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Effect of chemical preservatives on the shelf life of tomato juice

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Abstract:
This research conducted with the fully fresh, ripe and sound tomato (Lycopersicon esculentum Mill.) was collected then washed, peeled, seeds removed and transferred to the juice extractor. Sugar, preservatives were added to the extracted filtrated juice. Then the juice was heated, cooled and bottled for preservation. Tomato juice was prepared with three different treatments. Among the three treatments T1, T2 and T3 sample were prepared with no preservative, Na-benzoate preservative and potassium meta-bisulphite (KMS) preservative respectively. The organoleptic observation of this tomato juice was studied for 60 days storage period. Chemical analysis and sensory tests were carried out during the 30 days at an interval of 15 days to assess the effect of chemical additives on the shelf life of tomato juice. Negligible Change in chemical constituents except vitamin C was observed in the prepared juice throughout the 30 days storage period. Color was gradually faded and slightly off flavor develops at the end of the storage periods. The treatment T2 secured highest score for color, flavor, taste and overall acceptability and ranked as "Like very much" by a taste testing panel. Tomato juice prepared with no preservative (T1) spoiled after 45 days storage and juice prepared with KMS preservative (T3) spoiled after 60 days storage. Total number of viable bacteria was highest in tomato juice treated with no preservative (T1) and KMS preservative (T3). Tomato juice with Na-benzoate preservative (T2) contained least viable bacteria which was better than T1 and T3 sample. Considering all the parameters, Na-benzoate tends to be better additives than potassium meta-bisulphite (KMS) for preservation of tomato juice.

Keywords: Tomato, Preservative, Sensory Analysis, KMS, Storage.

1.0. Introduction:

In modern day diets, vegetable juice places a very important role in many cities all over the world. It became important in recent years due to overall increase in natural juice consumption as an alternative to the traditional caffeine containing beverages such as coffee, tea, carbonated soft drinks. Making juice is an easy way to add more vegetable to any diet. Consuming fruits and vegetables promote health, energy, quality of life. A diet that is rich in fruits and vegetables has been associated with protection against cardiovascular disease [8] and several common cancers [34]. Fruits and vegetables juice have significant improved blood profile in people affected by hyper-cholesterolemia as well as promote detoxification of human body [10].

Tomato is one of the most popular and demanding vegetables because of its vitamin and valuable nutrients content. Tomato (Lycopersicon esculentum) belongs to the family Solanaceae. Its center of origin is presumed to be in the present state of Mexico [15]. It is believed that the tomato was introduced Indian subcontinent during the British resume. Now days in Bangladesh, good amount of tomato grows. In 2016-17, 389 thousand metric tons tomato produced in Bangladesh (BBS, 2017).

Tomato is highly perishable vegetables and rapidly deteriorates after ripening. Due to lack of processing, storage and transport facilities, 35 percent of the total production of fruit and vegetables are spoiled and lost after harvest [1]. In season, price and demand of tomatoes are very low which causes economic loss for the farmer. On the other hand, during end period of harvesting season the tomato price becomes 2 to 3 times higher. Hence the short preservation like making tomato juice, tomato puree, cocktail, paste, ketchup, sauce, jelly, soups, powder and Tomato chutneys etc. will help the grower by reducing economic loss.

Tomato juice is a ready to drink tomato products. The quality attributes (flavor, color, consistency and nutritive value) of tomato juice are influenced by variety, climate, cultural practice, harvesting procedure, degree of ripeness, length of storage before processing, washing, sorting and steps of the processing procedure. The effects of length and temperature of storage period and the relationship of oxygen, light, sugar, pH and ascorbic acid change in color of juices. Storage temperature and oxygen were the most specific for color injury of both juices and pigments. Exposure to light caused little deterioration in color. Tomato juice is also susceptible to microbial spoilage and readily spoiled by the bacterial growth and activities. Considering all the above factors an attempt was made to prepare the tomato juice from different chemical preservatives namely Potassium meta-bisulphite, Na-Benzoate and to study their effect on the shelf life to fulfill the following specific objectives;

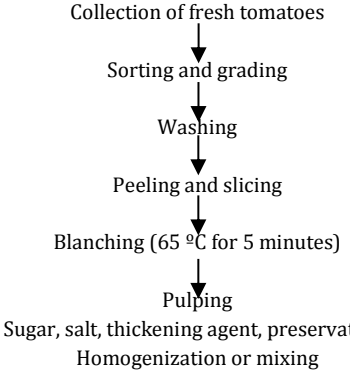
1. To study the proximate composition, shelf life and sensory attributes of prepared tomato juice.
2. To examine the effect of chemical preservatives namely Potassium meta-bisulphite, Na-Benzoate on the physiochemical characteristics of tomato juice.

2.0 Materials and Methods

This Research was performed in the laboratory of the Department of Food Processing and Preservation and some analysis were conducted in Agricultural-chemistry laboratory, Hajee Mohammad Danesh Science and Technology University, Dinajpur. The mature tomatoes, sugar preservatives and other ingredients were collected from local market of Dinajpur. Chemicals and reagents in the study were used from laboratory stockanalytically graded with the highest purity.

2.1 Methodology

Tomato Juice was prepared according to the method by [25].



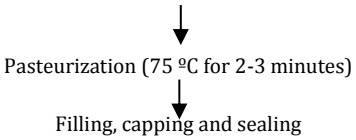


Figure 1: Schematic illustration of the experimental design

The experiment was examined with three samples which vary with the different preservatives. Basic treatments for preparation of Tomato juice shown in table 1

Table 1 Basic treatment for preparation of tomato juice

Ingredient	Sample T_1	Sample T_2	Sample T_3
Juice (ml)	60%	60%	60%
Water(ml)	40%	40%	40%
Sugar(gm)	20%	20%	20%
Preservatives(gm)	Control (No preservatives)	Na-Benzoate 300 ppm	KMS 300 m

2.2. Proximate analysis
The proximate analysis of moisture and ash was carried out by using AOAC standard method [6]. Another analysis protein content, Acidity and vitamin C content (Ascorbic acid) were determined by using Kjeldahl method and Rangana method [32] respectively. Total soluble solids (TSS) and pH also determined by using refractometer (Model no. HI 96801, ROMANIA) and pH meter. The minerals content was determined by the method of Pearson [30].

2.3. Estimation of total sugar

2.3.1. Reducing sugar
The reagents used for the estimation of reducing, non-reducing and total sugar were Fehling's solution (A), Fehling's solution (B), Methylene blue indicator, 45% Neutral lead acetate solution, 22% Potassium oxalate solution.

