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FORMULATION OF RTS FROM PINEAPPLE (ANANAS COMOSUS) AND ALOE VERA (ALOE BARBADENSIS) PULP: EVALUATION OF NUTRACEUTICAL PROFILE AND SHELF-LIFE STUDIES

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Abstract: Pineapple and aloe vera are rich sources of nutraceutical compounds such as flavonoids, ascorbic acid, and phenolic compounds with antioxidant properties, making them highly appealing to both consumers and processors. Aloe vera's beneficial qualities are attributed to its polysaccharide content. Ready-to-serve (RTS) beverages are popular non-fermented drinks that are appreciated for their flavor and therapeutic potential. This study focused on developing RTS drinks from blends of aloe vera and pineapple pulp with varying sugar content (10 °Brix, 12 °Brix, 14 °Brix, and 16 °Brix) and evaluating their physicochemical, microbiological, and sensory properties over 45 days. Storage at 9 °C resulted in a slight increase in TSS, titratable acidity, reducing sugar, and microbial load, while significant decreases were observed in total sugar, pH, phenolic content, and DPPH content. Among the formulations, the RTS beverage with 12 O Brix exhibited superior physicochemical and sensory qualities. Combining aloe vera and pineapple in RTS beverages offers not only an appealing flavor profile but also potential health benefits, making them an ideal choice for product innovation.

Keywords: *Ready-to-serve (RTS), TSS, nutraceutical, shelf-life, physicochemical properties.*

1. Introduction

Ready-to-serve (RTS) drink is a result of a combination of traditional and modern elements, seamlessly blending the best of both worlds (Vilas-Boas et al., 2022). This creative process yields a balanced composition that is highly palatable, nutritious, and health-supportive. Enriched with vitamins, minerals, and antioxidants, these juices contain vital micronutrients known to confer various health advantages, including mitigating the risks of cardiovascular diseases, diabetes, and cancer (Kaur et al., 2017). As highlighted by A. Yadev et al., these beverages serve as veritable storehouses of essential nutrients that are essential for healthy growth and development (A. Yadav et al., 2013).

Aloe vera juice is renowned for its multifaceted health benefits, including improved digestion, detoxification, aiding weight loss, and regulating blood sugar levels. Extensive studies have underscored its antioxidant properties, which may reduce inflammation and help prevent the onset of chronic diseases. Notably, research published in the International Journal of Environmental Science and Technology reveals the richness of aloe vera juice in vitamins, minerals, folic acid, and other essential compounds (Añibarro-Ortega et al., 2019). Moreover, the presence of key minerals such as calcium, iron, potassium, and sodium in aloe vera juice contributes to maintaining healthy blood pressure, muscle function, and nerve function. Furthermore, beneficial enzymes and amino acids found in aloe vera juice promote optimal digestion and facilitate nutrient absorption, as demonstrated by various studies. A finding from the American Journal of Clinical Nutrition demonstrates the lipid-lowering effects of aloe vera juice, which is particularly beneficial for individuals with high cholesterol (Alinejad-Mofrad et al., 2015). Additionally, research suggests that aloe vera juice exhibits antidiabetic properties, helping in the regulation of blood sugar and insulin levels (Rodríguez et al., 2010). Similarly, pineapple juice emerges as a nutritional powerhouse, brimming with essential vitamins, minerals, such as potassium, and antioxidants, as well as a variety of polyphenols and flavonoids (Valderrain-Rodríguez et al., 2017).

The objectives of this research were to investigate:

1. To produce a pineapple & aloe vera RTS beverage
2. To assess the effect of the storage period on total soluble solids acidity, DPPH content, total phenolic content, reducing sugar, and microbial characteristics of the beverage. Additionally, sensory evaluations were conducted over 45 days, both at 9 °C and room temperature.

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2. Experimental Methods

The present study, titled "Studies on beverages from aloe vera (*Aloe barbanensis*) and Pineapple (*Ananas comosus* L.) blends," was conducted at the Centre of Food Science and Technology, Banaras Hindu University, Varanasi (U.P.), India, during the academic year 2015–2016. The following sections detail the materials used in the experimental methodology employed in this study:

Aloe Vera Pulp Preparation

Aloe vera leaves selected for this study were required to be healthy, undamaged, free of mildew and rot, and 3–4 years old to ensure the concentration of active components. Conventional hand filleting techniques were employed to remove the pulp to prevent contamination of the internal fillet with yellow sap. Portions of the rind containing a significant amount of mucilage were discarded during this process. It is imperative to complete this procedure within 36 h of procuring the leaves to preserve the integrity of the active constituents. Subsequently, the pulp was heated for l0 min at 60–65 °C to ensure sanitation. The heated pulp was then minced using a hand beater. Aloe vera juice was obtained by filtering the mashed pulp through a muslin cloth (R. Yadav et al., 2013).

