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Studies on the Preparation and Shelf Life of Aloe Vera Juice

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MMH and MMK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MAA managed the analyses of the study. Authors MEJ and FA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The study was carried out for the preparation of aloe vera juice. Four different formulations of juices were prepared. The products were analyzed for their storage studies, microbiological study and sensory evaluation. The storage studies were done at room temperature (28-30ºC) and refrigeration temperature (4-6 ºC) at 7-, 15-, 30- and 45-days interval. At room temperature it was observed that pH and TSS was increased, pH changed ranging at 0.12-0.23%. The acidity of the sample was decreased during with increasing this storage period. It was observed that vit-C degradation occurred in all sample at room temperature storage. The sample containing 35% water + 300 PPM KMS (sample B) lost highest amount of vit-C compare to others sample. All the samples preserved

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with KMS were found good in their color, flavor, sourness and sweetness but sedimentation was observed after 30 days of storage. The highest sedimentation was observed in the juice containing highest amount of aloe vera juice. The rate of sedimentation increased with increasing the storage period. At both storage conditions the fugal growth was visible after 30 days. The microbiological analysis after 45 day of storage period showed that the total viable count (Bacteria) was less in the sample containing 30% water + 200 PPM KMS (sample A) in comparison to another sample containing 35% water + 300 PPM KMS (sample B), 30% water + 300 PPM KMS (sample C) and 35% water + 200 PPM KMS (sample D). The sensory attributes of aloe vera juices were evaluated and found the sample A and sample C obtained highest color, flavor and overall acceptability.

Keywords: Alo vera; juice; shelf-life and storage stability.

1. INTRODUCTION

Aloe vera, often referred to as Aloe barbadense, is a succulent that is indigenous to tropical areas and has long been used as a remedy. Aloe vera is a typical xerophyte with thick fleshy, strangely cuticularized spiny leaves [1]. Aloe vera has been used for its healing abilities for thousands of years. Its applications have been recorded in ancient cultures of India, Egypt, Greece, Rome, and China. In biblical times the Egyptians hailed aloe vera as the plant of immortality [2]. When the aloe plant leaves are cut, a clear, thick, sticky liquid called aloe vera juice can be extracted. Aloe vera has a number of uses and mainly they are used as a food preservative and medicine [3]. For the last decades, the use of Aloe vera gel has been extended to the food industry, mainly as a resource of functional ingredients, especially used for the preparation of healthy food drinks and other beverages, including Aloe vera juice [4]. Additionally, it can be added to juices or elixirs for better digestion and other internal health advantages or blended into smoothies.

Today, Aloe vera has attracted significant interest because of its nutritional and medicinal characteristics, and its potential as a generator of economic activity in arid and semi-arid areas [5]. An aloe vera juice beverage is a clear or nearly clear unfermented liquid which is developed from the removal of the sweet watery sap from live fruits. In the food industry, Aloe vera juice has been used for the preparation of soft drinks with healthy nutritional qualities and tonics containing amino acids and minerals [6]. Aloe vera juices are becoming popular in comparison with synthetic beverages evidently because of their taste, flavor, nutritive value and their storage stability. The beverage product Juice has a good demand in this subcontinent as well as many other foreign countries. The period of time a product can be maintained in storage before losing its fitness for use, consumption, or sale. The shelf life of aloe vera juice will vary depending on what kind of being used [7]. If it has bought aloe vera juice at the store, it should last a long time, as pre-processed juice is usually treated with preservatives to extend its shelf life. This helps to prevent the juice from decomposing once it's exposed to oxygen. The product claims must be tested by intensive clinical trials, verified and certified by the Government regulatory authorities to build consumer confidence and safety of the aloe vera products.

2. MATERIALS AND METHODS

The study was conducted in the laboratory of the Department of Food Technology and Rural Industries under the Faculty of Agricultural Engineering and Technology and Professor Muhammad Hossain Central Laboratory, Bangladesh Agricultural University, Mymensingh.

