macerated sample was taken and homogenized with few quantity of distilled water and then transferred to 250 ml volumetric flask. The sample was neutralized with 0.1 N NaOH and decolorized by adding 2 ml lead acetate. After shaking, the sample was allowed to stand for 10 min. The excess lead was removed by adding potassium oxalate and final volume was made up to 250 ml with distilled water.

Assay

The neutralized and decolorized sample was filled in burette and titrated against mixture of Fehling's solution as did for standardization of Fehling's solution. The per cent reducing sugar present in sample was determined by using following formula.

$$\text{Reducing sugar (\%)} = \frac{\text{Fehling's factor} \times \text{Dilution of sample made} \times 10}{\text{Titre value of sample} \times \text{Wt. of sample} \times 100}$$

6. Total sugar

For the estimate of total sugars, the titrate obtained in the estimation of reducing sugars was used. An aliquot from the filtrate was taken. 10 ml of dilute HCl was added and the inversion was carried out at room temperature for 24 h. Subsequently, contents were cooled and neutralized with 40% sodium hydroxide solution using phenolphthalein as indicator and the final volume was made.

The solution was filtered and titration was carried out using filtrate as detailed for reducing sugars. The total sugars content was expressed as percentage in terms of invert sugars according to the formula (Ranganna, 1986).

$$\text{Total sugar (\%)} = \frac{\text{Glucose equivalent} \times \text{Total vol. made up} \times \text{Vol. made after inversion}}{\text{Titre} \times \text{weight of sample} \times \text{aliquot taken for inversion}} \times 100$$

7. Ascorbic acid content

The ascorbic acid content was determined by blending a known volume of sample with 3 per cent meta phosphoric acid. After macerating the content were made up to a known volume in a volumetric flask with 3% meta phosphoric acid and filtered. A known amount of filtered aliquot was titrated against standard 2, 6dichlorophenol indophenols solution to a light pink colour which persisted for 15 seconds (AOAC, 2005) [14].

$$\text{Ascorbic acid (mg/100 g)} = \frac{\text{Titration reading} \times \text{Dye factor} \times \text{Volume made up}}{\text{Aliquot of extract} \times \text{Volume of sample taken}} \times 100$$

8. Determination of total phenolic content

Total phenolic contents (TPC) from the extracts were

quantified using Folin– Ciocalteu's method (Pinelo *et al.*, 2005) [15]. First, 5 ml Folin –Ciocalteu reagent was added to 1 ml sample in tube. Then, 4 ml of 7.5% (w/v) sodium carbonate was added to mixture. After 60 min of incubation at room temperature (32±1 °C), the absorbance was read at 765 nm against blank. The results were expressed as mg gallic acid equivalent per gram dry weight basis of fresh sample (mg GAE/g dw basis). The total phenolic contents in sample was calculated using following formula,

$$C = c V/m$$

Where

C = total phenolic content in sample (mg GAE/g dw basis)

c = concentration of gallic acid from standard calibration curve (mg/mL)

V = volume of extract (ml)

m = mass of extract (g)

9. Measurement of theoretical energy value

Energy value is determined theoretically by using values of crude protein, crude fat and total sugar content of sample and considering that 1 g of protein yields 4 Kcal energy, 1 g of fat yields 9 Kcal energy and 1 g carbohydrates yields 4 Kcal energy (Gopalan *et al.*, 2004) [16]. Total theoretical energy value was determined by calculating the described energy values for carbohydrates, protein and fat which gives energy value.

10. Microbial analysis of prepared RTS beverage

Microbial analysis is the perfect quality assessment protocol performed in food products. Microbial deterioration and possibility of the growth of pathogenic organism on the product was high hence it poses the highest food safety risk. The results obtained for each count was recorded as colony forming unit per ml of sample i.e. cfu/ml.