Preparation of Pineapple Pulp

Fresh, fully matured Kew variety of pineapple was used to extract the pulp. The fruits were squashed using a pulper to obtain the pulp. Following extraction, the pulp was carefully stored in stainless steel containers.

Preparation of RTS beverage

The RTS beverage was formulated by blending aloe vera pulp and pineapple pulp at different concentration levels, maintaining a ratio of 25% aloe vera pulp to 75% pineapple pulp. Additionally, the blended beverage included sodium benzoate at a concentration of 0.2% by weight per kilogram of pulp for preservation, as well as a suitable amount of edible color (2 drops) and water to achieve the desired consistency and visual appeal.

Figure 1. The pineapple aloe-vera RTS was developed in four samples (10,12,14, and 16 °Brix TSS)

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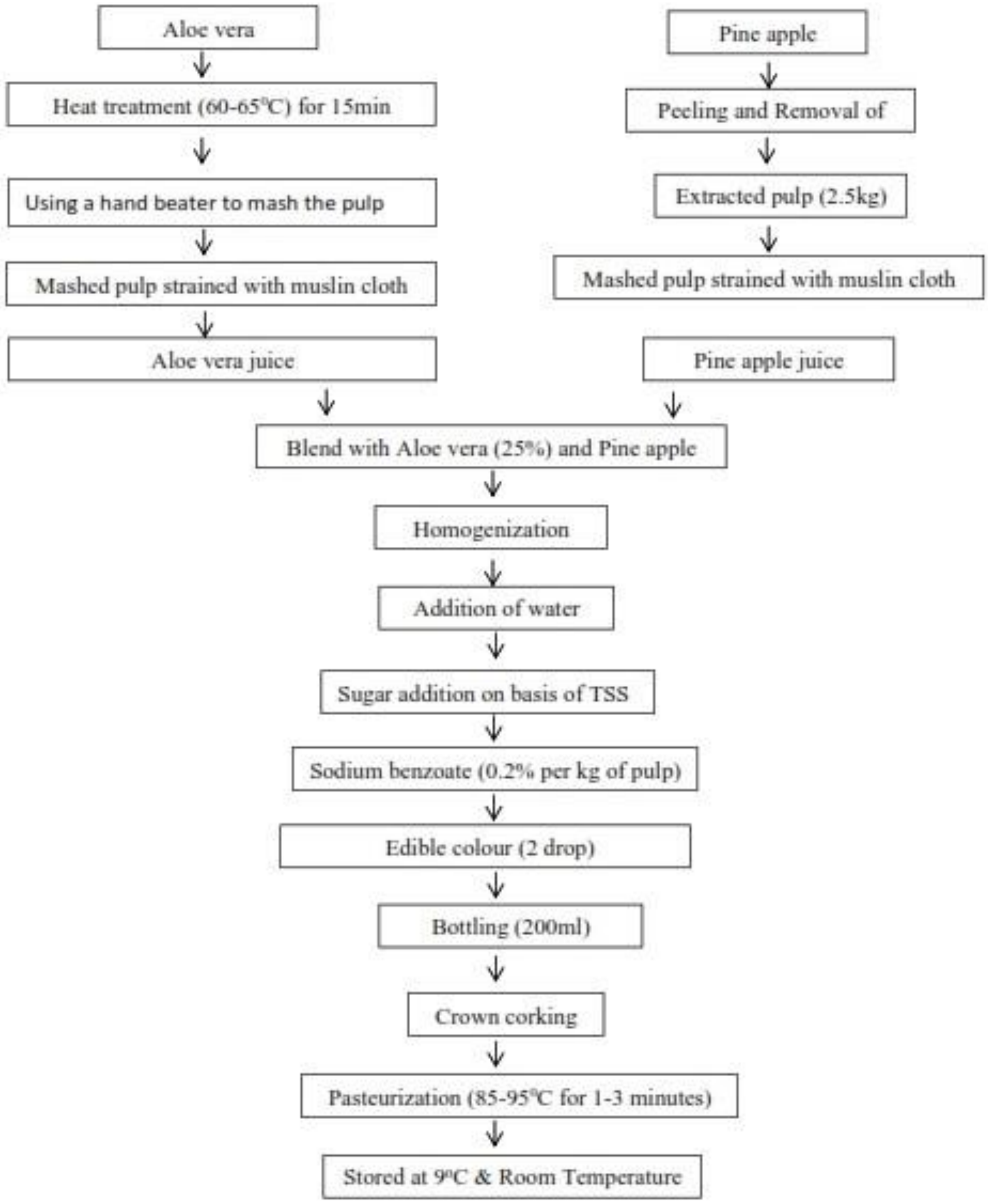