2.3.1.1. Standardization of Fehling's solution
10 ml of both Fehling's solution A and B were mixed together in a beaker. Standard sugar solution was taken in a burette.10 ml of mixed solution and 25 ml distilled water taken into 250 ml conical flask and heated on hot plate. When the solution began to boil, three drops of methylene blue indicator solution was added to it. Mixed solution was titrated by standard sugar solution. The end point was indicated by decolorization of the indicator. Fehling's factor was calculated by using the following formula:

$$\text{Fehling's factor} = \frac{\text{Titre} \times 2.5}{1000}$$

2.3.1.2. Preparation of the sample
10 gm of filtered juice and 100 ml of distilled water were mixed in homogenizer and transferred to 250 ml volumetric flask. Then the mixture was neutralized with 0.1N NaOH and 2 ml of lead acetate solution was added and followed to stand for 10 minutes. After that 5 ml potassium oxalate solution was added and made to a volume of 250 ml. Then the mixture was filtered and made the dilution.

2.3.1.3. Titration for reducing sugar
10 ml of mixed Fehling's solution was taken in a conical flask and 25 ml of distilled water was added to it. Purified juice was taken in a burette. Conical flask containing mixed Fehling's solution was added to the flask when boiling started and titrated with solution taken in the burette at the same time. The end point was indicated by decolorization of indicator. Percent reducing sugar was calculated by using the following formula:

$$\% \text{ Reducing sugar} = \frac{I \times D \times 100}{T \times W \times 100}$$

2.3.2 Non-reducing sugar
50 ml purified solution and 50 ml distilled water taken in a conical flask and 5 gm citric acid added to it. Then added sucrose and heated the flask for 10 minutes and finally cooled. 0.1 N NaOH solution used to neutralize the sample. The volume was made up to 100 ml with distilled water. For titrated mixed Fehling's solution similar procedure followed as for reducing sugar. Also invert sugar was calculated by using similar procedure as for reducing sugar from which the percent non-reducing sugar is calculated as follows:For titrated mixed Fehling's solution similar procedure followed as for reducing sugar. Invert sugar was calculated by using similar procedure for reducing sugar from which the percent non-reducing sugar is calculated as follows:

% Non-reducing sugar =% Invert sugar-%Reducing sugar

2.3.2.1 Estimation of total sugar
Total sugar can be calculated as follows:

% Total sugar = %Reducing sugar +%Non-reducing sugar.

2.4 Determination of minerals content in tomato juice
1 ml sample with 15 ml of diacid mixture (2:1) ($HNO3$: $HClO_4$) was taken in a beaker and boiled until the solution become clear. Cooled and made the volume in 100 ml.

2.4.1 Estimation of calcium
5 ml solution mixed with 20-25 ml hot distilled water. Added 10 drops of each solution of Potassium pherocyanite, OH- amine, hydrochloride, triethanolamine and 5 ml of NaOH buffer (10%) and 5-6 drops calcon indicator. test sample was titrated against EDTA (0.01M) solution from a burette until color changed from pink to blue.

1 ml 1 M Na_2-EDTA= 40.08 mg Ca

2.4.2 Estimation of magnesium
5 ml solution mixed with 20-25 ml hot distilled water. Then 10 drops of each solution of Potassium pherocyanite, OH- amine hydrochloride, triethanolamine, Na tungstate and 5 ml ammonium buffer and 5-6 drops EBT (Erriochrome Black-T) was added. Test sample was titrated against EDTA

(0.01M) solution from a burette until pink color completely turned to pure blue color.

1 ml 1 M Na_2-EDTA=24.305 mg of Mg

2.4.3 Estimation of Potassium

Exactly 1.907 g KCl and 300-400 ml distilled water was taken in a 1000 ml volumetric and shake to dissolve. Then the volume made up to the mark with distilled water. Concentration of that solution is made 100 ppm of potassium. 5 ml, 10 ml, 15 ml, 20ml, 25 ml, 30 ml, 40 ml, 50 ml and 60 ml of 100 ppm K solution was taken in several 100 ml volumetric flask and made volume up to the mark with distilled water.Finally, in Flame Emission Photometer showed the reading directly for unknown solution.

2.5 Microbiological test

2.5.1 Determination of total viable bacteria

Total viable count of microorganism present in the sample was followed according to the method "Recommended method for the microbiological examination of food" method [5].

2.5.2 Preparation of media

Peptone2.5 gm, Agar9.0gm, Beef extract1.5 gm, Sodium chloride (NaCl)1.0 gm, Distilled water500 ml taken in a conical flask and heated for proper mixing. During heating the mixture was rotted with a glass rod. After proper mixing, the head of the flask was blocked with cotton plug andaluminum foil. Then the conical flask with media was placed in autoclave for sterilization (Temperature: 121^{0}C, Pressure: 15 Ib/inch^2 and time: 15 mins.).

2.5.3 Preparation of dilution blank

In order to dilute the sample consecutively 1ml of the sample was diluted stepwise through a series of tubes containing 9 ml of distilled water. At first 9ml of the distilled water was taken in a sterile test tubes and then 1ml of the original sample (juice) was taken to the first test-tube with a sterile pipette. Water with the sample was vigorously shaken for homogenous distribution of the bacterial population in the solution. This tube was denoted as "A". From the tube "F-1" another 1ml aliquot was transferred to the second tube and this tube was denoted as "F-2". In this way "F-3", "F-4", "F-5", "F-6" was prepared until the desired dilution is achieved. Now the tube "F-1" has got the dilution 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} respectively.

The dilutions were as follows:

Tube No.	Dilution	Volume of original fluid per mi
1	1/10	0.1 or 10^{-1}
2	1/100	0.01 or 10^{-2}
3	1/1,000	0.001 or 10^{-3}
4	1/10,000	0.0001 or 10^{-4}
5	1/100,000	0.00001 or 10^{-5}
6	1/1,000,000	0.000001 or 10^{-6}

2.5.4 Procedure of plating

Now from the test-tube "F-1", 1ml of the sample solution was taken in a sterile Petridis containing 9 ml of agar medium. The agar with bacterial sample was mixed by rotating the Petridis. This Petridis was marked as "A". In this way "B", "C", "D", "E", "F" marked Petri dishes were prepared from the tubes "F-2", "F-3", "F-4"," F-5" and "F-6" respectively. Then these Petri dishes were placed on a level surface for few minutes for solidifying the agar medium.

2.5.5 Incubation and colony count

After solidification Petridis were placed in the incubator at 36-37^0 C for 20-24 hours, the over loaded Petri dishes were avoided and the Petri dishes containing countable colony were selected. Colonies were counted with the aid of a magnifying glass and finally the total number of bacteria per gram of sample was calculated by the following equation:

Colony count (per ml) = Number of colonies (per plate) × Reciprocal of the dilution.

2.6 Sensory evaluation of tomato juice

The sensory evaluation of tomato juice was evaluated for color, flavor, taste, texture and overall acceptability parameters by 20 tasters.For the evaluation of tomato juice were given to 20 panelist and randomly coded sample and asked to rate the given sample a 9 point hedonic scale with ratings of: 9 =Like extremely, 8 = Like very much, 7 = Like moderately, 6 = Like slightly, 5 = Neither like or unlike, 4 =Dislike slightly, 3 =Dislike moderately, 2 = Dislike very much, 1 = Dislike extremely. The results were evaluated by Analysis of Variance and Duncan's Multiple Range Test (DMRT) procedures of Statistical Analysis System (SAS, 1985).