(a) Total plate count

Microbial analysis was done to determine total plate count (TPC) of the samples on the nutrient agar media for bacterial count by the method recommended by Harrigan and McCance (1966) [17]. Nutrient agar media was prepared and the samples were serially diluted up to 10⁻⁵ dilution factor. 0.25 ml of the samples, suspended in saline solution, was transferred to the respective Petri dishes of nutrient agar media. Three replicates were taken for each dilution. The inoculated petri dishes were incubated for 48 hours at 37+1 °C and total colonies were calculated by the following formula.

$$\text{TPC (cfu/ml)} = \frac{\text{No. of colonies} \times \text{dilution factor}}{0.25}$$

(b) Yeast and mould count

Microbial analysis was done to determine total yeast and mould count of the samples on the potato dextrose agar media for yeast and mould count by the method recommended by Harrigan and McCance (1966). Potato dextrose agar media was prepared and the samples were serially diluted up to 10⁻⁵ dilution factor. 0.25 ml of the samples, suspended in saline solution, was transferred to the respective petri dishes of potato dextrose agar media. Three replicates were taken for each dilution. The inoculated petri dishes were incubated in a incubator for 48 hours at 37+1°C for counting of yeast and mould.

(c) Coliform count

The Coliform basically E. coli are the indicator microbes of

water contamination by faeces and therefore it is mandatory to examine the contamination. The Coliform gives red pink colonies on Violet Red Bile (VRB) agar during analysis. Using the pour-plate technique, appropriately 0.1 ml aliquots was taken in duplicate plates and tempered VRB agar was added. The agar was allowed to solidify and then overlay of about 5 ml of VRB agar was added. Allow agar to solidify. Plates were inverted and incubated at 35°C for 24 hours. Red

colonies surrounded by a zone of precipitate and report as presumptive coli forms cfu/ml.

Result and Discussion

1. Sensory evaluation of kiwi fruit RTS incorporated with lemongrass: Sensory attributes such as colour, appearance, flavour, taste, mouthfeel and overall acceptability were evaluated using 9 point headonic scale results are presented in table.

Table 2: The sensory evaluation of kiwi fruit RTS incorporated with lemongrass

| Sr. no. | Samples | Sensory attributes | | | | | |
|---------|----------------|--------------------|------------|---------|---------|------------|-----------------------|
| | | Colour | Appearance | Flavour | Taste | Mouth feel | Overall acceptability |
| 1. | T ₀ | 8.8 | 8.9 | 8.4 | 8.3 | 8.8 | 8.6 |
| 2. | T ₁ | 8.6 | 8.3 | 8.7 | 8.2 | 7.9 | 7.8 |
| 3. | T ₂ | 8.7 | 8.2 | 8.2 | 8.7 | 8.9 | 8.5 |
| 4. | T ₃ | 8.3 | 7.8 | 8.3 | 7.9 | 7.7 | 8.0 |
| SE ± | | 0.06971 | 0.04988 | 0.079 | 0.03468 | 0.03361 | 0.03328 |
| CD @ 5% | | 0.20984 | 0.15014 | 0.23781 | 0.1044 | 0.10118 | 0.10018 |

Sensory evaluation of the samples revealed that, all the samples were found to be acceptable. Sample T₂ got highest score (8.5) followed by T₃ (8.0) after the T₀ control sample (8.6). The sample T₂ containing 2 per cent concentrated lemongrass extract was found to be statistically significant

over sample T₃ containing 3% concentrated lemongrass extract. Considering all the above parameters the selected sample (T₂) was found to be statistically significant over the all samples.