The process flow chart for the production of Pineapple Aloe Vera RTS (R. Yadav et al., 2013)

Determination of Physicochemical Quality of Pineapple Aloe Vera RTS

pH

The pH of the pineapple-aloe vera RTS solution was determined by measuring the logarithm of its hydrogen ion concentration.

pH = -log (H⁺)

Where H^+ = hydrogen ion concentration (g/L).

An electronic pH meter was employed to measure pH readings, following the method outlined in the previous study (Ranganna,2001). Calibration of the electronic pH meter was performed using standard buffer solutions with pH values of 7 and 4. The pH function selector switch was set, and the reading was taken after allowing the digital display to stabilize.

Total Soluble Solids (TSS) Measurement

The total soluble solids (TSS) of the aloe vera RTS were determined using a hand refractometer, as recommended by a previous study (Srivastava et al., 2004). The degree Brix (°Brix) was calculated based on the refractometer reading using the following equation:

TSS (°Brix) = Refractometer reading

Acidity Measurement

The acidity levels of several samples were determined following the method outlined by Ranganna (**Ranganna, 2001).** A 5 mL sample of aloe vera RTS was dissolved in 501 mL of distilled water. Subsequently, a 20 mL aliquot of this solution was withdrawn and titrated with 0.1 N NaOH, with phenolphthalein serving as the indicator. The appearance of a pink color indicated

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the endpoint of the titration. The volume of NaOH used was recorded, and the acidity was calculated as a percentage of total acids using the following equation:

$$\textbf{Acidity}\ (\%) = \frac{\textbf{Vol. of NaOH used (mL)} \times \textbf{Norm. of NaOH} \times \textbf{Equi. wt. of citric acid} \times \textbf{100}}{\textbf{Vol. of sample (mL) titrated}}$$

Reducing Sugar Measurement

Principle

The determination of reducing sugar in the samples was based on the principle described by Miller, employing Dinitro salicylic acid (DNS) (Miller, 1959). When a reducing sugar reacts with a basic solution, it forms an aldehyde or ketone. The reduced form of 3, 5-dinitro salicylic acid (DNS) reacts with water in the presence of the aldehyde group of glucose. It catalyzes the reaction to form 3-amino-5 nitro salicylic acid and liberate oxygen gas as a by-product.

The absorption of light at a wavelength of 540 nm is affected by the formation of 3-amino-5-nitro salicylic acid. The quantity of reducing sugar present is directly proportional to the absorbance, as determined using a spectrophotometer.

Total Phenolic Content

Sample Preparation

A sample of 1 mL of fresh juice was diluted to 25 mL and then centrifuged at 4 °C for 20 min at 5000 rpm. The supernatant solution obtained was then used for further analysis.

Determination of Total Phenolic Content

The Folin-Ciocalteu's reagent was diluted with distilled water to a 1/100 concentration, and 1.0 mL of this diluted reagent was added to the tubes. Subsequently, 0.2 mL of the diluted sample extract was transferred to the tubes. After waiting for 10 min, 0.8 mL of a 7.5% w/v sodium carbonate solution was added to the sample. The absorbance was measured at 743 nm after allowing the sample to equilibrate at room temperature for 30 min. The total phenolic content of the fruit juice was expressed in terms of milligrams of gallic acid equivalent (GAE) per 100 mL of juice. The concentration of polyphenols in the sample was calculated using a gallic acid standard curve with a range of 0.2–4 mg/L.