2.7 Studies on storage stability of prepared tomato juice

Processed tomato juice was store at refrigeration temperature (4°C) in glass bottle, shelf life of tomato juice was assessed by objective and subjective tests at different time intervals. The moisture content, pH, acidity, TSS, vitamin C, color, flavor, texture and fungal growth were observed initially for 1 month.

2.8 Statistical analysis

Data were analyzed using statistical software R (windows version 2.13.1). a single factor analysis of variance was carried out. Significant difference was estimated using Duncan Multiple Range Tests (DMRT). Differences were considered to be significant at ≤ 0.05.

3.0. Result and Discussion

The tomato juice is perishable and subjected to quick deterioration during storage. The present study was undertaken to assess the effect of different preservatives on the tomato juice during storage at refrigeration temperature.

3.1. Proximate composition of tomato juice

The juice samples were analyzed for physical (moisture, pH, acidity, total sugar and total soluble solid), chemical (protein, ash, vitamin C) and mineral

(calcium, magnesium, potassium) properties. The approximate composition of tomato juice per 100 g showed in the Table 2.

Table 2 Approximate composition of tomato juice per 100 g

Components	Fresh Pulp	Processed Juice
Moisture	93.50	81.28
Ash	0.28	0.248
Acidity	0.424	0.312
pH	3.85	4.69
Vitamin C	16.75	12.25
Total soluble solid	2.00	13.00
total sugar	3.65	8.00

3.2. Chemical analysis for tomato juice

Physical properties of tomato juice immediate after preparation shown in table 3

Table 3 Physical properties of tomato juice immediate after preparation i.e., 0th day

Sample	Treatment	Moisture (%)	pH	TSS (%)	Acidity (%)	Total sugar (%)
T_1	Control	81.28^c	4.69^a	13.13^a	0.248^a	6.44^c
T_2	Na-benzoate	83.15^b	4.68^a	13.46^a	0.31^a	8.06^b
T_3	KMS	83.22^a	4.69^a	13.22^a	0.255^a	9.66^a
LSD		0.063	0.089	0.641	0.06	1.28
CV %		0.02	0.96	2.42	10.67	0.43

a, b, c means bearing the same superscript within the column do not differ significantly (p≥0.05).

The prepared tomato juice was tested for chemical composition immediately after preparation, i.e. 0th day. The moisture content of samples T_1, T_2 and T_3 were 81.28%, 83.15%, 83.22%, respectively. The highest amount of moisture content in samples T_3 was treated with KMS compare to other samples. This result is close to tomato juice (86.32% moisture) obtained by Jannat *et al.,* (2016). There was significant different between the sample T_1, T_2 and T_3. The moisture content was found for tomato juice reported ranged from 93.8% to 94.1% depending on the varieties [24]. The small variation may be due to technical error in measuring procedure and the difference is statistically insignificant. The high moisture content of these fruit juices gives great impact on energy density (amount of energy in a given weight of food (kcal/g)) as water adds substantial weight to the food without adding energy and this may give the consumers a better satiety without increase their energy intake [28].

The pH of sample T_1, T_2 and T_3 were 4.69, 4.68 and 4.69, respectively. The highest amount of pH in sample $T_1$was treated with no preservatives other than two samples. There was no significant difference between the samples. Several researchers have reported fruit and vegetable juices with different pH values. Tomato juice has been reported to contain a pH range of 3.7 – 4.5 by Frazier and Westhoff (1995). Akusu *et a.,* (2016) found that pineapple juice has a pH value of 3.97. Adubofuor *et al.,* (2010) reported a range of 4.82 – 4.99 for cocktail juices, Ndife *et al.,* (2013) observed a range of 3.23 – 4.08 for different brands of orange juices.

The total soluble solid of sample T_1, T_2 and T_3 were 13.13, 13.46 and 13.22° Brix, respectively. The highest amount of pH in sample $T_2$was treated with Na-benzoate compare to other two samples. There was no significant difference between the samples.

The acidity of sample T_1, T_2 and T_3 were 0.248%, 0.31% and0.255%, respectively. The highest amount of acidity in sample T_2 was treated with Na-benzoate compare to other two samples. There was no significant difference between the samples. The result is similar that is reported by Herna´ndez *et al., (*2007b) was obtained 0.30 ± 0.09 g/100 g acidity.

The total sugar of sample T_1, T_2 and T_3 were 6.44%, 8.06% and9.66%, respectively and showed significant difference in all the samples. Ndife *et al.,* (2013) reported a range of 9.15-14.25% total sugar for different brands of orange juice. El-Sheikha *et al.,* (2010) reported a Recommended Dietary Allowances (RDA) of 130g/day for total sugars, consumption of tomato fruit juice will contribute about 14.88%. Chemical & mineral properties of tomato juice immediate after preparation was shown in table 4.

Table 4 Chemical & mineral properties of tomato juice prepared immediate after preparation i.e., 0th day

Sample	Treatment	Protein (%)	Ash (%)	Vitamin-C mg/100 g	Ca mg/100ml	Mg mg/100ml	K mg/100ml
T_1	Control	0.85^a	0.27^a	12.25^b	9.13^b	11.25^a	222.4^a
T_2	Na-benzoate	0.84^a	0.27^a	15.62^a	10.08^a	11.80^a	228.8^a
T_3	KMS	0.85^a	0.28^a	15.75^a	10.03^a	11.13^a	226.3^a
LSD		0.11	0.063	0.58	0.92	0.72	8.93
CV %		5.95	2.31	2.00	4.64	3.15	1.98

Protein content of the different concentration of tomato juice was low, ranging from 0.85 – 0.85% for samples T_1 and T_3, respectively (Table 3.3). Sample T_3 was observed to have higher value compared to other samples and there was no significant difference.

The ash content was observed in sample T_1 (0.27%), T_2 (0.27%), T_3 (0.28%). There was no significant difference between samples T_1, T_2 and T_3. Akusu *et al.,* (2016) found the value 0.30% for tomato juice.

The vitamin C of sample T_1, T_2, T_3 were 12.25, 15.62, 15.75 mg/100 g, respectively. The highest amount of vitamin C in sample T_3 was treated with KMS other than two samples. There was significant difference between the samples. Apart from samples T_1 (12.25 mg/100 g), there was no significant difference between sample T_2 (15.62 mg/100 g) and T_3 (15.75 mg/100 g) for vitamin C. The values found in the present study were comparable to the ones reported by Kotkov *et al.,* (2011) (12-17 mg/100 g) and lower than that reported by Ilahy *et al.,* (2011) and Juroszek *et al.,* (2009).

Calcium content of different concentration of tomato juice has been shown in Table 3.4. Higher calcium value was found in sample T_2 (10.08%) as compared to sample T_1 (9.13%) and T_3 (10.03%). Apart from samples T_1, there was no significant difference between sample T_2 (10.08 mg/100 ml) and T_3 (10.03 mg/100 ml) for calcium. Calcium content of different varieties of tomato juice ranged 9.85-12.21mg/100ml reported by Ensminger *et al.,* (1983).