2.1 Sample Collections, Chemicals, Solvents and Ingredients

The fresh matured aloe vera leaves were collected from the local market. The aloe vera leaves were cleaned thoroughly with fresh water. Water was glass distilled unless otherwise mentioned, and all chemicals and solvents used in the study were of AR grade. Sugar was procured from the local market. Potassium metabisulphite (KMS), carboxymethyl cellulose (CMC), citric acid and other materials required were used from the laboratory stock.

2.2 Methods

2.2.1 Formulations

The formulations of Aloe vera juices were designed as different sets of ingredients and coded as A, B, C, and D.

Table 1. Formulations of aloe vera juices

Ingredients	Formulations			
	A	B	C	D
Aloe vera (g)	700	650	700	650
Sugar (g)	115	120	115	120
Citric acid (mg)	3.54	3.54	3.54	3.54
KMS (PPM)	200	300	300	200
CMC (mg)	4.00	4.00	4.00	4.00
Water (ml)	184	229	184	229

2.3 Reception of Raw Material

After being harvested, aloe vera leaves must be transported from the field to the laboratory for processing in refrigerated vans. The leaves should be sound, undamaged, mold free and mature (0.5-1 years) in order to keep all the active ingredients in full concentration [8]. The treatment of the leaves after harvesting is a significant component that affects the final product's composition because the dissolution of the gel matrix begins immediately after cutting owing to natural enzymatic processes and the activity of bacteria that are typically present on the leaves. The quality of the finished product can decrease as a result. Therefore, the freshly picked leaves must be refrigerated within 6 hours or they must be fed directly to laboratory processing.

2.4 Preparation of Aloe Vera Juice

Fresh and matured aloe vera leaves were cleaned to remove dirt, residual skin and other undesirable materials. It was washed thoroughly, peeled and cut into slices and blanched in boiling water to make the texture soft. After cooling it was blended in an electric blender. Then calculated amount of sugar, citric acid was mixed properly to make the product TSS 12-14%, acidity 0.7-0.8% and mixed with required amount KMS. Then stored at room temperature (28-32°C) and refrigeration temperature (4-6°C).

2.5 Storage

For the protection of the delicate bioactive components, aloe vera juice is stored in amber-colored glass bottles. Two of the most crucial environmental factors that influence product quality are temperature and relative humidity. These two parameters can also affect the amount of the volatile substance of the juice absorbed by the packaging material [9].

2.6 Chemical Analysis

The fresh aloe vera leaves and-processed aloe vera juices were analyzed for moisture, ash, vitamin C, pH, β -carotene, total soluble solids, titratable acidity, reducing sugar, non-reducing sugar and total sugar content as per the methods of Rangana [10].

2.6.1 Moisture content

Five grams of juice was taken in porcelain crucibles and oven dried at 80°C until the weight become constant. Percent moisture content was calculated according to the following formula.

$$\% \text{ Moisture} = \frac{\mathrm{IW}-\mathrm{FW}}{\mathrm{IW}} \times 100 \quad (1)$$

Where,

IW= Initial weight of Aloe vera samples
FW= Final weight of oven dried sample

2.6.2 Ash Content

Five grams of sample was taken in dry, clean porcelain crucibles and burned using an electric heater. Then the crucibles were placed into a muffle furnace at constant temperature of 550°C for 4 hours. The sample was then cooled in a desiccator and weighed. Ash percent was calculated as follows:

$$\% \text{ Ash} = \frac{AW}{IW} \times 100 \quad (2)$$

Where,

AW =Weight of ash and
IW= Initial weight of Aloe vera

2.6.3 Vitamin C

Vitamin-C content was determined using the methodology followed by Jahan et al. [11].

2.6.4 Total soluble solids

Abbey Refractometer was used for determination of total soluble solids content. One drop aloe vera juice was placed on the prism of the refractometer and covered with the lid and the reading was taken from the refract meter scale directly.

2.6.5 pH

The pH of the aloe vera juice was measured by using pH meter at an ambient temperature.

