

Nutritional and Compositional Changes in α -Tomatine Rich Ready-To-Serve Beverage from Matured Green Tomato (*Solanum lycopersicum*)

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ABSTRACT: The study aimed to develop and evaluate the storage stability of α -Tomatine rich ready-to-serve (RTS) beverages from green tomato juice at low temperatures (LT, 4 \pm 1 $^{\circ}$ C) up to 90 days. The results indicated the α -tomatine content in green tomato juice and RTS beverage was found to be 43.23 \pm 3.59 mg/100 mL and 3.94 \pm 0.21 mg/100 mL, respectively. The stability of the product stored in a Brown glass bottle (BB) container was excellent with better retention of α -Tomatine (2.31 \pm 0.29 mg/100 mL), total phenolic content (1.42 mg GAE/100 mL), ascorbic acid (17.66 mg/100 mL), total chlorophyll (1.07 mg/100mL) and viscosity (15.46 cp), with high sensory scores (7.6) as compared to the quality of the product (with 6.8 sensory scores) stored in a white glass bottle (WB) container. The microbial counts of products stored in both containers after 90 days are indicated within the permissible limit.

Keywords: α -tomatine; chlorophyll; green tomato juice; RTS Beverage; storage stability; overall acceptability

INTRODUCTION

Tomato is the world's second-largest vegetable cultivated after potato, and it tops the list of canned vegetables. The previous studies showed that tomatoes are a rich source of vitamins, carotenoids, and antioxidants (ascorbic acid, α -carotene, chlorogenic acid, rutin, plastoquinones, tocopherol, and xanthophylls). Tomatoes are also rich in carbohydrates, proteins, fiber content, flavor compounds, calystegines, glycoalkaloids (tomatine and dehydrotomatine), and minerals (iron, phosphorus, copper, iron, chromium, and potassium) (Kozukue and Friedman, 2003; Dzyadevych et al., 2004). Tomatine, a glycoalkaloid, is a mixture of α -tomatine and dehydrotomatine with health benefits, mainly anti-carcinogenic (Lee et al., 2011) and anti-inflammatory in humans and antifungal and antimicrobial agent in the plants. It is present in high quantities in immature green tomatoes and degrades during ripening state.

Tomatine can help prevent all types of cancer (Lee et al., 2004), inhibiting prostate cancer cells (Lee et al., 2011; Friedman, 2013). Monotherapy with α -tomatine had a significant dose-dependent anticancer effect, which peaked at 1 mg/kg (Friedman et al., 2009). Tomatine is a potent inhibitor of both human colon and liver cancer cell lines (Itkin et al., 2013). Earlier reports suggest that tomatine is dominant in the green stage, and as the tomato fruit starts to mature, the red lycopene will show an increment and simultaneous decrease in tomatine content. Therefore, green tomatoes have high tomatine content (as high as 500 mg/kg), while red tomatoes are just around 5 mg/kg (Friedman, 2013).

So far, the researchers have not indicated developing a product enriched with the benefit of α -tomatine. Fruit beverages are one of the most popular categories of beverages that are consumed all over the world. These drinks are easily digestible, very refreshing that quench the thirst, and are appetizing with high nutrition properties. So these drinks are far more beneficial than most synthetic and carbonated beverages (Sharma et al., 2012). Fruit juices and

drinks are the most commonly processed food products appropriately used and preferred by all age group buyers. They have immense scope to meet the daily requirement of nutrients in a healthy diet (FSSAI, 2017). Consumers are now progressively shifting towards the drinking of natural fruit juice-based liquid refreshments due to medicinal importance (FSSAI, 2017).

Tomatine alone or tomatine-rich green tomato products could be beneficial for humans to treat various diseases reported above. Therefore, the present study aims to develop an α -tomatine rich ready-to-serve (RTS) beverage using matured green tomatoes. The formulated α -tomatine RTS will be evaluated for quality parameters in terms of physicochemical, microbiological, and sensory quality attributes under the influence of low-temperature storage conditions and types of storage containers.

MATERIALS AND METHODS

Chemicals

All chemicals and reagents used were of analytical grade and purchased from SRL Pvt. Ltd, Bengaluru, India. The standard α -Tomatine was purchased from T.C.I. (Japan). Methanol and Acetonitrile were of HPLC grade obtained from Merck (Darmstadt, Germany). Distilled water (Milford, MA) and Sep-Pak C.L.S. environmental cartridges from WATERS.