Magnesium content of the different tomato juice was ranging from 11.25 – 11.80 mg/100 ml for samples T_1 and T_2, respectively (Table 3.3). Sample T_2 was observed to have higher value compared to other samples and there was no significant difference. Watt and Merrill *et al.,* (1963) also found in a lower amount of magnesium in different varieties of tomato.
The highest potassium content was observed in sample T_2 (228.8mg/100ml) and lowest in sample T_1 with the value of 222.4 mg/100ml. There was no significant difference between samples T_1, T_2 and T_3.

3.3. Effect of chemical preservatives on the prepared tomato juice at various storage periods

3.3.1. Effect of chemical preservatives on moisture content

The Moisture content of chemically preserved tomato juice ranged from 81.28% – 83.22 %. Juice prepared with Na-benzoate (T_2) contained 83.15% moisture content on the day of preparation and 83.16% moisture content after 30 days storage. Juice prepared with KMS (T_3) contained 83.22% moisture content on the day of preparation and 83.16% moisture content after 30 days storage shown in Figure 2. Juice prepared with no preservatives (T_1) contained 81.28% moisture content on the day of preparation and 81.26% moisture content after 30 days storage. After 30 days of storage, moisture content reduced very small amount in juice prepare with Na-benzoate (T_2 sample) and Potassium meta-bisulphite (T_3 sample). But in case of Control samples, moisture content slightly reduced after 30 days storage. Moisture content remained more or less same through 30 days storage.

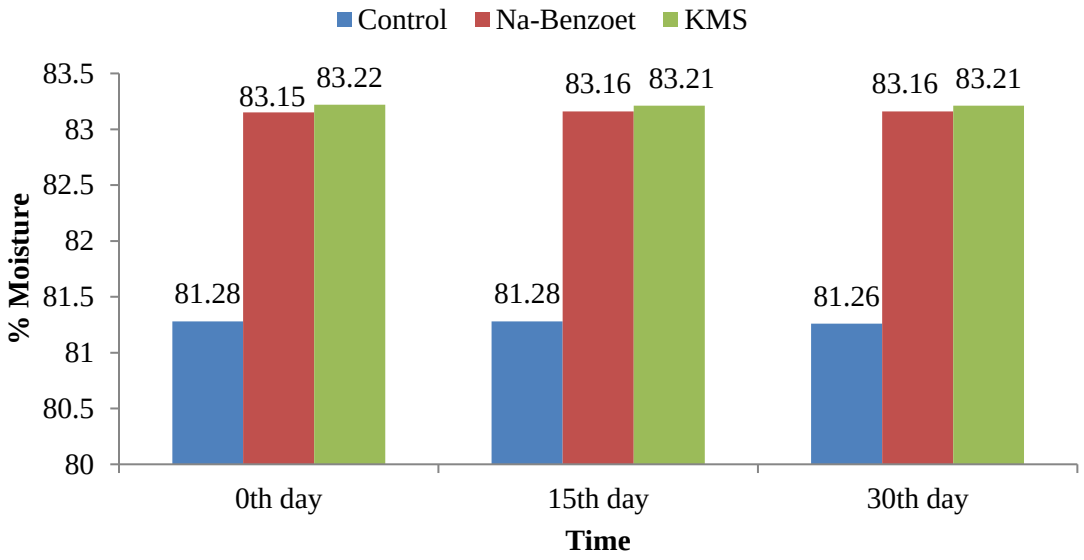


Figure 2: Effect of chemical preservatives on moisture content

3.3.2. Effect of chemical preservatives on pH

The pH of the examined tomato juices ranged from 4.43 to 4.69 shown in Figure 3. The pH decreased as the number of days of storage increased, indicating acidity in tomato juices. The use of sodium benzoate, potassium meta-bisulphite as preservatives significantly influenced the pH of tomato juice. The pH of samples was decreased after 15 days storage shown in figure 2. However, after 30 days storage, the pH of tomato juice preserved using potassium meta-bisulphite, Na-benzoate and the control sample slightly decreased. The slight reduction in pH, observed in sample T_1, T_2 and T_3 due to the biochemical degradation of sugars by colonizing microorganisms resulting in the production of acids. The work is similar to Shahnawaz *et al.,* (2013), Makanjuola *et al.,* (2013) and Ajibola *et al.,* (2009). They reported a decrease in pH with increasing storage periods in fruit juices. Mishra *et al.,* (2012) reported that pH values decreased from 4.02 to 3.41 in amla-grape blend juice during storage. Hosssain *et al.,* (2011) found that pH decreased from 4.30 to 2.90 in apples and apricot blend juice during storage. High acid and low pH may be due to production of acetic acid and lactic acid during storage.

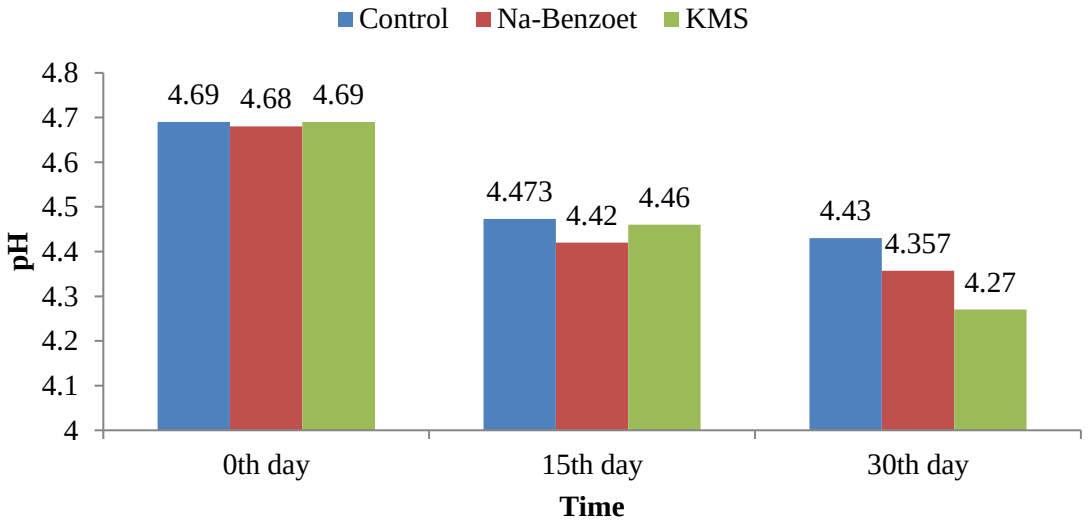


Figure 3: Effect of chemical preservatives on pH

3.3.3. Effect of chemical preservatives on TSS

The total soluble solids of chemically preserved tomato juice ranged from 12.16 – 13.13 ºBrix. The total soluble solids of samples T_1, T_2 and T_3 were 13.13°Brix, 13.46° Brix, 13.22°Brix in the preparation day storage. After 30 days storage the total soluble solids of samples T_1, T_2 and T_3 reduced to respectively 12.16° Brix, 13.05°Brix and 12.62° Brix shown in figure 4. After 30 days of storage the total soluble solids were the lowest in the control juice (12.16 °Brix) while the total soluble solids were the highest in the juice preserved with Na-Benzoate T_2 sample (13.05° Brix). These results are in agreement with the results obtained by Zeb *et al.,* (2009) during preservation of grape juice stored at room temperature for one month preserved with sodium benzoate and potassium sorbate. Similar results were also obtained by Hussain *et al.,* (2011) who worked on the storage of apple and apricot blended juice at refrigeration temperature for three months. Increase in soluble solids may be due to break down of polysaccharides into monosaccharide and oligosaccharides while decrease may be due to fermentation of sugars into ethyl alcohol, carbon dioxide and water [11].