2.6.6 Titratable acidity

Twenty grams of sample was blended and homogenized in a blender with distilled water. The blended materials were then filtered and transferred to a 250 ml volumetric flask and the volume was made up to the mark with distilled water. 5ml of solution was taken in a conical flask and titrated with 0.1N solution just below the end point, using phenolphthalein indicator. For accuracy, the titration was performed numerous times. Percent titratable acidity was calculated using the following formula-

$$\% \text{ Titratable acidity} = \frac{(T \times N \times V1 \times E)}{(V2 \times W \times 1000)} \times 100 \quad (3)$$

Where, T = Titre, N = Normality of NaOH, V1 = Volume made up, E = Equivalent weight of acid, W = Weight of sample taken for estimation and V2 = Volume of sample taken for estimation.

2.6.7 Sugars

2.6.7.1 Standardization of Fehling's solution

10 ml of both Fehling's solution A and Fehling's solution B were mixed together in a beaker. 10 ml of mixed solution was pipetted into a 250 ml conical flask and 25ml distilled water was added to it. Standard sugar solution was taken in a burette. The conical flask containing mixed solution was heated on a hot plate. When the solution began to boil, three drops of methylene blue indicator solution was added to it. The mixture was titrated using a normal sugar solution. Decolorization of the indication marked the termination point. Fehling's factor was calculated using the following formula:

$$\text{Fehling's factor} = \frac{\text{Titre} \times 2.5}{1000} \quad (4)$$

2.6.7.2 Preparation of sample

25 ml juice and 100 ml distilled water were mixed in a 250 ml volumetric flask. 2ml neutral lead acetate solution was added and a stand for 10 min. 5 ml potassium oxalate solution was added, made up to the volume with water and filtered.

2.6.7.3 Total sugar

50 ml of the clarified solution was taken in a 250 ml volumetric flask. 50 ml of water and 5g of citric acid were combined, and the inversion process was completed by gently boiling the mixture for 10 minutes before cooling. Transferred to a 250 ml volumetric flask and neutralized with 1N NaOH using phenolphthalein as indicator and made up to volume. 10ml of concentrated HCl was added to it. The mixture was allowed to stand for 24 hrs. at room temperature for inversion. Neutralized with NaOH, made volume up to 250 ml and then titrated against standard Fehling's solution. Percent total sugar was calculated using the titrate value obtained in the determination of total sugars after inversion.

2.6.7.4 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 heated on a heater. Three drops of methylene blue indicator were 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 the decolorization of indicator. Percent reducing sugar was calculated from the following formula:

$$\% \text{ Reducing Sugar} = \frac{(F \times D \times 100)}{(T \times W \times 1000)} \quad (5)$$

Where,

F = Fehling's factor, D = Dilution, T = Titer,
W = Weight of sample, Non-Reducing Sugar.

2.6.7.5 Non-reducing Sugar content was calculated as follows

% Non-Reducing Sugar =% Total Sugar -% Reducing Sugar (6)

2.8 Storage Studies

The aloe vera leaves were processed to aloe vera juices and packed in bottles. The bottles were stored in a shelf at room temperature and at refrigeration temperature for 45 days.

2.9 Microbiological Examination

Microbiological quality assessment of the developed samples was carried out by determining the standard plate count as per the AOAC [12].

2.9.1 Standard plate count

The plate count technique was used for the detection of viable bacteria in developed samples. The Standard Plate Count (SPC) or aerobic plate count is an indicator of the overall degree of microbial contamination of food. 1 ml of 10^{-1} and dilution up to 10 folds of the samples were inoculated using the pour plate method. The plates were then incubated at 37°C for 24 hours. After 24 hours of incubation in nutrient agar media, visible bacterial colonies were counted and expressed as cfu/g. A calculation was made for the number of cfu per gram as follows:

Standard plate count (cfu/gm) = Average number of colonies x Reciprocal of dilution used (7)

2.9.2 Observation of fungal growth

The fungal growth in different formulations was examined by visual observation at different storage periods.