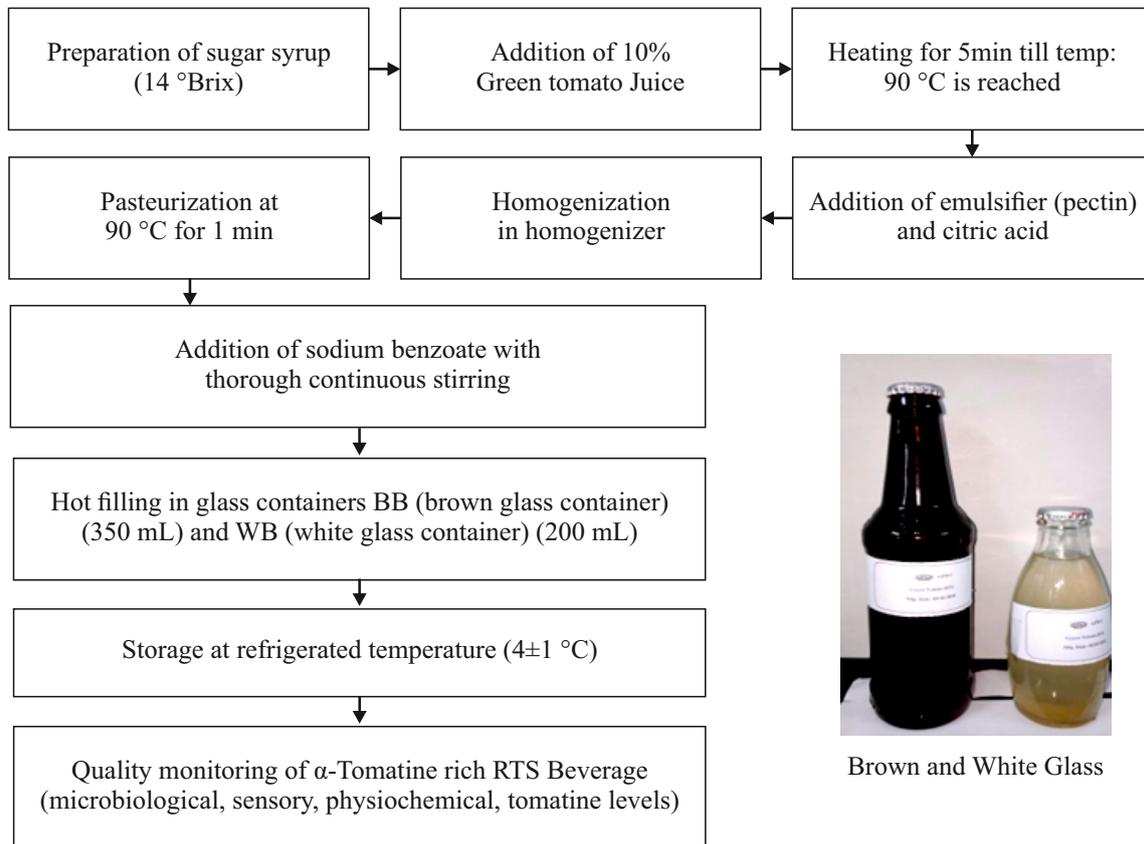
Sample Collection

The matured green tomato fruits (4-6 $^{\circ}$ Brix, 0.90 % acidity) were procured from Mysore (India), transported in corrugated fiber boxes, and overnight kept in a cold room to eliminate the field heat. The fruits were water washed, air-dried, and pulped using a power-driven pulper and juice extracted. This juice was then homogenized using a homogenizer (JM60B, Serial No: 504031, JM Series Colloid Miller, CHINA), and the same was used for the development of α -Tomatine rich ready to serve beverage.

Fruit pulping and preparation of RTS beverage formulation

The RTS beverage formulated as per the flowchart shown in Figure 1 with 10 % homogenized green tomato juice. The green tomato juice was obtained through a series of primary processing equipment (such as fruit miller, fruit pulper, and homogenizer). The TSS and % acidity of tomato juice were determined. The required quantity of sugar and citric acid

was added to adjust TSS (14 °Brix) and the acidity (0.3 %). The LMP (Low methoxy pectin) pectin (0.15 % w/w) was added to the above mix and pasteurized at 90 °C (holding time, 1 min). The sodium benzoate (120 ppm) as a preservative was added. Beverages were cooled down to 70 °C and immediately hot-filled in previously sterilized glass containers [BB (350 mL) and WB (200 mL)] and capped with presterilized lids to ensure the safety of the product.



Brown and White Glass

Figure 1. Flow chart showing the process of preparation of α-Tomatine rich RTS-beverage

Physicochemical Analysis

Color measurement

The color measurement was carried out in triplicate using a colorimeter (Shimadzu, Model: UV 2100). The hunter color values (L*, a* and b*) were measured where L* indicates brightness, a relates to greenness (-)/redness (+), and b* resembles blueness (-)/yellowness (+). The hue angle and chroma values were calculated by using L* a* and b* values based on the following formulae:

$$\text{Hue angle} = \text{TAN}^{-1} b/a \tag{1}$$

$$\text{Chroma value} = (a^2 + b^2)^{1/2} \tag{2}$$

Total soluble solids (TSS, °Brix) and moisture content

The TSS of the pulp and the product of green tomato were taken in triplicates. They were measured using a digital refractometer (Model: Hanna instruments-refractometer, Romania) and were expressed as °Brix. All observations were carried out in triplicates.

The moisture content of the pulp and the samples were determined by using a digital moisture analyzer (Model: IR-35, Denver Instrument, Germany) at 110±2 °C. Sample (2-3 g) was exposed to the IR RAYS, and the analysis was completed in 35-40 minutes and expressed in percentages.

pH and titratable acidity (%)

The pH of the sample was recorded using a pH meter (EUTECH Instruments-pH Tutor, Singapore). The calibrated electrode of the pH meter was inserted into the beaker with a 30 mL sample. The reading was taken thrice for each sample. The acidity of samples was estimated using 0.01N NaOH as per the protocol of Ranganna et al. (1999).

Sugars Estimation

The sugar contents in samples were determined as per the standard protocols of Lane and Eynon (AOAC, 1990) as reducing sugars (% RS), non-reducing sugars (% NRS), and total invert sugars (% IS).

Ascorbic acid content

The ascorbic acid content was determined using the titrimetric method as per AOAC (1995), with minor modification. Briefly, the sample (10 mL) was mixed with 4% oxalic acid (90 mL) solution and filtered. Further, the filtrate was diluted (1:3) with a 4% oxalic acid and titrated against 2, 6-dichlorophenolindophenol dye (0.02%) till endpoint (light pink color) persists for 15 s. The dye factor was calculated using 0.05% ascorbic acid solution. The ascorbic acid content was calculated as per the following formula:

Ascorbic acid (mg/100g) = $(0.5 / V_1) \times (V_2 / 15 \text{ mL}) \times (100 \text{ mL} / \text{Sample weight}) \times 100$

V_1, V_2 = Volumes of dye used for standard and samples, respectively.

Total Phenolic Content

The total phenolic content determination was done as per the modified protocol of Singleton et al. (1965). The sample (10 mL) was mixed with 80% ethanol (40 mL), concentrated using a rotary evaporator at 45°C, and extracted with ethyl acetate (10 mL, thrice). The extract was pooled, concentrated, and redissolved in 10 mL methanol. In a glass tube, the extracted sample (100 μ L) and various concentrations of the standard were mixed with distilled water (2.9 mL), and a Folin-Ciocalteu reagent (500 μ L) was added. The samples were incubated for 3 min at room temperature. Further 2 mL of Na_2CO_3 (20%) solution was added, and kept in the dark for 30 min. The absorbance of samples was taken at 765 nm with the UV-visible spectrophotometer (UV-1601, Shimadzu, Japan). A calibration curve of gallic acid was used for total phenolic calculation and reported as mg of gallic acid equivalent (GAE) per 100 mL sample.