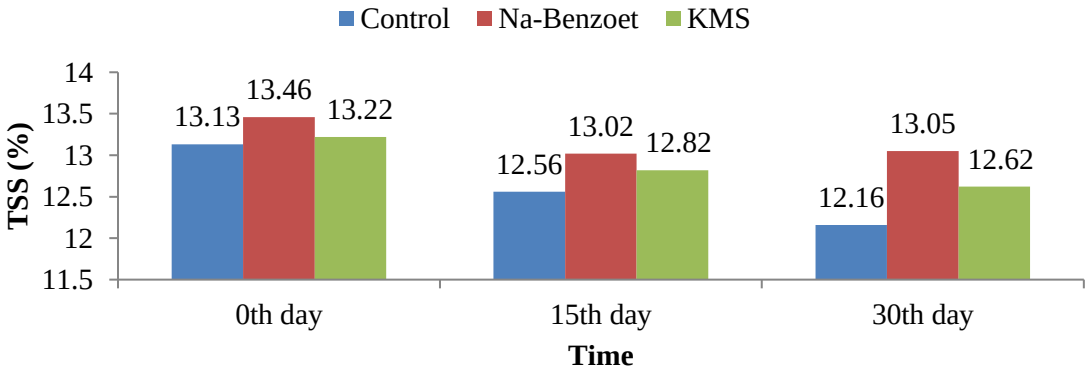


Figure 4: Effect of chemical preservatives on TSS

3.3.4. Effect of chemical preservatives on acidity

The total titrable acidity (TTA) of tomato juice ranged from 0.248 – 0.31 %. The TTA slightly increased as number of days of storage increased and was the highest in the potassium meta-bisulphite preserved sample and the lowest in control sample. The increase in the TTA of the juices might be due to fermentation activities by colonizing microorganisms [29]. In the preparation day, the total solids of samples T_1, T_2 and T_3 were respectively 0.248%, 0.31%, 0.255%. After 30 days storage the total solids of samples T_1, T_2 and T_3 were slightly increased respectively to 0.273%, 0.315%, 0.312% shown in Figure 5. This might be due to the high solubility index of sodium meta-bisulphite and sodium benzoate in water. This is in conformity with the report of Kaur and Aggarwal (2014) who evaluated the effect of different chemical preservatives on storage characteristics of bitter gourd. Wadikar *et al.,* (2010) reported that acidity increased from 0.25-0.36% during storage of cucumber-basil juice. Rahman and Hossain, (2011) found that acidity changed from 0.39% to 0.51% in apple and appricot blend juice during storage.

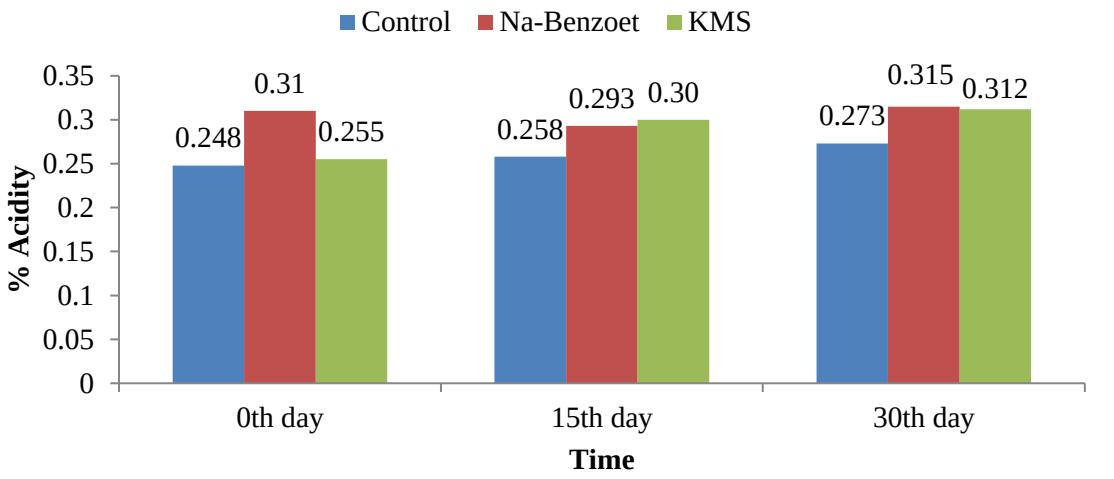


Figure 5: Effect of chemical preservatives on acidity

3.3.5. Effect of chemical preservatives on vitamin C

The ascorbic acid (vitamin C) contents of the samples are presented in Figure 6. The vitamin C content of the chemically preserved tomato juice ranged from 15.75 – 2.6 mg/100 g and decreased with increase in day of storage. This is in support of the report of Akinola *et al.,* (2017) on a juice blend of orange, watermelon, carrot and ginger. After the 30th days of storage, the vitamin C content was the highest in sodium benzoate (11.25 mg/100 g). In the preparation day, the vitamin C content of samples T_1, T_2 and T_3 were 12.25, 15.62 and 15.75 mg/100 g respectively shown in Figure 6. After 30 days storage the vitamin C of samples T_1, T_2 and T_3 were highly decreased to 2.6, 11.25 and 5.033 mg/100 g respectively. The results are in similar with the findings of Zeb *et al.,* (2009) who worked on the preservation of grape juice with sodium benzoate and potassium sorbate, stored at room temperature for one month, they observed decrease in vitamin C content of grape juice. The results of Ayub and Khan (2001) are in agreement with our results, who observed decrease in vitamin C content of pomegranate syrup, preserved under different light conditions and different packaging materials at room temperature for storage period of four months. The losses may be due to oxygen present in the product and headspace of the package. Hussain *et al.,* (2011) found that ascorbic acid changed from 5.67% to 3.64% in apple and appricot blend juice during storage. Ascorbic acid changed from 18.38 to 12.90 mg/100 ml juice in kinnow-anola-ginger blend juice [9].

