

# Development and Quality Evaluation of *Aloe Vera* and Pineapple Juice Blended Beverage

Sonali Biswas<sup>1</sup>, Dorcus Masih<sup>2</sup>, Madhulika Singh<sup>3</sup>, Chitra Sonkar<sup>4</sup>

<sup>1</sup>M.Tech, Department of Food Process Engineering, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad- 211007

<sup>2</sup> Assistant Professor, Department of Food Process Engineering, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad- 211007

<sup>3</sup>Head, Division of Biotechnology, CytoGene Research and Development, Lucknow.

<sup>4</sup> Assistant Professor, Department of Food Process Engineering, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad- 211007

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**Abstract** - Juices of pineapple (*Ananas comosus*) and *Aloe vera* were optimized to a blended beverage which was stored for 21 days in glass bottles (200 ml capacity) at refrigerated temperature. The protein content was observed to be increasing with the increasing amount of *Aloe vera*. The acidity increased (0.179-0.192) and pH of the juice decreased progressively during the storage period. The overall acceptability scores of more than 8.5 for juice samples up to 30% *Aloe vera* juice incorporation indicated the commercial scope for manufacturing good and nutritious pineapple juice blended with Heat pasteurization (97°C for 2-3 min) was more effective for inactivating the microbial flora. However the shelf life of juice was established within 14 days.

**Key Words:** *Aloe vera* juice, pineapple juice, blended beverage, shelf life, palatable health drink

## 1. INTRODUCTION

Fruit and vegetable beverages have higher nutritional, medicinal and calorific values compared to synthetic beverages. The Egyptians called *Aloe* "the plant of immortality." Today, the *Aloe vera* plant has been used for various purposes in dermatology (Surjushe *et al.*, 2008).

*Aloe vera* has also been commonly used to treat first and second degree burns, as well as sunburns and poison oak, poison ivy, and poison sumac infections and eczema (Mishra *et al.*, 2015). *Aloe vera* has the anti-bacterial property along with the anti-inflammatory property which helps to cure mouth and gum problems and severe gum diseases (Kumar *et al.*, 2010). Glucomannan. Acemannan the major carbohydrate fraction in the gel, is a water

soluble long chain mannose polymer which accelerate wound healing, modulate immune function and demonstrates antineoplastic and antiviral effects (Rahman *et al.*, 2015).

Pineapple [*Ananas comosus* (L.) Merr. Family: Bromeliaceae] is one of the most important commercial fruit crops in the world. It is known as the queen of fruits due to its excellent flavour and taste (Baruwa, 2013). Pineapple is the third most important tropical fruit in the world after Banana and Citrus (Bartholomew *et al.*, 2003). One healthy ripe pineapple fruit can supply about 16.2% of daily requirement for vitamin C (Hemlatha *et al.*, 2013). Vitamin C also retards the development of urinary tract infections during pregnancy and reduces the risk of certain cancers, including colon, esophagus and stomach (Debnath *et al.*, 2012). Excessive consumption of ripen pineapple cores formation of fiber balls in the digestive tract. It has huge nutritive value but it has also few side effects (Joy, 2010). The global functional beverages market drives due to the growth in health and wellness concerns increase in disposable incomes, consumer awareness, introduction of new flavored products meeting various nutritional and health requirements, obesity concerns, aging population and changing lifestyles (Kumar, 2015).

Fruit juice blends can be produced from various fruits in order to combine all the basic nutrients present in these different fruits for use when combined. It can improve the vitamin and mineral content depending on the kind and quality of fruits and vegetables used (De Carvalho *et al.*, 2007). *Aloe vera* has a great value in the society for studies demonstrate its analgesic, antidiabetic, antimicrobial, anti-inflammatory, wound healing, anti-proliferative, gastric

mucosal protection, hepato-protective, neuro-protective, hypolipidaemic, anti-leishmanial, radio protective and immune-modulatory properties based on scientific evidence (Malik, 2013). The interest and use of *Aloe vera* gel has increased dramatically in the field of health care and cosmetics (Devi *et al.*, 2005).