Determination of antioxidant activity

The samples were evaluated for scavenging ability using 1,1-diphenyl-2-picrylhydrazine (DPPH) radical. The DPPH radical scavenging antioxidant capacity assay was determined using a previously reported method (Gulcin et al., 2007). The equal volume of diluted sample and DPPH (0.1 mM) solution were mixed and kept in darkness for 30 minutes to complete the reaction. The antioxidant activity was expressed as percentage inhibition.

Estimation of α -Tomatine estimation by HPLC method

The α -Tomatine was determined by a modified method by Meher and Gaur (2003). Fresh tomatoes (100 g) were homogenized and extracted with 100 mL of methanol (thrice) using mortar and pestle. The extract was pooled and centrifuged at 2400 g for 10 min, and the supernatant was collected. The pellet was re-suspended in methanol and centrifuged again. Then, the combined supernatants were filtered through a filter, and the filtrate was adjusted to 40% methanol (v/v) with water. Subsequently, the solution was applied to the preconditioned Sep-Pak cartridge. The cartridge was washed with 10 mL of 40% methanol, and α -tomatine was extracted with 25 mL of 80% methanol. In the case of beverages, the extract was concentrated and injected. All steps were carried out at room temperature. The 10 μ L eluent was directly injected into the HPLC system. A mixture of 20 mM potassium dihydrogen phosphate (KH_2PO_4) solution in acetonitrile (25:75 v/v, pH 6.11) was used as an isocratic mobile phase for chromatographic separation. The flow rate was 1.0 mL/min, and the column temperature was set at 40 °C. The α -tomatine was detected at 208 nm, and the concentration was determined based on peak area calculation from the standard.

Total mineral content

10 mL of sample was added to preweighed silica crucible and concentrated on water bath till only solids remain. Then the sample was charred and placed in a muffle furnace at 550°C for 6-8 h till white ash is obtained. The mineral content was calculated by the following formula and expressed in percentage (%).

$$\% \text{Mineral Content} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100\%$$

Chlorophyll content

The Chlorophyll a, b, and total chlorophyll content was estimated as per the protocol of Ranganna (1999).

Viscosity determination

The viscosity is a critical constraint for adjudging the stability of beverages. The viscosity was measured using Brookfield viscometer (Model: RV DV-II+ Pro) with spindle (size- 2) at a speed of 100 rpm for a time period of 30 s. The readings were noted in centipoise (cp).

Microbiological Analysis

The beverage may get contaminated during the handling of raw material and processing steps. Therefore makes the finished product unfit for consumption. The microbiological analysis for prepared and stored beverages was carried out at systematic intervals by standard protocols suggested by APHA (1984).

Sensory Analysis

The sensory analysis of the samples was carried out during 90 days of LT storage (0, 15, 30, 45, 60, 75, and 90 days after storage) by trained panelists (n=15) using the 9-pointer hedonic scale. Each time, the products in six replicates (n=12) were evaluated for color, appearance, texture, flavor, taste, and overall acceptability. The numerical values were assigned to each point on the range where one indicates poor, and nine indicates excellent. These samples were randomly served to the panelists in white cups coded with three-digit random numbers. Each value of the sensory attributes data was presented as the mean of twelve observations recorded by all panelists.

Storage stability and Shelf-life extension Studies

RTS beverages were kept at refrigerated temperature (LT, 4 \pm 1 °C) for storage study. The quality of RTS beverages was monitored periodically (0, 15, 30, 45, 60, 75, and 90 days) for evaluation of product stability during 90 days of storage. The microbiological safety, sensory acceptability, physicochemical changes, and α -Tomatine levels were checked at the above regular intervals. The shelf life of the products were determined based on permissible limits of microbiological and the scores of all the sensory attributes.

Statistical Analysis

All experiments were analyzed in triplicate (n=3). The statistical analysis was implemented using SPSS (16.0) software, using analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) with a 95% confidence level (p <0.05).

RESULT AND DISCUSSION

Physicochemical characteristics of fruit juice

The results of the physicochemical characteristics of the green tomato juice (Table 1). The juice had a TSS of 4°Brix, and highly acidic (0.91 %). The total phenolic content, the ascorbic acid content, and the total sugars of juice were found to be 9 mg GAE/100 mL, 1.87 mg/100g, and 3.12 %, respectively. The juice of green tomato had α -Tomatine content of 0.40 \pm 0.4 mg per 100 mL. Based on the above results and the aim of the study, α -Tomatine rich RTS beverage was formulated as per the process flow chart (Figure 1) and aseptically hot filled in two types of glass containers (BB and WB) and stored at LT (4 \pm 2°C) condition, for physicochemical, microbiological, and sensory quality evaluation.

Table 1. Initial physicochemical quality attributes of the green tomato fruit juice

Parameters	Values
Hue angle (°)	15.00±0.05
Chroma value	48.00±0.05
Penetration force(N)	12.91±0.12
TSS (°Brix)	4.01±0.03
Moisture (%)	77.05±0.24
pH	4.21±0.014
Titration acidity (%)	0.91±0.03
Ascorbic acid (mg/100 g)	1.88±0.23
Total sugars (%)	3.12±0.02
Reducing sugars (%)	1.26±0.05
Sucrose (%)	2.39±0.04
Non-reducing sugars (%)	2.80±0.02
Total phenolics (mg GAE/100 mL)	9.00±0.53
α-Tomatine (mg/100 mL)	3.94±0.21
Sugar to acid ratio	31.57±0.02
Total mineral content (mg/100g)	279.00±0.13

Note: All the values are Mean±SD (n = 3)

Physical quality attributes of α-Tomatine rich ready to serve beverages

Changes in color

The color of beverages during storage (Table 2) showed significant ($p \leq 0.05$) changes in L^* values during 90 days of storage in BB ($4 \pm 2^\circ\text{C}$). The BB samples were found slightly darker in appearance than the WB samples regarding lightness (L^*). The a^* values also displayed a similar tendency. There was a trivial but significant increase in b^* values in BB samples, and the intensity of green color decreased on prolonged storage. In contrast, WB samples showed a decrease in chroma value indicates a decline in greenness. The color variation in WB stored samples might be due to the photo-oxidation or enzymatic degradation of chlorophylls during storage (Sindhupati and Prem Latha, 2013). The differences in the color values in WB samples were probably affected by low polyphenolic contents (Porto et al., 2017). The other reason could be the condensation and pigments destruction by Maillard reaction or melanoidins formation that causes deterioration in color (da Costa et al., 2017).