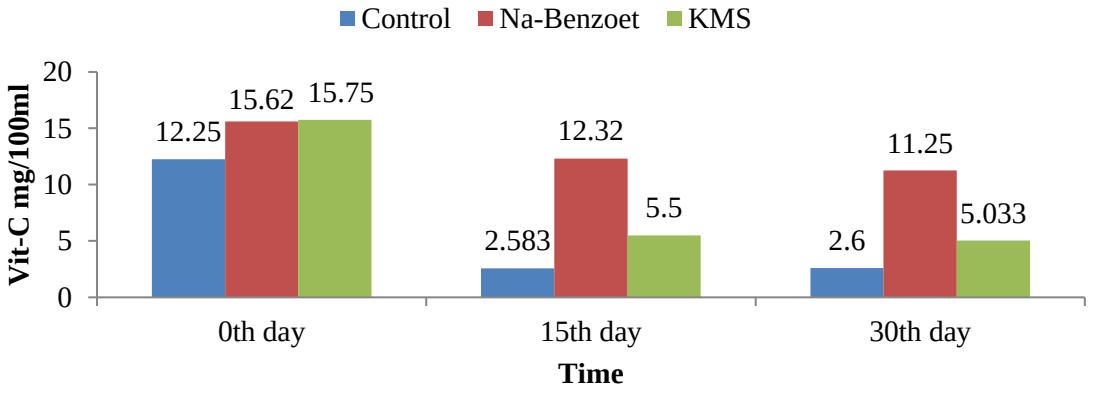


Figure 6: Effect of chemical preservatives on vitamin C

3.4. Storage studies

Three treatments of tomato juice were stored at refrigerated temperature (4°C). The color, flavor, texture, visual fungal growth was observed during the storage period of two months at refrigerated temperature (Table 5). In refrigerated temperature, there was no change in color, flavor and texture change up to 15 days of storage, then slightly changed. There was no fungal growth occurred up to two months for sample T_1, T_2 and T_3. Tomato juice treated with Na-benzoate (T_2) seems to be more stable as after 45 days storage. Tomato juice with KMS (T_3) was slightly pungent and sediment after 45 days storage and the juice treated with no preservatives was changed after 60 days storage. After two months of storage, color and flavor were slightly changed for T_2.

Table 5 Organoleptic evaluation of prepared tomato juice stored at refrigeration temperature

Storage period(days)	Treatment	Color	Flavor	Texture	Visual fungal growth
00	T_1	Natural	Natural flavor of Tomato juice	Uniform	No growth
	T_2	Natural			
	T_3	Natural			
15	T_1	No change	No off flavor	Uniform	No growth
	T_2	No change	No off flavor	Uniform	No growth
	T_3	No change	No off flavor	Uniform	No growth
30	T_1	slightly fade	slightly pungent	Uniform	No growth

	T_2	No change	No off flavor	Uniform	No growth
	T_3	No change	No off flavor	Uniform	No growth
45	T_1	Turbid/fade	Slightly pungent	slightly sediment	No growth
	T_2	No change	No off flavor	Uniform	No growth
	T_3	No change	Slightly pungent	Slightly sediment	No growth
60	T_1	Turbid/fade	Pungent	sediment	No growth
	T_2	Slightly Turbid	Slightly pungent	Slightly sediment	No growth
	T_3	Slightly Turbid	Slightly pungent	Slightly sediment	No growth

3.5 Microbial load calculation

Bacterial Growth: Microbiological activity, its multiplication and load were calculated (Table 6) during 30 days storage period for the juices from three samples. This study was performed by Colony count method and the dextrose tryptone Agar (DTA) media was used. After 48 hour of incubation colonies were counted. The total viable bacteria present in tomato juice were not uniform. Total viable bacterial count of the juice with no preservatives sample T_1 increased from 3.77 to 4.51 log cfu mL^{-1} after 30 days storage at refrigeration temperature. Total viable bacterial count of the juice with Na-benzoate sample T_2 increased from 3.53 to 3.88 log cfu mL^{-1} after 30 days storage at refrigeration temperature. Total viable bacterial count of the juice with KMS sample T_3 increased from 3.69 to 4.00 log cfu mL^{-1} after 30 days storage at refrigeration temperature. The result showed that among the three sample, sample T_3 contained least viable bacteria (3.88 log. cfu/ml) and sample T_1 contained highest viable bacteria (4.51 log cfu/ml) after 30 days at refrigeration temperature. From the above results, it is clear that the total viable bacteria count increased with the increase of storage period. Sample prepared with Na-benzoate T_2 contain less viable bacteria compare to other samples. Growth of bacteria in tomato juice at refrigeration temperature is shown in table 6.

Table 6 Growth of bacteria in tomato juice with three different additives at refrigeration temperature

Sample	Storage period	No, of total bacteria (cfu mL^{-1})	Total Count (log cfu ml^{-1})
	Initial	6×10^3	3.77
T_1 (Control)	15	1.1×10^4	4.04
	30	3.2×10^4	4.51
	initial	3.4×10^3	3.53
T_2 (Na-benzoate)	15	5.2×10^3	3.71
	30	7.6×10^3	3.88
	initial	5×10^3	3.69
T_3 (KMS)	15	7×10^3	3.84
	30	1.5×10^4	4.00

3.6. Sensory evaluation

A panel of 16 judges (teachers and students of faculty of Agricultural Chemistry) tested the color, flavor, taste and overall acceptability of tomato juice prepared with two preservatives Na-benzoate and KMS. The mean scores for color, flavor, taste and overall acceptability of different treatment of juice such as T_1, T_2 and T_3 are presented in Table 7.

Table 7 Mean score for color, flavor and taste and overall acceptability of Tomato juice

Treatment	**Sensory Attribute**			
Sample	**Color**	**Flavor**	**Taste**	**Overall Acceptability**
T_1 (Control)	5.1875^c	5.1250^c	5.1250^c	5.145^c
T_2 (Na-benzoate)	7.5625^a	7.2500^a	6.8125^a	7.208^a
T_3 (KMS)	6.3750^b	6.1875^b	5.9375^b	6.166^b
LSD (P<0.05)	0.5630	0.6117	0.6109	0.4487
CV%	12.40	13.88	14.40	10.15

a, b, c superscript indicates respectively the higher, medium and lower value. The test values along the different column carrying different superscripts are significantly different ($p < 0.05$).

Hedonic scale used: 9 = Like extremely; 8 = Like very much; 7 = Like moderately; 6 = Like slightly; 5 = Neither like nor dislike; 4 = Dislike slightly; 3 = Dislike moderately; 2 = Dislike very much; 1 = Dislike extremely.

Color:

A one way analysis of variance ANOVA (Appendix A.1 and Appendix A.1.1) was carried out forcolor preference and results revealed that there was significant (P<0.05) difference in color acceptability among the tomato juice. The results of DMRT (Appendix A.1.2) showed that there was significant difference for color difference among the three samples T_1, T_2 and T_3 (Table 7). In case of color preference among the treatment, the treatment T_2 was more acceptable than treatment $T_1$and T_3. Treatment T_2 secured the highest score 7.5625 and ranked as "Like very much". Treatment T_1 ranked as “Neither like nor dislike” and T_3 are ranked as "Like slightly" securing score 5.1875 and 5.9375, respectively.