2.10 Sensory Evaluation

Sensory properties like color, flavor, and overall acceptability of Formulated juices were evaluated by 13 semi-trained panelists on a 9-point hedonic rating scale [13]. The scale is Like extremely (9), Like very much (8), Like moderately (7), Like slightly (6), Neither like nor dislike (5), Dislike slightly (4), Dislike moderately (3), Dislike very much and 1 = Dislike extremely. Statistical analysis, including variance analysis and Duncan's Multiple Range Test (DMRT), was performed on the collected data to assess preference differences among the panelists.

3. RESULTS AND DISCUSSION

During the off-season, processed aloe vera juices can be used. The purpose of the study was also to identify a good formulation for making aloe vera juice.

3.1 Chemical Composition of Fresh Aloe Vera Juice

Table 2. Approximate composition of fresh aloe vera and processed aloe vera juice

Components	Fresh Aloe vera juice	Processed Aloe vera juice
Moisture (%)	95.7	91.3
Ash (%)	0.36	0.23
Vitamin C mg/ 100 g	2.56	2.45
TSS (%)	1.56	15
Acidity (%)	1.68	1.52
Reducing sugar (%)	0.26	5.23
Non-reducing sugar (%)	0.56	4.69
Total sugar (%)	0.82	9.92
pH	4.36	4.2

3.2 Storage Studies

The Effect of Storage on the Proximate Composition of juices:

3.2.1 Acidity

Acidity for all formulations during storage were determined and the results were shown in Table 3 and Table 4. From the analysis, it was found that the acidity of juices gradually decreased. Ranganna [10] recommend that the acidity of various fruit juices was within the range of 0.12% to 0.23% which was lower than the range of acidity of formulated juice. Talib [12] also found that the titratable acidity of all RTS formulations was increased with the storage period. However, at and after 60 days, the acidity begins to cross over 0.4% in all products, which made them unacceptable.

3.2.2 Total soluble solids (TSS)

TSS initially adjusted in formulations A, B, C, and D showed a negligible change throughout the 45 days storage period at room temperature and refrigeration temperature. Ranote et al. [14] also observed a negligible change in TSS of processed fruit pulp during prolonged storage. It is due to the conversion of carbohydrates and acids into sugar. Talib [12] also reported that TSS was found to increase. This may be attributed to the acidic hydrolysis of sugars and polysaccharides. The change in TSS of PRT0 was reported to be negligible while more changes were reported in PRT3 (30% aloe vera).

3.2.3 pH

The pH of all juices was represented in Table 3 and Table 4. Slight variations in pH were observed throughout the 45 days storage period in all the formulations. Samples A, C, and D had the highest pH than sample B. The change in pH is associated with a number of reasons; it might be due to the effect of heat treatment on the biochemical condition of fruits and vegetables and the slower rate of respiration and metabolic activity [13]. Talib [12] also found that pH values for aloe vera and pear juice were found to be 4.34 and 4.56 respectively.

3.2.4 Studies on sedimentation of bottled aloe vera juice during the storage period

The sediment settles gradually on the bottom of the bottles. At 0 days the total juice was emulsified. The total height of the total juice in the bottle was 18 cm. After 7 days of storage, clear juice was observed in the upper portion of the bottles. With the increase of storage period the height of clear juice increased and hence the height of sediment decreased i.e., the sediment settled at the bottom of the bottles. If it would shake before use then it would be seen to be fresh homogenous juice. Rangana [10] suggested some useful methods for fruit juice clarification. By using peptic enzyme, tannin, and gelatin, or by the combination of these two or by centrifuging and filtering the juice might be successfully clarified.

3.2.5 Influence of storage period on flavor

At room temperature flavors of the sample A and sample A were changed after 30 days and sample B and sample C were changed after 45 days. At refrigeration temperature flavors of the samples A, C, and D were changed after 45 days and sample B remain good. It is shown in Table 3 [15].

3.2.6 Observation of fungal growth in formulated Aloe vera juices

The fungal growth in the formulated aloe vera juices at different storage periods was examined through visual observation and the results found are shown in Table 3 and Table 4. Up to 15 days of storage no fungal growth was observed [16].