Changes in viscosity

The BB samples exhibited a slight increase in viscosity (Table 2) compared to WB stored samples. The increase in viscosity could be due to the interactions of various molecules like emulsifier (pectin), acidulent (citric acid), sugar, and liquid phase of the product. The hydrogen bonds between hydroxyl groups of solutes could also be accountable for an increase in product viscosity and important for the level of viscosity. The pectin, citric acid, and sugar might have a role in modifying the green tomato RTS beverage rheology during storage and preventing sedimentation (Kaul et al., 2009).

Chemical quality attributes of α-Tomatine rich ready to serve beverages

Changes in total soluble solids

The results showed significant changes in TSS for WB stored samples, while BB samples had a slight increase in TSS (Table 2). The increase in TSS of WB samples could be due to the polysaccharide hydrolysis into simple sugars and other constituents (Sindhupati & Bhardwaj et al., 2011). The increase in TSS could also be due to photolysis of chlorophyll and degradation of ascorbic acid. A similar trend in TSS was also observed in our previous report on cape gooseberry RTS drinks during storage (Hemalata et al., 2018) and mix formulation of cape gooseberry, sweet lime, amla, and ginger (Lokesh and Sangma, 2017).

Changes in pH

The pH of products is directed by inherent as well as added organic acids during the product preparation. The acid content of mature green tomatoes and added citric acid in RTS preparation helps enhance the flavor and preservation of the product. The decrease in pH was significant ($p \leq 0.05$) for both BB and WB stored samples (Table 2). The WB samples recorded lower pH than the BB during storage could be due to photolytic degradation, chemical and enzymatic changes in the beverage. This reduction in the pH affected the condensation of pectin molecules. It further reduced the hydrophilic portions and increased the tendency for gel formation like a network in the liquid phase, enhancing the viscosity of beverages (Yadav et al., 2010).

Table 2. Changes in Physico-chemical quality attributes of α -tomatine rich RTS beverage during Storage at LT (4 \pm 2 $^{\circ}$ C).