## 2. MATERIALS AND METHODOLOGY

In this section we discussed about the materials and methodology used for the development and quality testing of blended juice.

### 2.1 Raw Materials

Fleshy green *Aloe vera* leaves were collected from a local garden in Lucknow. Golden brown pineapple was collected from a local fruit market in Lucknow City. Synthetic color, flavor, salt and low calorie sugar was bought from a local store.

### 2.2 Development of blended juice

The juice of *Aloe vera* was extracted by cold extraction method as earlier described by Mishra *et al* (2013). The peel of the pineapple was removed using a sharp knife, was cut into small chunks followed by blending. The juice was then filtered using muslin cloth, pasteurized and stored. Four treatments were prepared by adding *Aloe vera* juice in different ratio (table 1), along with the addition of black salt, sugar, pineapple flavor and yellow color. The blends were pasteurized at 97°C for 2-3 min, cooled and stored at a refrigerated temperature. Flowchart of the complete process is depicted in figure 1.

**Table -1:** Blending ratio of pineapple and Aloe vera juices

Sr. No	Treatment Name	Blend ratio
1.	T <sub>0</sub>	Pineapple 100 : Aloe 0
2.	T <sub>1</sub>	Pineapple 90 : Aloe 10
3.	T <sub>2</sub>	Pineapple 80 : Aloe 20
4.	T <sub>3</sub>	Pineapple 70 : Aloe 30

### 2.3 Organoleptic Evaluation

Sensory evaluation (color, flavor, taste and overall acceptability) of pineapple juice without added preservatives were carried out using a 9 point hedonic scale as previously done by Islam *et al* (2014).

### 2.4 Physicochemical Analysis

#### 2.4.1 pH

pH was measured for all the samples by dipping the probe into the sample solution and recording the reading (AOAC 1985).

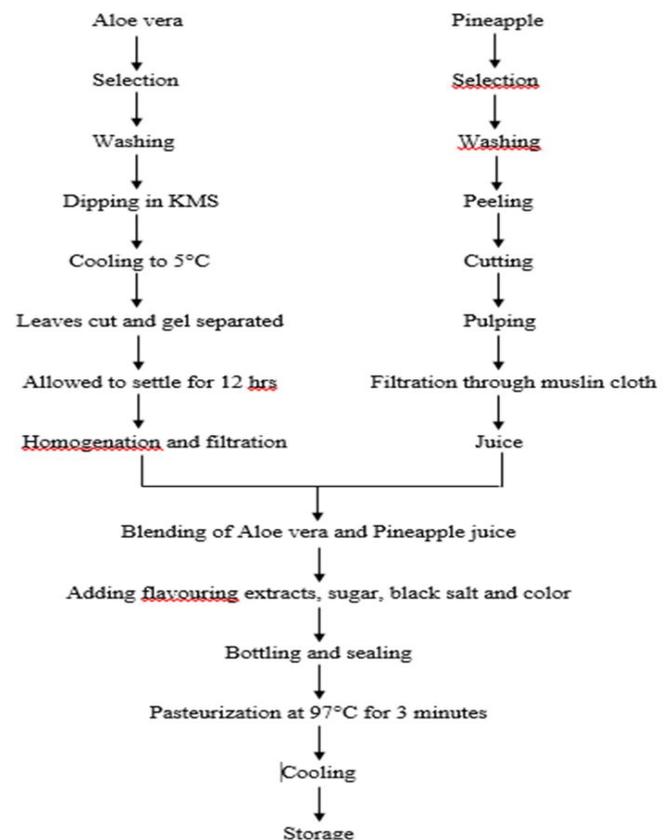
#### 2.4.2 Determination of titratable acidity