3.2.7 Microbiological study of the formulated aloe vera Juice after 45 days storage

Table 5 shows the amount of microbial load during the 45 days of storage. The microbial study showed that the total viable count was higher ($45x10^3$ cfu/ml**)** in sample D containing 35% water + 200 PPM KMS and lower ($37x10^3$ cfu/ml**)** in sample A containing 30% water + 200 PPM KMS.

3.2.8 Vitamin-C degradation at storage period

During storage, it has been seen that vitamin C decreased with the increase of storage period. Ascorbic acid prolongs the shelf life of a product by reacting with residual oxygen and retarding the development of off-flavor [17]. The ascorbic acid reduced remarkably when increasing storage time (0- 45 days) and the reduction was prominent with different treatments. The loss of vitamin C is dependent on temperature and storage time. Sufi [17] found that vitamin-C content decreased from 35.1 to 2.8 mg per 100 gm in the guava juice-based carbonated beverage for the storage period of 35 days at 20-25°C. Fig. 1 showed that vitamin C gradually decreased with the increase of storage period. Talib [18] reported more similar result that is ascorbic acid content of aloe vera based RTS was noticed to be decreased over 90 days period, owing to storage loss. The ascorbic acid loss was also noticed more in high aloe vera content soft drinks.

3.3 Sensory Evaluation

A two-way analysis of variance indicated that all the sensory attributes of different aloe vera juice sample were significantly different at level ($p = 0.05$) of statistical significance. The sensory scores for alo vera was shown above Fig. 2. The color of juice sample A was most preferred than

Table 3. Shelf-life studies of aloe vera juice at room temperature (28-32°C) Storage

Storage period (Days)	Sample	pH	TSS (%)	Acidity (%)	Sedimentation	Flavor Change	Fungal growth
0	A	4.29	14	1.53	Clear	Good	Not visible
	B	4.27	14	1.52	Clear	Good	Not visible
	C	4.25	13	1.50	Clear	Good	Not visible
	D	4.24	12	1.52	Clear	Good	Not visible
7	A	4.30	14.2	1.51	Clear	Good	Not visible
	B	4.30	14.5	1.50	Clear	Good	Not visible
	C	4.28	13.8	1.49	Clear	Good	Not visible
	D	4.25	13	1.50	Clear	Good	Not visible
15	A	4.32	14.8	1.48	Slightly cloudy	Slightly	Slightly visual
	B	4.32	15.2	1.48	Clear	change	Not visible
	C	4.30	14.5	1.47	Clear	Good	Not visible
	D	4.29	14	1.49	Slightly cloudy	Good Change	Not visible
30	A	4.35	15.1	1.47	Slightly cloudy	Change	Slightly visual
	B	4.35	15.6	1.47	Clear Slightly	Good	Not visible
	C	4.32	14.9	1.46	cloudy Slightly	Good	Slightly visual
	D	4.30	14.2	1.48	cloudy	Change	Slightly visual
45	A	4.35	15.2	1.46	Cloudy	Change	Visual
	B	4.35	15.7	1.46	Cloudy	Change	Visual
	C	4.32	15	1.45	Cloudy	Change	Visual
	D	4.30	14.7	1.47	Cloudy	Change	Visual

Sample A = 30% water + 200 PPM KMS; Sample B = 35% water + 300 PPM KMS; Sample C = 30% water + 300 PPM KMS; Sample D = 35% water + 200 PPM KMS

Table 4. Shelf-life studies of aloe vera juice at refrigeration temperature (4-6°C)