Flavor:

A one way analysis of variance ANOVA (Appendix A.2 and Appendix A.2.1) was carried out forflavor preference and results revealed that there was significant (P<0.05) difference in color acceptability among the tomato juice. The results of DMRT (Appendix A.2.2) showed that there was significant difference for flavor difference among the three samples T_1, T_2 and T_3 (Table 7). In case of flavor preference among the treatment, the treatment T_2 was more

acceptable than treatment $T_1$and T_3. Treatment T_2 secured the highest score 7.2500 and ranked as "Like moderately". Treatment T_1 ranked as “Neither like nor dislike” and T_3 are ranked as "like slightly" securing score 5.1250 and 6.1875, respectively.

Taste:

A one way analysis of variance ANOVA (Appendix A.3 and Appendix A.3.1) was carried out fortaste preference and results revealed that there was significant ($P<0.05$)difference in taste acceptability among the tomato juice. The results of DMRT (Appendix A.3.2) showed that there was significant difference for taste difference among the three samples T_1, T_2 and T_3 (Table 7). In case of taste preference among the treatment, the treatment T_2 was more acceptable than treatment $T_1$and T_3. Treatment T_2 secured the highest score 6.8125 and ranked as "Like moderately". Treatment T_1 ranked as “Neither like nor dislike” and T_3 are ranked as "like slightly" securing score 5.1250 and 5.9375, respectively.

Overall Acceptability:

It was apparent from the results of the ANOVA (Appendix A.4 and Appendix A.4.1), that there was significant ($p<0.05$) difference in overall acceptability of the treatment tested as the calculated F (47.304) greater than the tabulated F value (2.960). This indicates that so far as overall acceptability is concern the treatments were not equally acceptable. Significant difference exist when F(Calculated)>F(Tabulated). It can be seen from Table 7 that the treatment T_2 is the most acceptable product receiving 7.208 out of 9.0 compared to the other treatment and ranked as "Like moderately". Treatment T_3 securing 6.166 ranked as "like slightly ". However T1securing 5.145 ranked as “Neither like nor dislike ".
Tomato juice T_2containing Na-benzoate preservative secured the highest score for color, flavor, taste and overall acceptability among all the treatment and was closely followed by fruit juice T_3 having KMS preservatives after 30days storage. So, treatment T_2 tomato juice may be regarded as the best juice among the four treatments.

4.0. Summary and Conclusion

To assess the physic-chemical properties and the effect of chemical preservatives on the shelf life of prepared tomato juice, three treatments namely, T_1, T_2 and T_3 were considered for study prepared with 60% tomato juice, 40% water, 20% sugar and 300 ppm sodium benzoate and KMS preservatives. Sample T_1, T_2 and $T_3$was prepared with no preservatives, Na-benzoate, KMS respectively. The shelf life and consumer`s acceptability of the prepared mixed juice were studied. The juice was stored at refrigerated temperature (4^0 C) for two months.

The physical and chemical analysis (Moisture, TSS, acidity, pH, vitamin C, Protein, Total sugar, mineral content such as potassium (K), calcium (Ca), magnesium (Mg) of the prepared tomato juice for treatment T_1, T_2, T_3were done immediate after preparation i.e. 0^{th} day. The effect of chemical preservatives on Moisture, TSS, acidity, pH, vitamin C were found at an interval 0, 15, 30 days storage period. On the day of preparation the **Treatment T_1** contained Moisture 81.28%, pH 4.693, TSS 13.13%,acidity 0.2480%, vitamin C 12.25 mg/100 g, total sugar 6.44%.**Treatment T_2** contained Moisture 83.15%, pH 4.677, TSS 13.46%,acidity 0.3117%, vitamin C 15.62 mg/100 g, total sugar 8.06%. **Treatment T_3** contained Moisture 83.22%, pH 4.687, TSS 13.22%,acidity 0.2553%, vitamin C 15.75 mg/100 g, total sugar 9.66%. After 30 days storage the **Treatment T_1** contained Moisture 81.26%, pH 4.443, TSS 12.16%, acidity 0.2730%, vitamin C 2.26 mg/100 g. **Treatment T_2** contained Moisture 81.16%, pH 4.357, TSS 13.05%, acidity 0.3147%, vitamin C 11.25 mg/100 g. **Treatment T_3** contained Moisture 83.21%, pH 4.273, TSS 12.62%, acidity 0.3117%, vitamin C 5.033 mg/100 g. Negligible Change in physical and chemical constituents except vitamin C was observed in the prepared juice throughout the 30 days storage period.The statistical analysis of the score response by the taste-testing panelists on the sensory attributes of juice revealed that color, flavor, taste and overall acceptability of the differently treated juice were significantly ($p<0.05$) different. It was found that color, flavor, taste and overall acceptability of tomato juice of treatment T_2 (tomato juice prepared with Na-benzoate) was more acceptable than other treatments.
Immediately after preparation of juice, total number of viable count was not uniform. It also showed that after 30 days intervals revealed that total colony increased slightly with the increase of storage period. After 30 days storage, number of total bacteria was less in tomato juice treated with Na-benzoate compare to other treatments. After 60 days sedimentation problem occurs in the samples. This may be due to improper capping, coarse homogenization etc. The study demonstrated that the tomato juice was effective to maintain the edible quality during two months of storage at refrigerated temperature (4^0C). Use of preservatives (Na-benzoate) was also effective against microbial growth to prevent spoilage.

The experimental finding concludes that, it is possible to prepare tomato juice in Bangladesh those cannot be preserved for long time. The tomato juice with a simple preservatives sodium benzoate and packed in glass bottles manually can be easily preserved for two months in refrigerated temperature without any spoilage and less taste, color and flavor. Every year in Bangladesh a large amount of tomatoes are damaged due to inadequate processing and preservation facilities. The juice preparation is a simple technique for preservation and suitable for cottage and small scale enterprises.

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Appendices

Table A.1: Rating score for color of Tomato juice

No. of Panelists	Sample T1	Sample T2	Sample T3
1	7	8	7
2	7	7	8
3	6	7	6
4	5	7	6
5	5	8	7
6	4	8	7
7	5	8	7

8	4	8	7
9	5	7	6
10	5	8	6
11	5	8	6
12	6	8	6
13	6	8	7
14	4	7	5
15	5	7	5
16	4	7	6
Total	83	121	102
Mean	5.1875	7.5625	6.375

Hedonic scale used: 9 = like extremely; 8 = like very much; 7 = like moderately; 6 = like slightly; 5 = neither like nor dislike; 4 = dislike slightly; 3 = dislike moderately; 2 = dislike very much; 1 = dislike extremely.

Table A.1.1: ANOVA (Analysis of variance) for color of tomato juice

		Sum of Squares	df	Mean Square	F	Sig.
Color	Between Groups	45.125	2	22.563	36.100	.000
	Within Groups	28.125	45	.625		
	Total	73.250	47			

Table A.1.2: DMRT (Duncan's Multiple Range test) test for color of tomato juice

Color				
Duncan[a]				
		Subset for alpha = 0.05		
Sample	N	1	2	3
Sample 1	16	5.1875		
Sample 3	16		6.3750	
Sample 2	16			7.5625
Sig.		1.000	1.000	1.000
Means for groups in homogeneous subsets are displayed.				
a. Uses Harmonic Mean Sample Size = 16.000.				