Titratable acidity (as % citric acid) was determined by titrimetric method (Ranganna 1986). Titratable Acidity Determination test is intended for measuring the Titratable Acidity of Pineapple Juice. Results are reported as g of citric acid/100 mL, equivalent to % citric acid. Results are expressed as percentage acid:

$$\text{Percentage acid} = \frac{\text{Titre} \times \text{Acid factor} \times 100}{10 (\text{ml juice})}$$

#### 2.4.3 Determination of antioxidant activity by reducing power assay

Antioxidant activity was determined by reducing power assay as described by Oyaizu (1986). 100 µl of blended treatments were taken in different test tubes and mixed with 900 µl distilled water. 1 ml of methanol was added to all the test tubes. To each test tube, 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of 1% Potassium ferricyanide was added. Tubes were then placed in a boiling water bath for 20 min at 50°C, cooled rapidly and mixed with 2.5 ml of 10% trichloroacetic acid and 0.5 ml of 0.1% ferric chloride. The amount of Iron (II) ferricyanide complex was determined by measuring the formation of Perl's Prussian blue at 700 nm after 10 min.



**Figure-1:** Layout of the development of Aloe vera and pineapple juice blended juice

#### 2.4.4 Protein estimation by Lowry's assay

Lowry's assay is generally the most performed colorimetric assay for the determination of total protein was described by Lowry *et al* (1951). 400 µl of blended treatments were taken in different test tubes and mixed with 600 µl distilled water. Then, 5 ml of Reagent D was added to each test tube and placed in dark at room temperature for 10 minutes. After that 0.5 ml of Folin's reagent was added to the test tubes, again incubated at room temperature for 30 minutes and OD was recorded at 600 nm.

#### 2.4.5 Determination of total phenolic content by Folin Ciocalteau method

The total phenolic content estimation was carried out using Follin-Ciocalteu method as described by Singleton *et al* (1999). 0.1 ml Follin's reagent was mixed to the different blends taken in separate test tubes and incubated at room temperature in dark for 15 minutes. After incubation 2.5 ml of 7% Na<sub>2</sub>CO<sub>3</sub> was added to the tubes and again incubated in dark for 30 minutes. OD was recorded at 760 nm using UV visible spectrophotometer (2202).

#### 2.4.6 Microbiological evaluation

For the assessment of microbiological quality of juice, Enumeration of Aerobic Plate Count (APC), total fungal count were performed according to ISO standard method (ISO-4833:2003(E)). NAM (APC), PDA (Total Fungal Count) and MacConkey media (Total coliform Count) were prepared, autoclaved (at 15 psi for 15 minutes) and poured into autoclaved petriplates. The media were left to solidify in the laminar air flow. Juice samples were serially diluted in sterile (0.1%) peptone water and spread over the solidified agar plates. The plates were incubated for 24 hours at 37°C. After incubation the number of colonies were counted and recorded.

#### 2.4.7 Shelf life analysis

Shelf life is a product of physical, microbiological and chemical processes, triggered by any one of a multitude of contributing factors. Quality test for each parameter such as pH, titratable acidity, antioxidant activity, protein content and microbial analysis were performed till day 21 of blend preparation at regular interval of 7 days.

#### 2.4.8 Statistical analysis

Statistical analysis of the data was done on Excel sheet by one factor analysis of variance (Snedecor and Cochran, 1989), which is a special case of analysis of variance (ANOVA), for one factor of interest. It is a generalization of Student's t test for independent samples to situations with more than two groups.

### 3. RESULTS AND DISCUSSION

#### 3.1 Organoleptic evaluation

There was not a great difference in taste in all the blends as the non-bitter *Aloe vera* juice was quite tasteless. Amongst all the blends, blend T<sub>1</sub> with pineapple-*Aloe vera* juice (90:10) was found to be most acceptable. The scores of all the blends are depicted in table 2. Similar studies were found and Rahman *et al* (2015) recorded sensory attributes of the fixed fruit jam made from *Aloe vera*, pineapple and mango and Tiwari *et al* (2015) recorded the observation on preparation and storage of blended RTS beverage from bael and *Aloe vera*.