Storage period (Days)	Sample	pH	TSS (%)	Acidity (%)	Sedimentation	Flavor Change	Fungal growth
0	A	4.29	14	1.53	Clear	Good	Not visible
	B	4.27	14	1.52	Clear	Good	Not visible
	C	4.25	13	1.50	Clear	Good	Not visible
	D	4.24	12	1.52	Clear	Good	Not visible
7	A	4.30	14.1	1.50	Clear	Good	Not visible
	B	4.30	14.4	1.49	Clear	Good	Not visible
	C	4.28	13.7	1.48	Clear	Good	Not visible
	D	5.0	13.2	1.49	Clear	Good	Not visible
15	A	4.33	14.7	1.47	Clear	Good	Not visible
	B	4.31	15	1.47	Clear	Good	Not visible
	C	4.30	14	1.45	Clear	Good	Not visible
	D	5.1	13.8	1.47	Clear	Good	Not visible
30	A	4.35	15	1.44	Slightly cloudy	Slightly Change	Slightly visual
	B	4.36	14.9	1.44	Clear	Good	Not visible
	C	4.33	14.6	1.43	Clear	Good	Not visible
	D	5.2	14.2	1.45	Slightly cloudy	Slightly Change	Slightly visual
45	A	4.37	15.2	1.42	Slightly cloudy	Change Slightly	Slightly visual
	B	4.38	15.4	1.40	Slightly cloudy	Change Slightly	Not visible
	C	4.35	15	1.42	Slightly cloudy	Change	Slightly visual
	D	5.3	14.5	1.43	Cloudy	Change	Visual

Sample A = 30% water + 200 PPM KMS; Sample B = 35% water + 300 PPM KMS; Sample C = 30% water + 300 PPM KMS; Sample D = 35% water + 200 PPM KMS

Table 5. Total number of viable bacteria (cfu/ml) after incubation 48 h at 32°C

Sample	Number of Bacterial colonies (cfu/ml)
A	$37x10^3$
B	$39 x10^3$
C	$40 x10^3$
D	$45x10^3$

Sample A = 30% water + 200 PPM KMS; Sample B = 35% water + 300 PPM KMS; Sample C = 30% water + 300 PPM KMS; Sample D = 35% water + 200 PPM KMS

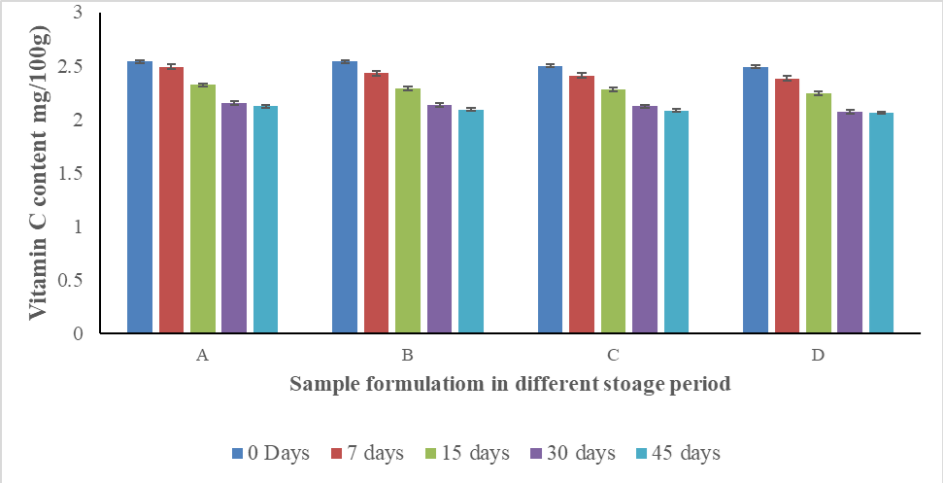

Fig. 1. Vitamin C content of ready-to-drink Aloe vera juice of different formulations at different storage periods at room temperature

Sample A = 30% water + 200 PPM KMS; Sample B = 35% water + 300 PPM KMS; Sample C = 30% water + 300 PPM KMS; Sample D = 35% water + 200 PPM KMS