Table A.1.3: Duncan's Multiple Range Test (DMRT) for color LSD value =0.5630, P<0.05

Treatment code	Original order of means	Treatment Code	Ranked order of means
T_1	5.1875[c]	T_2	7.5625[a]
T_2	7.5625[a]	T_3	6.3750[b]
T_3	6.3750[b]	T_1	5.1875[c]

Table A.2: Rating score for flavor of Tomato juice

No. of Panelists	**Sample T1**	**Sample T2**	**Sample T3**
1	6	8	6
2	6	8	7
3	5	8	7
4	6	8	7
5	5	7	7
6	7	7	7
7	3	8	6
8	4	8	5
9	6	8	7
10	5	7	4
11	5	7	6
12	5	7	6
13	5	7	6
14	5	6	6
15	4	6	6
16	5	6	6
Total	82	116	99
Mean	5.125	7.25	6.1875

Hedonic scale used: 9 = like extremely; 8 = like very much; 7 = like moderately; 6 = like slightly; 5 = neither like nor dislike; 4 = dislike slightly; 3 = dislike moderately; 2 = dislike very much; 1 = dislike extremely.

Table A.2.1: ANOVA (Analysis of variance) for flavor of tomato juice

		Sum of Squares	df	Mean Square	F	Sig.
Flavor	Between Groups	36.125	2	18.063	24.492	.000
	Within Groups	33.188	45	.738		
	Total	69.313	47			

Table A.2.2: DMRT (Duncan's Multiple Range test) test for flavor of tomato juice

Flavor				
Duncan[a]				
		Subset for alpha = 0.05		
Sample	N	1	2	3
Sample 1	16	5.1250		
Sample 3	16		6.1875	
Sample 2	16			7.2500
Sig.		1.000	1.000	1.000
Means for groups in homogeneous subsets are displayed.				
a. Uses Harmonic Mean Sample Size = 16.000.				

Table A.2.3: Duncan's Multiple Range Test (DMRT) for flavor LSD value =0.6117, P<0.05

Treatment code	Original order of means	Treatment Code	Ranked order of means
T_1	5.1250[c]	T_2	7.250[a]
T_2	7.2500[a]	T_3	6.1875[b]
T_3	6.1875[b]	T_1	5.1250[c]

Table A.3: Rating score for taste of Tomato juice

No. of Panelists	**Sample T1**	**Sample T2**	**Sample T3**
1	6	7	7
2	6	8	7
3	5	6	7
4	6	7	8
5	6	6	6
6	7	7	7
7	4	7	5
8	4	7	5
9	4	7	5
10	5	7	6
11	4	7	5
12	6	7	6
13	4	6	5
14	5	6	5
15	5	7	6
16	5	7	5
Total	82	109	95
Mean	5.125	6.8125	5.9375

Hedonic scale used: 9 = like extremely; 8 = like very much; 7 = like moderately; 6 = like slightly; 5 = neither like nor dislike; 4 = dislike slightly; 3 = dislike moderately; 2 = dislike very much; 1 = dislike extremely.

Table A.3.1: ANOVA (Analysis of variance) for taste of tomato juice

		Sum of Squares	df	Mean Square	F	Sig.
Taste	Between Groups	22.792	2	11.396	15.481	.000
	Within Groups	33.125	45	.736		
	Total	55.917	47			

Table A.3.2: DMRT (Duncan's Multiple Range test) test for taste of tomato juice

Taste				
Duncan[a]				
		Subset for alpha = 0.05		
Sample	N	1	2	3
Sample 1	16	5.1250		
Sample 3	16		5.9375	
Sample 2	16			6.8125
Sig.		1.000	1.000	1.000
Means for groups in homogeneous subsets are displayed.				
a. Uses Harmonic Mean Sample Size = 16.000.				

Table A.3.3: Duncan's Multiple Range Test (DMRT) for taste LSD value =0.6109, P<0.05

Treatment code	Original order of means	Treatment Code	Ranked order of means
T_1	5.1250[c]	T_2	6.8125[a]
T_2	6.8125[a]	T_3	5.9375[b]
T_3	5.9375[b]	T_1	5.1250[c]

Table A.4: Rating score for overall acceptability of Tomato juice

No. of Panelists	Sample T1	Sample T2	Sample T3
1	6	8	7
2	6	8	7
3	5	7	7
4	6	7	7
5	5	7	7
6	6	7	7
7	4	8	6
8	4	8	6
9	5	7	6
10	5	7	5
11	5	7	6
12	6	7	6
13	5	7	6
14	5	6	5
15	5	7	6
16	5	7	6
Total	83	115	100
Mean	5.1875	7.1875	6.25

Hedonic scale used: 9 = like extremely; 8 = like very much; 7 = like moderately; 6 = like slightly; 5 = neither like nor dislike; 4 = dislike slightly; 3 = dislike moderately; 2 = dislike very much; 1 = dislike extremely.

Table A.4.1: ANOVA (Analysis of variance) for overall acceptability of tomato juice

		Sum of Squares	df	Mean Square	F	Sig.
Overall acceptability	Between Groups	34.032	2	17.016	47.304	.000
	Within Groups	16.187	45	.360		
	Total	50.220	47			

Table A.4.2: DMRT (Duncan's Multiple Range test) test for overall acceptability of tomato juice

Overall_acceptability				
Duncan[a]				
		Subset for alpha = 0.05		
Sample	N	1	2	3
Sample 1	16	5.1458		
Sample 3	16		6.1667	
Sample 2	16			7.2083
Sig.		1.000	1.000	1.000
Means for groups in homogeneous subsets are displayed.				
a. Uses Harmonic Mean Sample Size = 16.000.				

Table A.4.3: Duncan's Multiple Range Test (DMRT) for overall acceptability LSD value =0.4487, P<0.05

Treatment code	Original order of means	Treatment Code	Ranked order of means
T_1	5.1458[c]	T_2	7.2083[a]
T_2	7.2083[a]	T_3	6.1667[b]
T_3	6.1667[b]	T_1	5.1458[c]

Table A.5: Testing for Tomato Juice

Name of Taster: …….. Date:

Please test these samples and check how much you like or dislike each one on four sensory attributes such as Color, Flavor, Texture and Taste. Use the appropriate scale to show your attitude by checking at the point that best describes your feeling about the sample. Please give a reason for this attitude. Remember you are the only one who can tell what you like. An honest expression of your personal feeling will help me.

Sample ID	Color	Flavor	Taste	Overall acceptability
Sample T1				
Sample T2				
Sample T3				

Extra comments on each sample if any:

N.B. Overall Evaluation:

Hedonic scale used: 9 = like extremely; 8 = like very much; 7 = like moderately;6 = like slightly; 5 = neither like nor dislike; 4 = dislike slightly;3 = dislike moderately; 2 = dislike very much; 1 = dislike extremely.