**Table -2:** Sensory scores of the developed blends

	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
Color	9.0	8.5	8.5	8.0
Flavor	9.0	9.0	8.9	8.0
Taste	8.5	9.0	8.9	8.7
O.A	9.0	9.0	9.0	8.9

O.A= Overall acceptability

Treatment T<sub>1</sub> was found to be most acceptable blend, therefore its sensory quality was observed throughout storage period as depicted in Table 3. It was noticed that by day 14, the sensory quality of the blend fell tremendously. The blend tasted quite good till day 7. Organoleptic parameters like color, flavor, and taste of the juices were acceptable up to 14 days of storage which was less than previous studies of orange and pineapple juice studies by Masih D and Jan Awsi (2012), Islam M.A *et al* (2014) where the product was acceptable up to 21days of storage.

**Table -3:** Sensory Quality of T<sub>1</sub> during storage

TREATMENTS	0 <sup>th</sup> Day	7 <sup>th</sup> Day	14 <sup>th</sup> Day	21 <sup>st</sup> Day
Color	8.5	8.5	8.5	8.5
Flavor	9.0	8.4	7.5	7.5
Taste	9.0	7.0	5.0	5.0
O.A	9.0	7.5	5.0	4.2

#### 3.2 Physio- Chemical Analysis

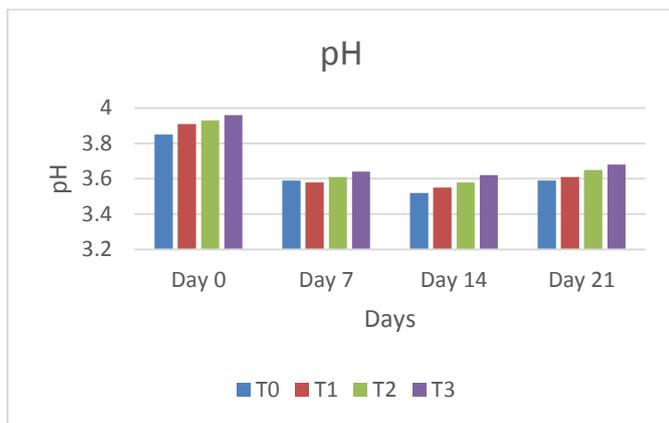
##### 3.2.1 pH

Pineapple juice being a very acidic fruit has a very low pH, therefore as the amount of pineapple juice decreases in the respective blends, the pH increases which can be seen in the table 4. With the increase in concentration of *Aloe vera* from T<sub>0</sub> to T<sub>3</sub>, the pH value is seen to be increasing. This is due to high pH of *Aloe vera* juice. The pH lies in a very acidic range as observed by Mishra *et al* (2013) who studied the physico-chemical properties of bael and aloe vera blended beverages.

**Table -4:** Sensory Quality of T<sub>1</sub> during storage

TREATMENTS	pH
T <sub>0</sub>	3.85
T <sub>1</sub>	3.91
T <sub>2</sub>	3.93
T <sub>3</sub>	3.96

It was observed that with time the pH of the blends kept on decreasing (figure 2). This might be due to the production of acids by the developing microbes in the juice. T<sub>3</sub> always had the highest pH due to the least pineapple juice concentration amongst all the blends. Pure pineapple juice showed a very acidic in nature with a pH of 3.5. Similar result was found by Dhaliwal and Hira (2001), Masih D and Jan Awsi (2012), Islam M.A et al (2014).