Sensory Evaluation

The sensory evaluation was conducted for the freshly formulated RTS beverage and repeated at intervals of 15 days. Various sensory parameters, including taste, color, flavor, texture, and overall acceptability were assessed. A panel comprising 35 semi-trained individuals, including teachers, students, and laboratory staff, was assembled to carry out the analysis. The evaluation was conducted using a 9-point Hedonic scale, where a rating of 9 indicated "Extremely like" and a rating of 1 indicated "Extremely dislike" (Nazni & Mythili, 2013).

Statistical Analysis

In this research analysis, experimental values were studied in triplicate and outlined as the mean value ± standard deviation (SD). The comparison of mean ratios was conducted using one-way ANOVA, followed by Duncan's test ($p < 0.05$) to assess significant differences among the groups.

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3. Results and Discussion

Parameters	Sample	Storage period (days)							
		0		15		30		45	
		9 °C	RT	9 °C	RT	9 °C	RT	9 °C	RT
(a) T.S.S.(°Brix)	A_{10}	10.29 ±0.18	10.29 ±0.18	10.28 ±0.06	10.37 ±0.03	10.75 ±0.10	10.83 ±0.03	10.94 ±0.05	10.98 ±0.03
	A_{12}	12.00 ±0.29	12.00 ±0.0	12.62 ±0.07	12.83 ±0.03	12.91 ±0.10	13.04 ±0.07	13.32 ±0.10	13.56 ±0.08
	A_{14}	14.00 ±0.1	14.00 ±0.0	14.40 ±0.1	14.78 ±0.03	14.71 ±0.1	14.97 ±0.02	14.93 ±0.1	15.29 ±0.07
	A_{16}	16.00 ±0.0	16.00 ±0.0	16.35 ±0.1	16.70 ±0.5	16.85 ±0.05	17.42 ±0.21	17.04 ±0.06	17.93 ±0.03
(b) Titrable acidity (% citric acid)	A_{10}	0.17 ±0.2	0.17 ±0.2	0.20 ±0.15	0.24 ±0.15	0.27 ±0.2	0.30 ±0.2	0.30 ±0.15	0.36 ±0.15
	A_{12}	0.19 ±0.2	0.19 ±0.24	0.21 ±0.21	0.27 ±0.37	0.27 ±0.21	0.29 ±0.34	0.34 ±0.22	0.39 ±0.03
	A_{14}	0.17 ±0.02	0.17 ±0.02	0.21 ±0.02	0.24 ±0.02	0.27 ±0.02	0.29 ±0.02	0.30 ±0.03	0.35 ±0.02
	A_{16}	0.19 ±0.04	0.19 ±0.02	0.22 ±0.03	0.24 ±0.03	0.29 ±0.03	0.31 ±0.03	0.37 ±0.03	0.39 ±0.03
(c) pH	A_{10}	4.4 ±0.25	4.4 ±0.25	4.2 ±0.2	3.9 ±0.2	4.0 ±0.25	3.8 ±0.25	3.7 ±0.25	3.4 ±0.3
	A_{12}	4.4 ±0.25	4.4 ±0.25	4.0 ±0.15	3.9 ±0.2	3.9 ±0.25	3.8 ±0.25	3.5 ±0.3	3.3 ±0.2
	A_{14}	4.4 ±0.25	4.4 ±0.25	4.2 ±0.15	4.1 ±0.2	3.9 ±0.25	3.8 ±0.15	3.6 ±0.3	3.2 ±0.25
	A_{16}	4.4 ±0.25	4.4 ±0.25	4.0 ±0.3	3.7 ±0.25	3.7 ±0.25	3.6 ±0.36	3.4 ±0.35	3.2 ±0.21
(d) Reducing sugar (g/100 mL)	A_{10}	10.02 ±0.25	10.02 ±0.25	11.46 ±0.2	12.74 ±0.2	12.37 ±0.25	13.13 ±0.25	14.1 ±0.25	15.65 ±0.3
	A_{12}	10.41 ±0.25	10.41 ±0.25	12.94 ±0.15	13.69 ±0.2	13.36 ±0.25	15.03 ±0.25	14.79 ±0.25	15.26 ±0.2
	A_{14}	10.72 ±0.25	10.72 ±0.25	14.38 ±0.15	15.55 ±0.2	14.79 ±0.25	15.95 ±0.15	14.9 ±0.3	16.25 ±0.25
	A_{16}	11.85 ±0.25	13.69 ±0.25	14.87 ±0.3	15.89 ±0.25	16.21 ±0.25	17.32 ±0.36	16.3 ±0.35	17.45 ±0.25

Note: Data are mean ± SD (n = 3). Similar letters in each row at $p < 0.05$ were not significantly different.