Parameters	Sample code	Storage period in days						
		0	15	30	45	60	75	90
Color Hue angle ($^{\circ}$)	BB	88.14 \pm 0.05 ^a	88.28 \pm 0.28 ^b	88.33 \pm 0.01 ^c	88.36 \pm 0.05 ^d	89.38 \pm 0.01 ^d	89.41 \pm 0.01 ^e	89.46 \pm 0.02 ^f
	WB	87.14 \pm 0.05 ^a	87.28 \pm 0.28 ^b	87.33 \pm 0.01 ^c	87.36 \pm 0.05 ^{cd}	86.38 \pm 0.01 ^d	86.41 \pm 0.01 ^d	86.46 \pm 0.02 ^f
Chroma value	BB	9.32 \pm 0.05 ^a	9.06 \pm 0.05 ^b	9.05 \pm 0.15 ^c	8.82 \pm 0.23 ^d	8.69 \pm 0.17 ^e	8.59 \pm 0.06 ^f	8.50 \pm 0.11 ^g
	WB	8.61 \pm 0.04 ^a	8.43 \pm 0.00 ^b	8.23 \pm 0.23 ^c	7.73 \pm 0.10 ^c	7.57 \pm 0.17 ^d	7.29 \pm 0.10 ^d	6.56 \pm 0.19 ^e
TSS ($^{\circ}$ Brix)	BB	13.62 \pm 0.01 ^a	13.69 \pm 0.02 ^c	13.75 \pm 0.05 ^c	13.85 \pm 0.03 ^c	13.93 \pm 0.11 ^c	14.03 \pm 0.17 ^a	14.12 \pm 0.17 ^a
	WB	13.6 \pm 0.09 ^a	13.8 \pm 0.05 ^c	13.9 \pm 0.16 ^c	14.01 \pm 0.06 ^c	14.31 \pm 0.01 ^c	14.48 \pm 0.02 ^a	14.6 \pm 0.10 ^a
pH	BB	3.01 \pm 0.01 ^a	2.88 \pm 0.03 ^c	2.69 \pm 0.01 ^c	2.64 \pm 0.01 ^c	2.64 \pm 0.06 ^{cb}	2.62 \pm 0.06 ^c	2.61 \pm 0.06 ^c
	WB	3.23 \pm 0.02 ^a	3.07 \pm 0.01 ^c	3.02 \pm 0.07 ^c	2.70 \pm 0.06 ^c	2.67 \pm 0.02 ^c	3.02 \pm 0.01 ^a	3.20 \pm 0.03 ^a
Titration acidity (%)	BB	0.33 \pm 0.01 ^a	0.31 \pm 0.02 ^a	0.29 \pm 0.01 ^c	0.27 \pm 0.05 ^c	0.28 \pm 0.02 ^c	0.27 \pm 0.01 ^c	0.26 \pm 0.02 ^c
	WB	0.34 \pm 0.02 ^a	0.33 \pm 0.03 ^c	0.32 \pm 0.04 ^c	0.31 \pm 0.02 ^c	0.67 \pm 0.01 ^c	0.25 \pm 0.01 ^a	0.24 \pm 0.03 ^a
Reducing sugar (%)	BB	0.97 \pm 0.02 ^e	1.06 \pm 0.01 ^f	2.26 \pm 0.02 ^e	2.57 \pm 0.01 ^d	2.93 \pm 0.02 ^c	3.41 \pm 0.03 ^b	3.82 \pm 0.04 ^a
	WB	0.98 \pm 0.05 ^e	1.24 \pm 0.10 ^f	1.63 \pm 0.03 ^c	2.02 \pm 0.02 ^d	2.65 \pm 0.06 ^c	3.93 \pm 0.06 ^b	4.43 \pm 0.05 ^a
Total invert sugar (%)	BB	9.55 \pm 0.05 ^a	9.47 \pm 0.06 ^a	9.13 \pm 0.01 ^b	9.11 \pm 0.10 ^b	8.77 \pm 0.06 ^c	8.60 \pm 0.04 ^d	8.53 \pm 0.03 ^d
	WB	9.55 \pm 0.05 ^a	9.31 \pm 0.01 ^b	9.04 \pm 0.07 ^c	8.92 \pm 0.06 ^c	8.67 \pm 0.08 ^d	8.21 \pm 0.03 ^e	7.40 \pm 0.13 ^f
Sucrose (%)	BB	8.15 \pm 0.03 ^a	7.98 \pm 0.63 ^b	6.52 \pm 0.01 ^c	6.28 \pm 0.14 ^c	5.54 \pm 0.04 ^c	4.93 \pm 0.10 ^f	4.45 \pm 0.06 ^g
	WB	8.15 \pm 0.04 ^a	7.67 \pm 0.09 ^b	7.04 \pm 0.06 ^c	6.54 \pm 0.07 ^d	5.72 \pm 0.15 ^e	4.07 \pm 0.05 ^f	2.80 \pm 0.17 ^g
Total sugar (%)	BB	10.51 \pm 0.06 ^c	10.53 \pm 0.05 ^c	11.39 \pm 0.01 ^d	11.62 \pm 0.05 ^c	11.70 \pm 0.10 ^f	12.01 \pm 0.03 ^b	12.34 \pm 0.34 ^a
	WB	10.52 \pm 0.05 ^f	10.54 \pm 0.11 ^f	10.67 \pm 0.09 ^e	10.95 \pm 0.05 ^d	11.31 \pm 0.30 ^f	12.13 \pm 0.08 ^b	12.50 \pm 0.03 ^a
Reducing sugar (%)	BB	0.97 \pm 0.02 ^e	1.06 \pm 0.01 ^f	2.26 \pm 0.02 ^e	2.57 \pm 0.01 ^d	2.93 \pm 0.02 ^c	3.41 \pm 0.03 ^b	3.82 \pm 0.04 ^a
	WB	0.98 \pm 0.05 ^e	1.24 \pm 0.10 ^f	1.63 \pm 0.03 ^c	2.02 \pm 0.02 ^d	2.65 \pm 0.06 ^c	3.93 \pm 0.06 ^b	4.43 \pm 0.05 ^a
Sugar : acid ratio	BB	30.27 \pm 1.48 ^c	31.75 \pm 1.33 ^c	38.19 \pm 0.03 ^d	41.16 \pm 0.40 ^c	42.92 \pm 0.3 ^c	44.73 \pm 0.63 ^b	46.99 \pm 0.32 ^a
	WB	33.21 \pm 0.212 ^c	32.84 \pm 0.69 ^c	32.89 \pm 0.03 ^{dc}	31.16 \pm 0.32 ^d	39.72 \pm 0.8 ^c	47.93 \pm 0.73 ^d	49.17 \pm 0.25 ^c
Chlorophyll a (mg/100 mL)	BB	4.80 \pm 0.09 ^a	4.78 \pm 0.09 ^a	4.73 \pm 0.15 ^a	4.42 \pm 0.30 ^b	3.43 \pm 0.22 ^c	2.98 \pm 0.09 ^d	2.98 \pm 0.20 ^d
	WB	4.81 \pm 0.03 ^a	4.85 \pm 0.54 ^a	4.43 \pm 0.31 ^a	3.71 \pm 0.16 ^b	3.05 \pm 0.33 ^c	2.26 \pm 0.08 ^d	0.32 \pm 0.18 ^e
Chlorophyll b (mg/100 mL)	BB	3.46 \pm 0.03 ^a	3.27 \pm 0.06 ^{ab}	3.03 \pm 0.05 ^b	2.22 \pm 0.34 ^c	2.10 \pm 0.05 ^c	1.91 \pm 0.09 ^d	1.52 \pm 0.16 ^e
	WB	3.44 \pm 0.03 ^a	2.58 \pm 0.54 ^b	2.21 \pm 0.35 ^{bc}	2.02 \pm 0.06 ^{cd}	1.72 \pm 0.15 ^{de}	1.33 \pm 0.13 ^e	0.24 \pm 0.05 ^f
Total chlorophyll (mg/100 mL)	BB	8.32 \pm 0.05 ^a	8.06 \pm 0.05 ^b	7.79 \pm 0.15 ^c	6.62 \pm 0.23 ^d	5.69 \pm 0.17 ^e	4.59 \pm 0.06 ^f	4.50 \pm 0.11 ^g
	WB	8.31 \pm 0.00 ^a	7.43 \pm 0.00 ^b	6.63 \pm 0.23 ^c	5.73 \pm 0.19 ^d	4.77 \pm 0.17 ^e	3.59 \pm 0.10 ^f	0.56 \pm 0.19 ^g
Viscosity (mPas ⁻¹)	BB	15.14 \pm 0.05 ^c	15.28 \pm 0.28 ^d	15.33 \pm 0.01 ^c	15.36 \pm 0.05 ^{cb}	15.38 \pm 0.01 ^b	15.41 \pm 0.01 ^b	15.46 \pm 0.02 ^a
	WB	15.10 \pm 0.02 ^c	15.20 \pm 0.2 ^d	15.33 \pm 0.02 ^d	15.46 \pm 0.05 ^c	15.48 \pm 0.01 ^b	15.51 \pm 0.01 ^a	15.56 \pm 0.02 ^a

Note: (n = 3; Mean \pm SD), values in rows with different superscripts (a–g) have significant differences at p<0.05 by DMRT Test

Changes in acidity

The acidity is considered an essential attribute of RTS beverage and the first preference for consumer acceptance. The analysis showed that the acidity of RTS beverages decreased with storage time (Table 2). The acidity has decreased progressively in BB (0.33 % - 0.26 %) and WB (0.34 % - 0.24 %) samples during storage. The changes might be due to the hydrolysis of the polysaccharides (Hemalata et al., 2018). In WB samples, the acidity declined up to 60 days, and then there was a considerable increase in acidity. Pawar et al. (2011) also reported a similar trend in their study to check the effect of heat processing on the quality characteristics of custard apple during storage.