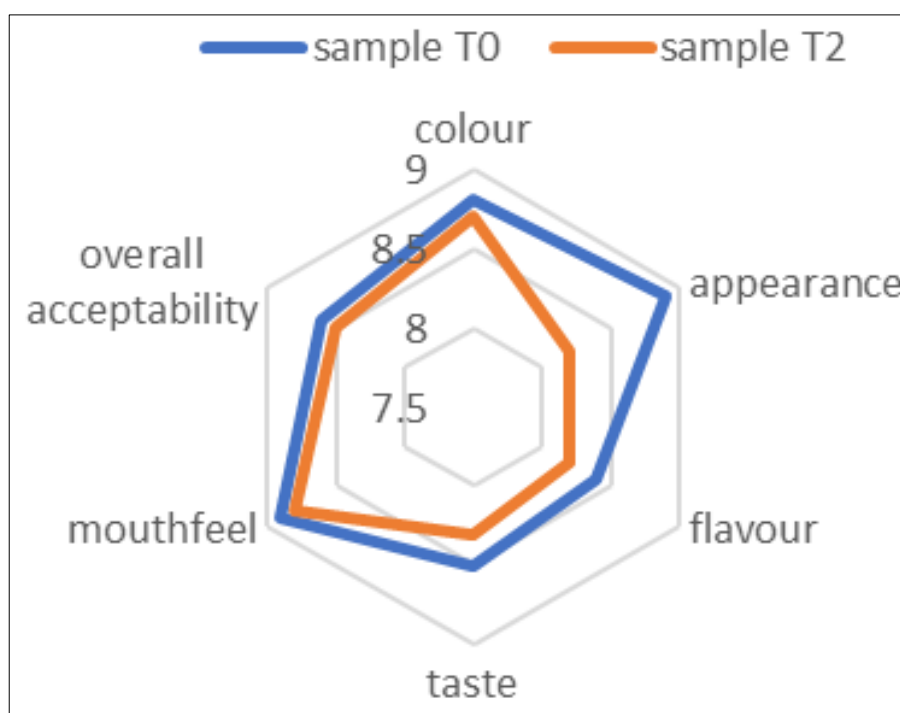


Fig 1: Sensory evaluation of RTS with different concentration of lemongrass

2. Effect of addition of guar gum on sensory evaluation of selected sample: Guar gum is added as stabilizer in RTS beverage to avoid the stabilization. Effect of guar

gum at different concentration on the sensory parameters of selected sample (T₂) were evaluated using 9 point headonic scale. Results recorded in the following table.

Table 3: Effect of addition of guar gum on sensory evaluation of selected sample (T₂)

| Sr. no. | Samples | Sensory attributes | | | | | |
|---------|---------|--------------------|------------|---------|---------|------------|-----------------------|
| | | Colour | Appearance | Flavor | Taste | Mouth feel | Overall acceptability |
| 1. | O | 8.7 | 8.2 | 8.2 | 8.7 | 8.9 | 8.5 |
| 2. | A | 7.5 | 7.9 | 7.9 | 8.0 | 8.5 | 7.9 |
| 3. | B | 8.5 | 8.1 | 8.1 | 8.3 | 8.7 | 8.3 |
| 4. | C | 7.6 | 7.3 | 7.3 | 7.3 | 7.2 | 7.3 |
| SE ± | | 0.03294 | 0.03191 | 0.03219 | 0.03479 | 0.03399 | 0.06332 |
| CD @ 5% | | 0.09917 | 0.09607 | 0.0969 | 0.10472 | 0.10233 | 0.1906 |

*Each value is an average of three determinations

From the table it was investigated that increase in concentration of stabilizer i.e. guar gum affects the sensory parameters of the RTS beverage. Taste and flavour were majorly affected *viz* as it gives slight starchy taste and flavour to the beverage. It was also observed that, it affect the colour of the beverage to give slight whitish colour with the addition of guar gum.

The Sample (B) with 0.2 per cent of guar gum and 2 per cent of lemongrass concentrated extract statistically significant over sample C containing 0.3 per cent guar gum. Considering all the above parameters the sample (B) was found to be statistically significant over the all samples.

On the basis of the sensory evaluation Sample B with 0.2 per cent guar gum has been selected for the further chemical and microbial analysis.

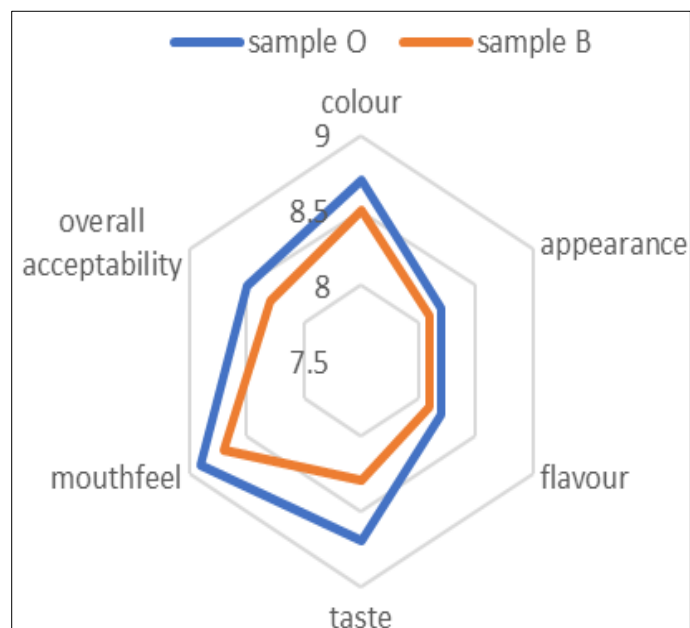


Fig 2: Sensory evaluation of selected RTS beverage with different concentration of guar gum

3. Chemical composition of RTS beverage

TSS, pH, titrable acidity, total sugar, reducing sugar, ascorbic acid and phenolic content was analysed. Effect of lemongrass extract on chemical composition RTS beverage were studied and compared with the control sample. Results were recorded in the below table.