Table 1. Effect on T.S.S, acidity, and reducing sugar during the storage period

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Parameters	Sample	Storage period (days)							
		0		15		30		45	
		9 °C	RT	9 °C	RT	9 °C	RT	9 °C	RT
(e)Total phenolic content (mg GAE/100 mL)	A_{10}	49.02 ±0.21	46.85 ±0.28	43.04 ±0.21	37.2 ±0.14	39.63 ±0.12	36.28 ±0.31	37.84 ±0.22	32.11 ±0.14
	A_{12}	35.50 ±0.24	37.13 ±0.11	27.50 ±0.24	32.44 ±0.11	35.96 ±0.21	33.54 ±0.17	33.54 ±0.41	29.78 ±0.48
	A_{14}	49.65 ±0.21	30.25 ±0.2	41.90 ±0.17	22.97 ±0.15	37.66 ±0.19	32.08 ±0.26	33.21 ±0.36	29.17 ±0.31
	A_{16}	50.34 ±0.17	32.53 ±0.21	43.86 ±0.14	24.14 ±0.13	34.72 ±0.32	31.27 ±0.37	30.46 ±0.29	27.64 ±0.17
(f)DPPH content	A_{10}	52.36 ±0.06	52.28 ±0.15	50.28 ±0.88	42.35 ±0.13	47.13 ±0.64	32.65 ±0.21	41.03 ±0.25	25.65 ±0.15
	A_{12}	51.27 ±0.07	53.35 ±0.08	49.37 ±0.13	43.59 ±0.26	46.56 ±0.25	33.54 ±0.33	39.59 ±0.25	23.49 ±0.45
	A_{14}	55.05 ±0.05	55.25 ±0.1	51.97 ±0.34	45.72 ±0.57	47.37 ±0.58	34.39 ±0.44	40.69 ±0.2	26.96 ±0.15
	A_{16}	50.61 ±0.2	49.21 ±0.09	47.86 ±0.46	39.38 ±0.14	45.83 ±0.44	29.98 ±0.15	38.68 ±0.3	22.81 ±0.35

Note: Data are mean ± SD (n = 3). Similar letters in each row at $p < 0.05$ were not significantly different

Table 2. Effect on total phenolic and DPPH content during the storage period

Effect on Total Soluble Solids

Table 1 presents the experimental data for illustrating the changes in TSS of the pineapple aloe vera RTS beverage during storage. Figure 2a depicts a line graph illustrating the variations in the TSS for various pineapple-aloe vera RTS samples over the storage period. The findings indicate that the RST beverage exhibited significant levels of total phenol and flavonoids.

As observed in Table 1, the total soluble solids experienced a notable increase from 0 to 45 days of storage. The TSS of the four processed ready-to-serve beverage samples were maintained at 10, 12, 14, and 16 °Brix, respectively. Notably, sample A16 (RT) exhibited the highest increase in TSS, rising from 16 °Brix to 17.93 °Brix. Conversely, sample A10 (9 °C) showed the smallest rise, increasing from 10 °Brix to 10.94 °Brix.

Additionally, Kalra et al. (1991), Sagar (1995), Murari K. and Verma R.A (1989), and Saravanan K. et al. (2000) have reported a similar increase in TSS during storage. This occurrence could be attributed to the conversion of insoluble polysaccharides to sugar.

Effect of Titrable Acidity

The experimental findings regarding the change in titrable acidity of the pineapple aloe vera RTS beverage during storage are presented in Table 1. Figure 2b illustrates a line chart depicting the changes in titrable acidity of various RTS samples over the 45-day storage period. The data from Table 1 indicate a significant increase in titrable acidity over the storage duration. Notably, sample A10 (9 °C) exhibited the least increase in acidity, rising from 0.17% to 0.30%. This increase can be partly attributed to the intrinsic acid content of the beverage. Hamaran and Amuth (2007) observed similar trends in their study. They found that both ambient temperature (35–36 °C) and low temperature (3–5 °C) storage conditions led to an increase in the acidity values of banana and sapota beverages (A. Yadav et al., 2013).