Changes in ascorbic acid content

The ascorbic acid losses in beverages were detected in both BB and WB samples (Table 2). The BB samples showed retention of high ascorbic content when compared to WB. The degradation in WB stored RTS might be due to oxidation, mainly due to the presence of oxygen trapped and entry of light, which converts ascorbic acid into dehydroascorbic acid. The losses could be due to the presence of oxidizing enzymes, heat processing, and storage conditions (Nisar et al., 2015).

Changes in Sugar Content

The sugar analysis showed the reducing sugar (RS) was (p \leq 0.05) increased from 0.97 to 4.43 % in WB samples during long storage (Table 2). In comparison, the % RS in BB was increased from 0.98 to 3.82 % (p \leq 0.05). While the non-reducing sugar (NRS) significantly (p \leq 0.05) reduced regardless of storage temperature, and losses were perceived more in WB samples (9.55 to 7.4 %). The increase in

reducing sugar could be due to the conversion of NRS through the process of glucogenesis to reducing sugar (Jadhav et al., 2006) and also organic acid hydrolysis in the stored samples (Nidhi et al., 2008). The total reducing sugar (TRS) content was increased significantly (p \leq 0.05) during the storage (Table 2). This increase in TRS is possibly due to the hydrolysis of polysaccharides and disaccharides into soluble sugars and also could be due to starch hydrolysis in the presence of citric acid (Waskar and Gerande, 2003). Therefore the increase in RS content was observed in both WB and BB stored samples, irrespective of the storage condition.

Changes in chlorophyll pigments content

The chlorophyll content of the mature green tomato beverage showed a significant (p \leq 0.05) reduction in total chlorophyll content (Table 2). The samples BB had a decrease in total chlorophyll (8.28 to 4.50 mg/100 mL), chlorophyll-b (3.46 to 1.52 mg/100 mL), and chlorophyll-a (4.8 to 2.98 mg/100 mL) content. The high losses in chlorophyll content were found in WB samples. The total chlorophyll content diminished from 8.28 to 0.67 mg/100 mL, chlorophyll-b (3.46 to 0.24 mg/100 mL) and chlorophyll-a (4.8 to 0.32 mg/100 mL) in WB samples. This significant loss in total chlorophyll could be due to heat, photolytic reaction, acid degradations, oxidative reactions, and changes in pigments due to enzymatic destruction. The other reason for color degradation could be due to the epimerization of chlorophyll-a at room temperature and even faster during heating (Chen and Cheng, 1993; Wold et al., 2005).

Changes in total phenolic content and the antioxidant property

The total phenolics were gradually decreased in mature green tomato RTS beverage samples stored in BB and WB packaged samples during storage (Figure 2A). The BB samples showed high retention in total phenolic content (2.163 to 1.456 mg GAE/100 mL) than the WB samples (2.13 to 1.03 mg GAE/100 mL) throughout the storage. These changes showed that BB is a superior packaging bottle for long-duration storage of RTS beverages as it is opaque, and negligible light passes through it. The reduction in total phenolic content might also be due to photolytic and polymeric oxidation. Therefore, BB stored RTS had better retention of phenolics.

The decrease in phenolics accounts for a reduction of the antioxidant property of the RTS beverage (Figure 2B). No significant difference ($p \leq 0.05$) was observed in the antioxidant property of beverages stored in BB bottles

throughout the storage period. The decrease in the antioxidant property of juice in WB samples than in the BB might be due to photo-oxidation and losses of total phenolic content (Jakhar and Patal, 2012).

Changes in pH

The pH of products is directed by inherent as well as added organic acids during the product preparation. The acid content of mature green tomatoes and added citric acid in RTS preparation helps enhance the flavor and preservation of the product. The decrease in pH was significant ($p \leq 0.05$) for both BB and WB stored samples (Table 2). The WB samples recorded lower pH than the BB during storage could be due to photolytic degradation, chemical and enzymatic changes in the beverage. This reduction in the pH affected the condensation of pectin molecules. It further reduced the hydrophilic portions and increased the tendency for gel formation like a network in the liquid phase, enhancing the viscosity of beverages (Yadav et al., 2010).

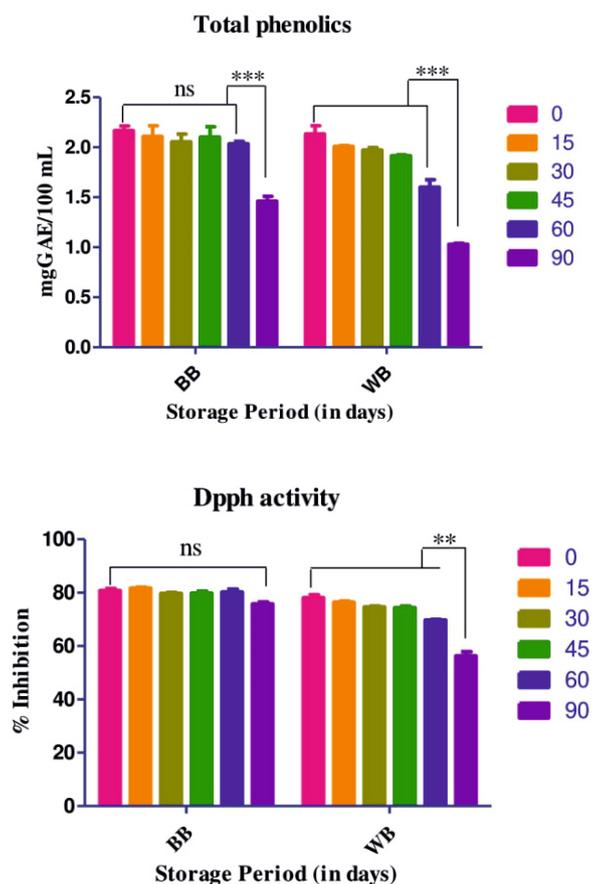


Figure 2A. Changes in total phenolic content and 2B. Changes in antioxidant property of α -Tomatine rich RTS stored in different containers at LT ($4 \pm 2^\circ\text{C}$) condition.