**Figure -2:** Graph depicting change in pH during storage

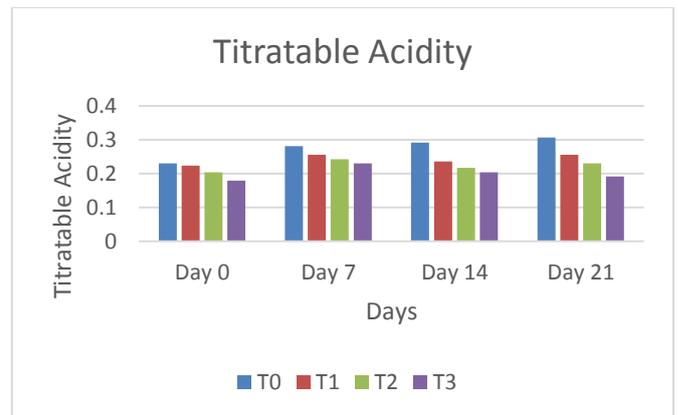
**3.2.2 Determination of titratable acidity**

pH and titratable acidity are inversely proportional to each other. The treatment with lowest pH value has highest acidity and vice-versa. The titratable acidity can be seen in table 5. Similar observations were recorded by Islam *et al* (2014) who carried out the analysis on mixed fruit juice from orange and pineapple.

**Table -5:** Titratable acidity of pineapple-Aloe vera juice blends

TREATMENTS	Titratable Acidity
T <sub>0</sub>	0.230
T <sub>1</sub>	0.224
T <sub>2</sub>	0.204
T <sub>3</sub>	0.179

It was observed that with time, the titratable acidity of the blends kept on increasing. It is because pH and titratable acidity are said to show an inverse relation to each other. T<sub>3</sub> being the least acidic in nature was observed to have the highest titratable acidity every time (figure 3).



**Figure -3:** Graph depicting change in titratable acidity during storage

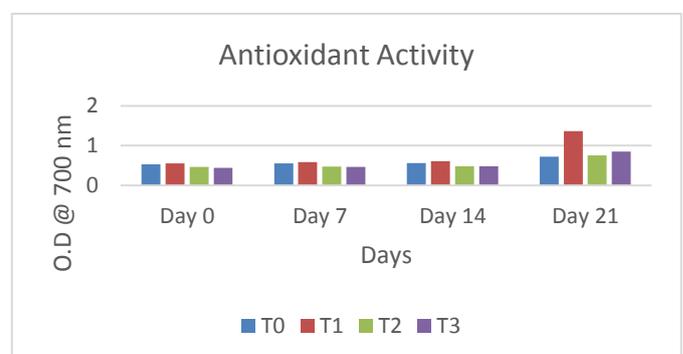
**3.2.3 Determination of antioxidant activity**

The blends were seemed to exhibit a very high antioxidant activity. Both the phenolic content and ascorbic acid content of fruits are responsible for their antioxidant activity. Amongst all the blends, T<sub>1</sub> (90:10 pineapple-aloe) showed highest antioxidant activity as shown in table 6. This might be due to high antioxidant activity of pineapple juice which is increased slightly with the supplementation of small amount of *Aloe vera* juice.

**Table -6:** Antioxidant activity of pineapple-Aloe vera juice blends

TREATMENTS	O.D at 700 nm
T <sub>0</sub>	0.53
T <sub>1</sub>	0.55
T <sub>2</sub>	0.46
T <sub>3</sub>	0.44

With passing week, antioxidant activity seemed to be increasing slightly (figure 4). This might be due to accumulation of substances produced by growing microbes.



**Figure -4:** Graph depicting change in antioxidant activity during storage

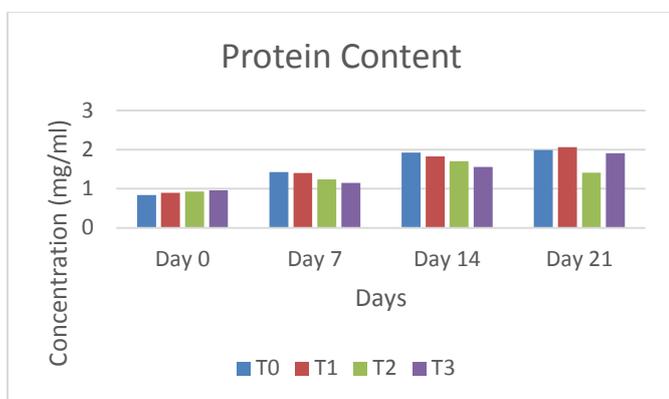
### 3.2.4 Protein estimation by Lowry's assay