Effect of pH

Experimental results for the change in pH of the RTS beverage during storage are presented in Table 1. Figure 2c depicts a line graph illustrating the pH variations of various RTS samples over 45 days. Sample A10 (9 °C) exhibited the least decline among the four samples, decreasing from 4.4 to 3.7. Similar trends were observed in the cases of banana and sapota beverages by Hamaran and Amuth (2007). Conversely, Bhatti (1975), Ullah (1975), and Tiwari (2000) reported an increase in pH. This gradual decline in pH is noteworthy as it acts as a preservative by inhibiting the growth of pathogenic organisms.

Effect on Reducing Sugar

Table 1 presents experimental data depicting the changes in reducing the sugar content of RTS beverages over time. Figure 2d illustrates the reduction in sugar content in several RTS beverages over the storage period through a line graph representation. It is apparent from Table 1 that the reduced sugar content

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significantly increased over the 45-day storage period. The highest increases were observed in samples A14 (RT) and A16 (RT), rising from 10.72% to 16.25% and from 13.69% to 17.45%, respectively. Sample A10 exhibited the smallest increase, rising from 10.02% to 14.1%. This increase in reduced sugar content can be attributed to the acid in the beverage hydrolyzing sucrose to produce glucose and fructose, leading to a simultaneous decrease in non-reducing sugars (Shahanas et al., 2019).

Effect on total phenolic content

Experimental data regarding the changes in phenolic content of the pineapple aloe vera RTS beverage during storage are presented in Table 2. Figure 2e depicts a line graph illustrating the variations in phenolic concentration among various RTS samples over the storage period. It is evident from Table 2 that the phenolic content experienced a significant decrease from 0 to 45 days of storage. Among the four samples, sample A10 (9 °C) exhibited the least amount of phenolic content loss, dropping from 49.02% to 43.4%. In recent years, there has been increased motivation among individuals to modify their diets in order to reduce susceptibility to or better manage certain health conditions. Consequently, research on antioxidants has increased due to concerns about improving health and the potential benefits of agricultural goods (Moore et al., 2005). Free radical damage to proteins, lipids, and nucleic acids has been linked to a variety of degenerative human diseases, including cancer, cardiovascular, and cerebrovascular diseases (Park et al., 2009).

Effect on total DPPH content

Compound 2,2-diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical compound commonly used to assess antioxidant activity. Spectrophotometric analysis using DPPH was conducted to quantify phenolic compounds, following the methodology described by Cuendet *et al.* (1997). Table 2 presents the experimental findings regarding the variation in DPPH content of pineapple-aloe vera RTS beverage over the storage period. Figure 2f depicts the fluctuation in DPPH content across various RTS samples over time, represented through a line graph. Observing Table 2, a noticeable decline in DPPH content is evident during the storage duration of 0 to 45 days. Notably, samples A10 (9 °C) and A16 (9 °C) exhibited the most significant reductions in DPPH concentration, decreasing from 52.36% to 41.03% and 50.61 to 38.68%, respectively.