Changes in α -Tomatine content of R.T.S. beverage during storage at L.T. ($4 \pm 1^\circ\text{C}$)

The green tomato juice and RTS beverages were analyzed for α -tomatine content (Figure 3). The α -tomatine content in green tomato juice was found to be 43.23 ± 3.59 mg/100 mL. The α -tomatine content of tomatoes was in range as obtained by Friedman (2009). The preliminary α -tomatine content of RTS beverage was found to be 3.94 ± 0.21 mg/100 mL. The reduction in α -tomatine content was attributed to processing loss (Tajner et al., 2011). The BB samples had a lesser decrease in α -tomatine content than the WB Beverage during

storage. The HPLC analysis of standards and samples were shown in Figure 3. During LT ($4 \pm 2^\circ\text{C}$) storage due to enzyme tomatinase, there is a change of α -Tomatine degrading to dehydrotomatine. Hence, there was a significant decrease in α -tomatine content at the end of the LT ($4 \pm 1^\circ\text{C}$) period of 90 days in both BB and WB samples (2.31 ± 0.29 mg/100 mL and 1.42 ± 0.34 mg/100 mL) respectively as compared to freshly prepared RTS beverages. Since the retention of α -tomatine was found more in BB than the WB at low temperatures, there were minor changes from the initial values.

Figure 3. HPLC chromatogram for α -Tomatine estimation throughout the study

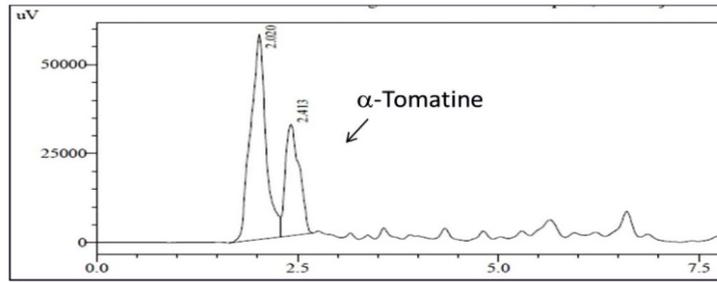


Figure 3A. Standard α -Tomatine chromatogram

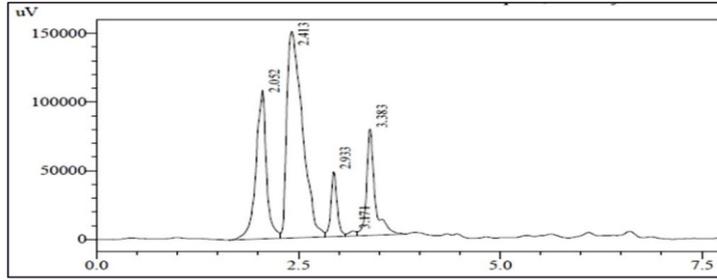


Figure 3B. The α -tomatine chromatogram of green tomato juice.

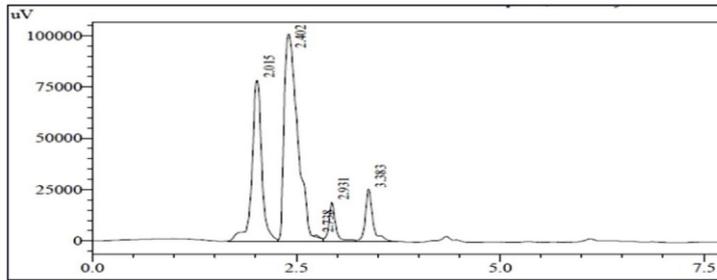


Figure 3C. Initial α -Tomatine chromatogram of RTS beverage (0th day).

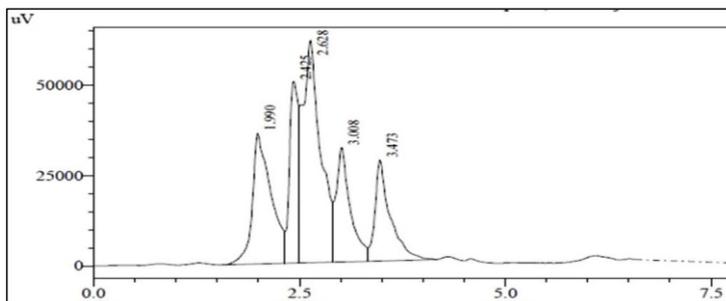
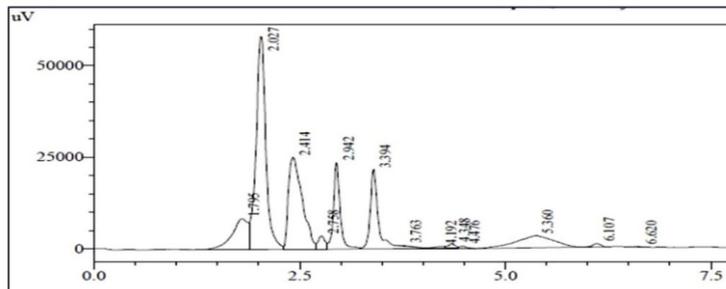


Fig 3D. The α -Tomatine chromatogram of BB and WB stored RTS beverage (45th day)

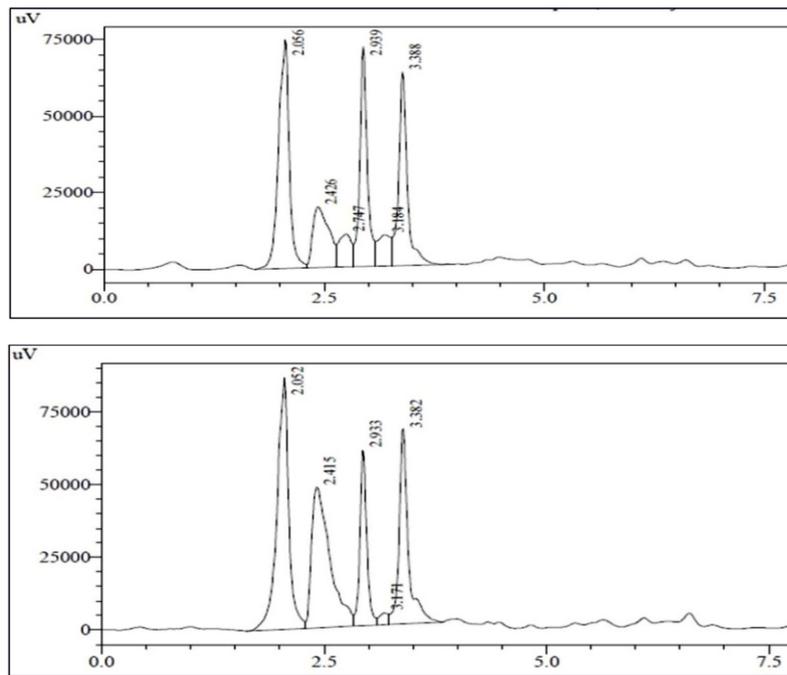


Figure 3E. The α -Tomatine chromatogram of BB and WB stored RTS beverage (90th day)