Table 4: Chemical composition of RTS beverage (B)

| Sr. no. | Chemical parameters | Results For final sample (B) |
|---------|-------------------------------------|------------------------------|
| 1. | TSS ($^{\circ}$ Bx) | 12.8 |
| 2. | pH | 3.2 |
| 3. | Titrable Acidity (%) | 0.384 |
| 4. | Total sugar (%) | 8.2 |
| 5. | Reducing sugar (%) | 6.4 |
| 6. | Ascorbic acid (mg/100ml) | 14.6 |
| 7. | Phenolic content mg (mg GAE/100 ml) | 102.9 |

*Each value is an average of three determinations

Results for the kiwi fruit – lemongrass RTS beverage from the above table TSS, pH and acidity found to be 12.8 Brix, 3.2 and 0.384 per cent respectively. kiwi fruit – lemongrass RTS beverage contained 8.2 per cent total sugars, while the reducing sugar contents were 6.4 per cent. Ascorbic acid (vitamin C) content is 14.6 mg/100ml which as an important nutrient that possesses antioxidant ability and provides the protection against free radicals Esteve *et al.* (2005) [18]. Phenolic content found to be 102.9 mg GAE/100ml.

4. Theoretical energy value of kiwi fruit-lemongrass RTS beverage

Table 5: Theoretical energy value of kiwi fruit-lemongrass RTS beverage

| Parameters | Values |
|------------------------------|---------------------|
| % Carbohydrates X 4 | 13.28 X 4 |
| % Protein X 4 | 0.0512 X 4 |
| % Fat X 9 | 0.0054 X 9 |
| Total energy (Kcal/100 ml) = | 53.37 (Kcal/100 ml) |

Results obtained by energy value calculation showed that kiwi fruit - lemongrass RTS beverage provide 53.37 (Kcal/100 ml). It is interesting to be mentioned it is a refreshing drink and also gives an additional benefits due to the presence of ascorbic acid and phenolic compounds due to the incorporation of lemongrass concentrated extract in kiwi fruit RTS.

5. Microbial analysis of kiwi fruit- lemongrass RTS beverage: The prepared kiwi fruit – lemongrass RTS beverage bottles were pasteurized and microbial examination of was carried out with respect to microbial load (TPC, cfu/ml), yeast and mould count and coliform count at the intervals of 30 days upto three months storage period. The results obtained are recorded in below table.

Table 6: Microbial analysis of selected RTS beverage (B) stored at refrigeration (4 $^{\circ}$ C) and at ambient temperature (27 $^{\circ}$ C)

| Storage period (days) | Room temperature (27 $^{\circ}$ C) | | | Refrigeration temperature (4 $^{\circ}$ C) | | |
|-----------------------|---|--|--|--|--|--|
| | Total plate count (cfu/ml $\times 10^3$) | Yeast and Mold (cfu/ml $\times 10^3$) | Coliform count (cfu/ml $\times 10^3$) | Total plate count (cfu/ml $\times 10^3$) | Yeast and Mold (cfu/ml $\times 10^3$) | Coliform count (cfu/ml $\times 10^3$) |
| 0 | ND | ND | ND | ND | ND | ND |
| 30 | 7 | ND | ND | 4 | ND | ND |
| 60 | 10 | ND | ND | 7 | ND | ND |
| 90 | 12 | 2 | ND | 9 | ND | ND |

It can be revealed from the above table that, microbial load of sample stored at ambient temperature (27 $^{\circ}$ C) was increased continuously with increasing time of storage as compared to storage at refrigerated temperature. The result found to similar with result reported by Afreen *et al.*, (2016) [19]. After 90 days of storage, the total plate count, yeast and mold count were 12 cfu/ml and 2 cfu/ml respectively at the room temperature. The total plate count was 9 cfu/ml at refrigeration temperature on

90th day of the storage whereas the yeast and mould were not detected. Also, coliform count was not detected in case of both room temperature and refrigeration temperature throughout the 90 days storage.

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