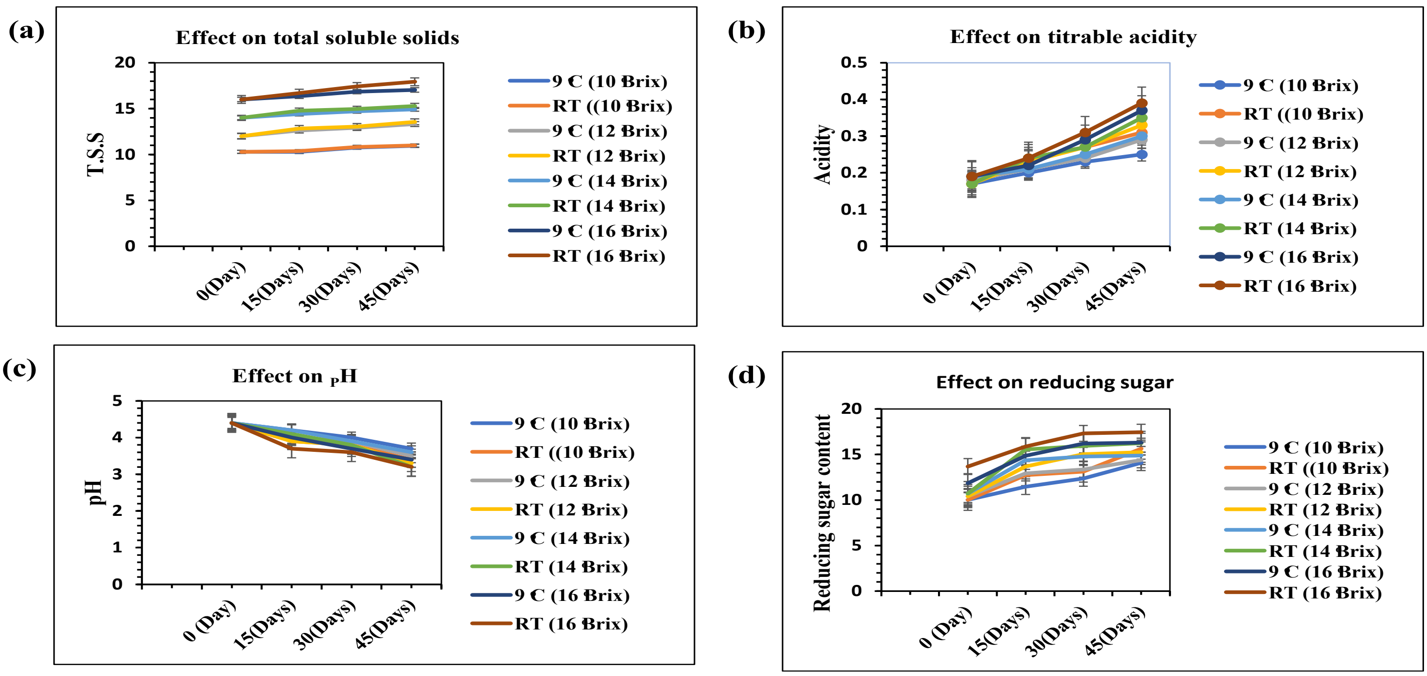

Figure 2. Graphical representation for effect on T.S.S, acidity, and reducing sugar during the storage period

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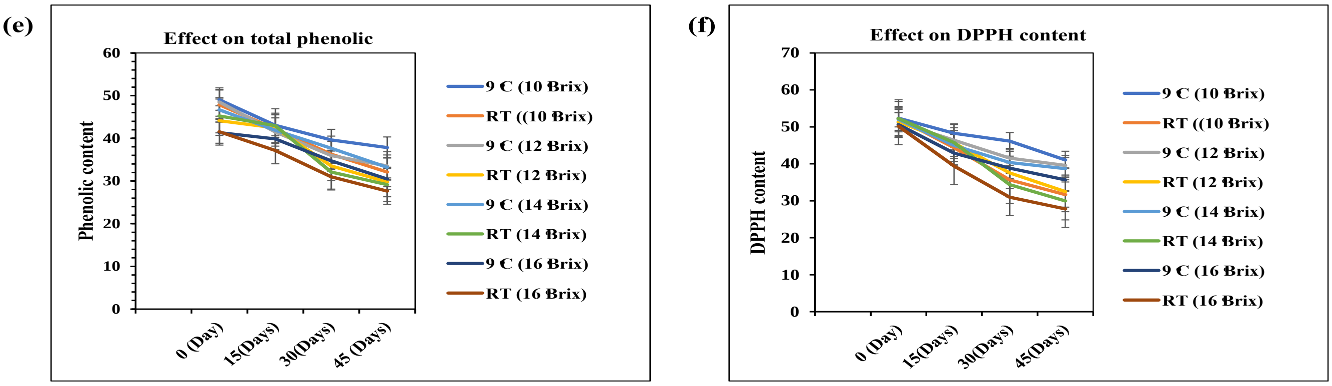

Figure 3. Graphical representation for effect on total phenolic and DPPH content during the storage period