Microbiological evaluation of beverages

The RTS beverages being high in water content and nutrients present, the growth of microorganisms restricted due to the low pH. Initially, there was no microbial growth observed. In BB stored samples, no bacteria, yeast, or mold growth is seen for up to 90 days. The quality of WB stored samples started deteriorating after a storage period of 75 days. The high bacterial growth (1.465 logs CFU/mL) was observed at the end of storage but within the permissible limit (FSSAI, 2017) and could be a reason for the decline in beverage color. The low counts observed in the BB and WB stored samples due to unfavorable conditions for multiply in the acidic beverages (pH <3.5). The coliform count was undetectable in all samples (both initially and after 90 days of LT storage). Therefore, high pasteurization temperature (90°C, 1 min) and low pH synergistically hampered the growth of microbes during storage.

Sensory quality evaluation

Figure 4 illustrates the sensory analysis of RTS beverages based on the mean scores for quality attributes. Initially, BB and WB got an average sensory score of 9.0 and 8.3, respectively, from the panelists.

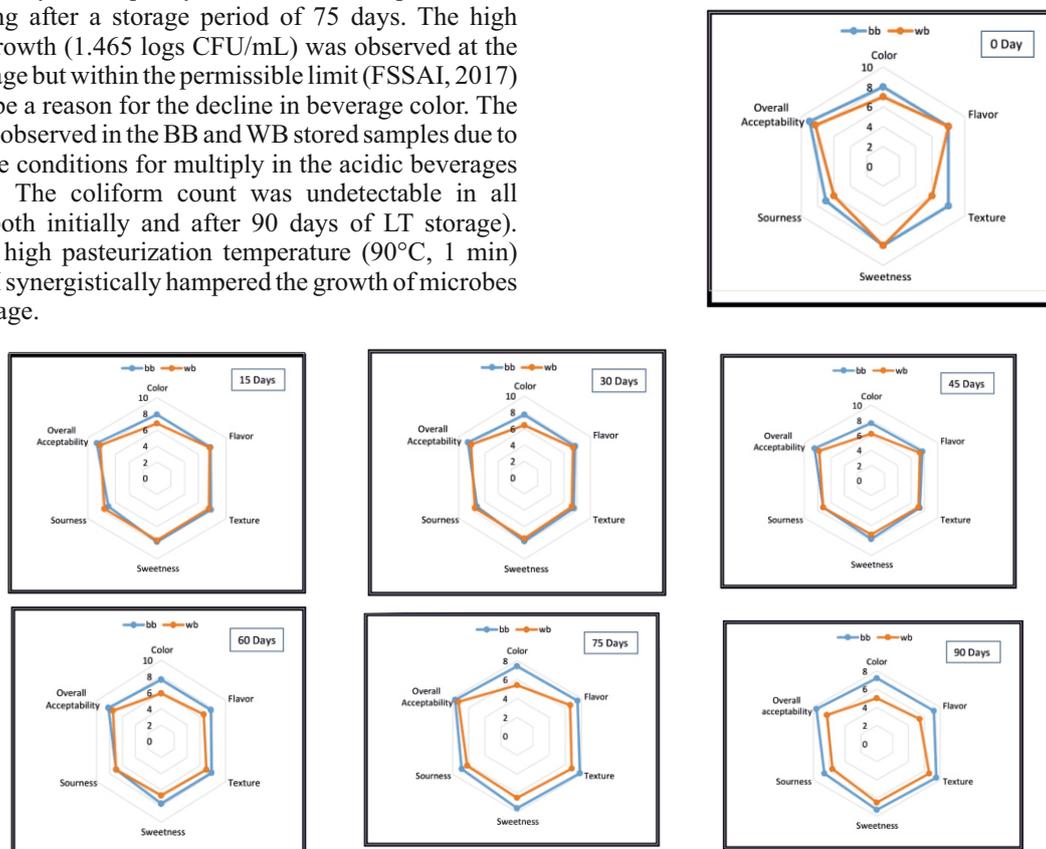


Figure 4. Sensory profile of BB and WB container packaged α -Tomatine rich RTS beverage during storage at LT (4±2 °C) condition.

On the 60th day, there were slight changes in sensory values for BB and WB stored samples with overall acceptability of 8.2 and 7.5, respectively, and also minor changes observed in other sensory quality attributes. After that, significant changes ($p \leq 0.05$) were observed in all the quality attributes of WB stored samples than the BB. At the end of storage (90th day), the overall acceptability for BB stored samples was 7.6, whereas WB stored samples had only 6.3. Even though the storage conditions were the same, sensory scores indicate that BB bottles have a better-preserving effect than WB stored samples in prolonged storage (90 days).

CONCLUSION

The study demonstrated the development of α -tomatine rich (3.94 ± 0.21 mg/100 mL) from matured green tomato juice. The stability of the product stored in a Brown glass bottle (BB) container was excellent with better retention of α -Tomatine (2.31 ± 0.29 mg/100 mL), total phenolic content (1.42 mg GAE/100 mL), ascorbic acid (17.66 mg/100 mL), total chlorophyll (1.07 mg/100 mL) and viscosity (15.46 cp), with high sensory scores (7.6) up to 90 days of storage than the quality of the product (with 6.8 sensory scores) stored in a white glass bottle (WB) container. The microbial counts of products stored in both containers after 90 days are indicated within the permissible limit. Therefore, α -tomatine rich beverages with other vital nutrients can be recommended as a health drink for consumption and export purposes.

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