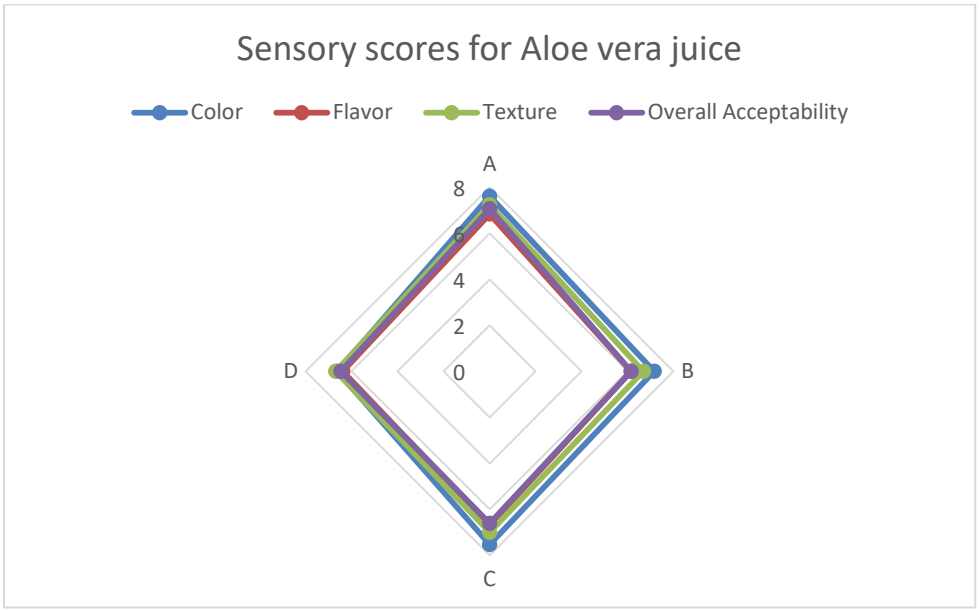

Fig. 2. Sensory scores for alo vera juice

other juice formulation. Talib [18] also found a similar result for color of different formulation aloe vera juice that is 6.85-7.08. Flavor preferences among the samples, showed that the flavor of juice sample A obtained the highest score (6.641) and was significantly different from

the other sample B and D secured the score 6.158, and 6.386 respectively. This research is in line with the findings of Talib [18] who reported 6.77-7.01 score out 9 for flavor of different formulation of aloe vera juice. For texture purpose, there is no significant deference (P=0.05) in texture acceptability. Sample A secured the highest score (7.237) and the sample C and D secured the lowest score (6.693) during the storage period. From the results of ANOVA, it was apparent that there was significant (P=0.05) difference in overall acceptability among the developed juice. Sample A and sample C were significantly different from other two sample B and D. The juice sample A was statistically acceptable with ranked as like very much and secured the highest score (7.07) among the juice sample. Similar score also found by Talib [18] and reported overall acceptability ranges from 6.87-6.99.

4. CONCLUSION

It has been shown that fresh aloe vera juice contained moisture content 95.7%. TSS 1.56%, pH 4.36, total sugar 0.82%, reducing sugar 0.26%, non-reducing sugar 0.56%, ash 0.36%, acidity 1.68%, and ascorbic acid 2.56 mg per 100g. The samples A and B showed the minimum total viable count of bacteria. No fungal growth was observed up to 15 days of storage. After 45 days whitish structure was observed at the surface of the juices. Except for vitamin C, there were very few changes in the prepared juices' composition over the course of storage. Remarkable decrease of vitamin C was found in the samples during storage period and TSS increased slightly and acidity decreased slightly. A statistical analysis of the score response by the taste testing panelists on the sensory attributes on juices revealed that all the products were accepted by the panelists and score was from 6.15 to 7.61. The chemical analysis of the products was done and the results were found satisfactory. Only vitamin-C of the formulated juices was very lower than the fresh aloe vera. Sedimentation was observed in the bottles during storage period. The preservative (KMS) was effective against microbial growth to prevent spoilage of the bottled juices. The use of CMC decreases the cloudiness of different formulations. Citric acid was used to lower the pH. More research work would be needed to avoid fading of color and sedimentation and to minimize the loss of vitamin-C content to prepare juice under different storage period. Every year in Bangladesh a large amount of aloe vera is spoiled during peak season due to inadequate processing and preservation facilities. The study was carried out to affect the preservatives on aloe vera juice. Thus, by processing and preserving aloe vera as juice may encourage more production of Aloe vera which will provide better nutrition to the people of Bangladesh.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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