The protein content of blends showed a slight increase with the increase in the quantity of Aloe vera juice. Blended juices contain higher protein content and it increases with the increase in *Aloe vera* concentration as shown in table 7. This may be due to increasing quantity of *Aloe vera* in the blends. Similar amounts of protein content of 0.872 mg/ml were reported in pineapple pulp extract previously in work done by Srinath *et al* (2012) on Isolation and characterization of Bromelain from pineapple (*Ananas Comosus*) and comparing its anti-browning activity on apple juice with commercial antibrowning agents.

**Table -7:** Protein content of pineapple-*Aloe vera* juice blends

TREATMENTS	Protein content (mg/ml)
T <sub>0</sub>	0.84
T <sub>1</sub>	0.90
T <sub>2</sub>	0.93
T <sub>3</sub>	0.96

The change in protein content with storage is given in figure 5. The protein content increased with every passing week, this might be due to the increasing number of microbes resulting in the production of extra proteinaceous substances.



**Figure -5:** Graph depicting change in Protein content during storage

### 3.2.5 Determination of total phenolic content by Folin Cioalteau method

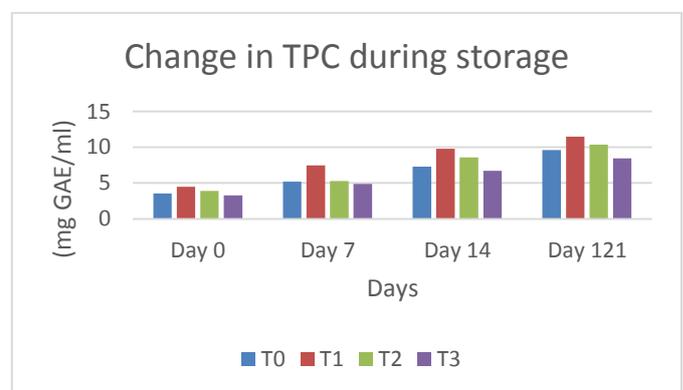
TPC was estimated for all the blends out of which, treatment with 90:10 pineapple-*aloe vera* juice was found to contain the highest amount of phenolics. T<sub>1</sub> shows the highest phenolics, this may be due to highest quantity of pineapple juice supplemented with a little amount of *Aloe vera* (10%) as seen in table 8. Similar results were reported for pineapple juice in previous work by Mahdavi *et al* (2010) on determination and comparison of total

polyphenol and vitamin c contents of natural fresh and commercial fruit juices.

**Table -8:** TPC of pineapple-*Aloe vera* juice blends

TREATMENTS	Total Phenolic Content (mg GAE/ml)
T <sub>0</sub>	3.55
T <sub>1</sub>	4.50
T <sub>2</sub>	3.90
T <sub>3</sub>	3.30

The total phenolic content also increased (figure 6) every week similar to the protein content.



**Figure -6:** Graph depicting change in TPC during storage

### 3.2.6 Microbiological evaluation

The blends were found to be absolutely safe for consumption till day 14, except treatment T<sub>3</sub> which had the highest *Aloe vera* concentration amongst all was safe for consumption till day 21. This proves that the shelf life of the blend is affected by concentration of *Aloe vera*, which showed antimicrobial effect in the blended juice. The microbiological analysis for the treatments showed no growth for bacteria, coliforms and yeast and moulds on the first day of preparation which is similar to the *Aloe* and *Pineapple* jam study performed by Joy K *et al* (2013).