Variation in Microbial Load Of Pineapple Aloe-Vera RTS During Storage

Table 4 presents experimental data tracking the microbial load changes in pineapple aloe vera RTS beverages over the storage period. Figure 5 complements this data with a line graph illustrating microbial load changes across various RTS samples during storage. Microbial analysis at a 10^{-3} dilution was conducted after 0 to 45 days of storage. Notably, sample A12 (RT) had the highest bacterial count, escalating from 0.65 x 10^{-3} to 1.45 x 10^{-3}, while sample A16 (9 °C) displayed the lowest bacterial contamination, increasing from 0.97 x 10^{-3} to 1.06 x 10^{-3}. Throughout the observed period, there was a slight increase in the microbial load of the ready-to-serve beverage. However, it is noteworthy that the microbiological growth was inhibited to some extent, likely due to the preservative characteristics of sugar. The presence of fruit pulp, serving as a substrate for bacterial development owing to its carbohydrate content, influenced the microbial dynamics, as indicated by both the physical and chemical results. These findings align with the observations of Dhamsaniya & Varshney (2013), confirming the effect of the storage conditions and beverage composition on microbial growth in RTS beverages.

Storage period (in days)	Sample & Storage temperature (M)							
	A_{10}		A_{12}		A_{14}		A_{16}	
	9 °C 10^{-3} Dilution (CFU/mL)	RT 10^{-3} Dilution (CFU/mL)	9 °C 10^{-3} Dilution (CFU/mL)	RT 10^{-3} Dilution (CFU/mL)	9 °C 10^{-3} Dilution (CFU/mL)	RT 10^{-3} Dilution (CFU/mL)	9 °C 10^{-3} Dilution (CFU/mL)	RT 10^{-3} Dilution (CFU/mL)
0 (Day)	0.22 ± 0.04	0.24 ± 0.04	0.18 ± 0.04	0.19 ± 0.04	0.15 ± 0.02	0.2 ± 0.04	0.17 ± 0.05	0.16 ± 0.05
30 (Days)	0.25 ± 0.05	0.37 ± 0.06	0.27 ± 0.05	0.41 ± 0.06	0.27 ± 0.08	0.32 ± 0.03	0.36 ± 0.04	0.39 ± 0.06
45 (Days)	0.31 ± 0.02	0.42 ± 0.08	0.34 ± 0.03	0.46 ± 0.4	0.333 ± 0.06	0.45 ± 0.03	0.39 ± 0.05	0.51 ± 0.07

Table 4. Changes in microbial load of pineapple aloe-vera RTS during storage.

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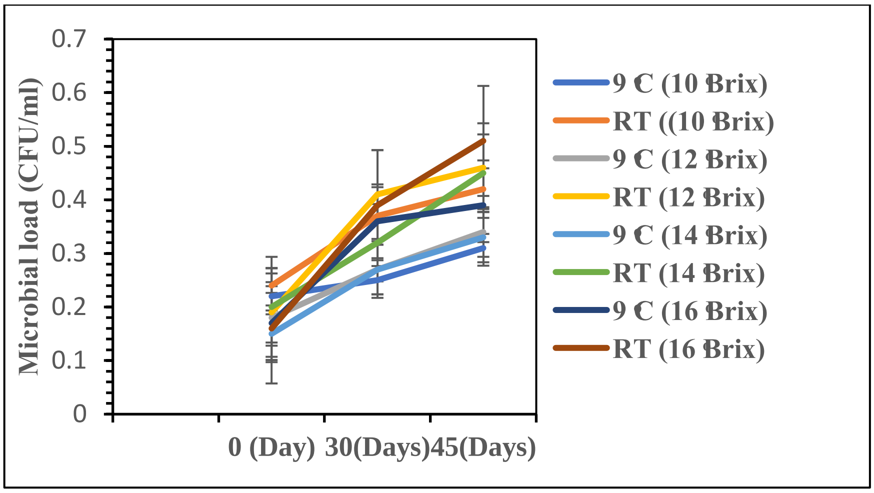

Figure 4. Microbial load of pineapple aloe-vera RTS during storage

4. Conclusion

The current study highlights the positive attributes associated with Ananas Comosus beverages, including an enhanced phytochemical profile, improved storage stability, and improved physicochemical, sensory, and microbiological qualities of the product. The formulated beverage contains four different TSS levels and is subjected to storage conditions at both room temperature and 9 °C, revealing various compositional changes over time.

However, observations indicate that over the 45-day storage period, these beneficial compounds experience gradual degradation, particularly when stored at 9 °C and room temperature. Despite this degradation, the RTS beverage comprising *Ananas Comosus* and *Aloe Barbadensis* presents itself as a promising product for consumers, offering numerous nutraceutical and functional benefits.

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