#### Total Plate Count

**Table -9:** Total Plate Count during storage

TREATMENTS	0 <sup>th</sup> Day (cfu/ml)	7 <sup>th</sup> Day (cfu/ml)	14 <sup>th</sup> Day (cfu/ml)	21 <sup>st</sup> Day (cfu/ml)
T <sub>0</sub>	Nil	0.28×10 <sup>2</sup>	3.04×10 <sup>2</sup>	2.27×10 <sup>3</sup>
T <sub>1</sub>	Nil	0.14×10 <sup>2</sup>	1.16×10 <sup>2</sup>	2.72×10 <sup>3</sup>
T <sub>2</sub>	Nil	0.66×10 <sup>2</sup>	0.92×10 <sup>2</sup>	1.73×10 <sup>3</sup>
T <sub>3</sub>	Nil	0.16×10 <sup>2</sup>	1.14×10 <sup>2</sup>	0.53×10 <sup>3</sup>

The Total Plate Count of the juice must not exceed  $10^3$ . Hence, we can say that the blends were fit for consumption till day 14 (table 9).

#### Yeast and Mould Count

All the counts were in the acceptable range. Hence, from yeast and mold point of view, the blends were completely safe till day 21 (table 10).

**Table -10:** Yeast and Mold Count during storage

TREATMENTS	0 <sup>th</sup> Day (cfu/ml)	7 <sup>th</sup> Day (cfu/ml)	14 <sup>th</sup> Day (cfu/ml)	21 <sup>st</sup> Day (cfu/ml)
T <sub>0</sub>	Nil	$0.02 \times 10^2$	$0.02 \times 10^2$	$0.16 \times 10^2$
T <sub>1</sub>	Nil	$0.02 \times 10^2$	$0.01 \times 10^2$	$2.2 \times 10^2$
T <sub>2</sub>	Nil	$0.02 \times 10^2$	$0.04 \times 10^2$	$0.3 \times 10^2$
T <sub>3</sub>	Nil	$0.02 \times 10^2$	$0.14 \times 10^2$	$0.04 \times 10^2$

#### Coliform Count

From day 0 to day 21, the colony forming units of coliforms were found to be negligible. Hence, the blends were in an excellent condition till 21 days with respect to coliforms (table 11).

**Table -11:** Total Coliform Count during storage

TREATMENTS	0 <sup>th</sup> Day (cfu/ml)	7 <sup>th</sup> Day (cfu/ml)	14 <sup>th</sup> Day (cfu/ml)	21 <sup>st</sup> Day (cfu/ml)
T <sub>0</sub>	Nil	<1	<1	<1
T <sub>1</sub>	Nil	<1	<1	<1
T <sub>2</sub>	Nil	<1	<1	<1
T <sub>3</sub>	Nil	<1	<1	<1

#### 4. CONCLUSIONS

It was concluded that the treatment T<sub>2</sub> [pineapple: Aloe vera (80:20)] were found to be most effective juice blend for minimum change in pH (3.93 to 3.65), protein (0.93 to 1.41 mg/ml), antioxidant activity (0.46-0.75) and can be stored up to 14 days. Almost all the blends had almost equally good sensory characteristics and can be accepted in the market as a palatable healthy drink. On the basis of the results of the conducted experiments, it was concluded that the storage life of the blend can be increased by increasing the Aloe vera content. T<sub>3</sub> [pineapple: Aloe vera (70:30)] having the highest Aloe vera concentration was microbiologically safe up to 21 days of storage while the rest blends were microbiologically safe for consumption till day 14 of storage at chilled temperatures. Sensory quality was also good till the end of the storage. On the basis of above results revealed in the present study it may be concluded that the formulation of mixed blend juice beverage is possible to satisfy consumer taste and preferences along with imparting health benefits.

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**Assistant Professor**

Department of Food Process Engineering, SHIATS-DU Allahabad.

E-mail : dorcus\_1234@rediffmail.com

E-mail (Off) : dorcus.masih@shiats.edu.in

**BIOGRAPHIES**


M.Tech in Food Technology (Food Process Engineering) from SHIATS-DU Allahabad. B.tech in Biotechnology from Amity University, Lucknow.

e-mail id: shobiswas@gmail.com