



Effect of storage on the physiochemical and antioxidant properties of *Aloe vera* drink

Muhammad Abuzar Masood¹, Faiz-ul-Hassan Shah², Shahid Bashir³, Rabiya Jamil⁴

^{1,2,3} University Institute of Diet and Nutritional Sciences, The University of Lahore, Punjab, Pakistan

⁴ Department of Food Science and Nutrition, Government College University, Faisalabad, Punjab, Pakistan

Abstract

Aloe vera is rich source of phytochemicals which are effective against many diseases e.g. diabetes, skin problem and certain type of cancer. In this study functional drink was prepared with *Aloe vera* gel leaf.

Keywords: storage stability, functional drink, *Aloe vera*, antioxidant activity

1. Introduction

Aloe vera name is taken from the Arabic dictionary which means "Alloeh" that mean is "shining bitter substance" and the name "Vera" is derived from the Latin dictionary that mean is "true". This plant has a history dating back to biblical time. *Aloe vera* has the quality of perennial succulent xerophyte. *Aloe vera* can survive in low rainfall areas and dry areas due to its property of water storage tissues in it which does not allow the plant to dry. *Aloe vera* plant has benefits associated towards polysaccharides enclose in the inner a part of the leaves referred to as *Aloe vera* gel [1]. Almost 250 plus species of *Aloe vera* are grown worldwide. But only two of them are grown commercially named as *Aloe barbadensis* Miller and *Aloe arborescens*. *Aloe vera* cannot survive in freezing temperature areas but can grow easily in tropical sand and warm areas. *Aloe vera* additionally referred to as the name of "the silent healer", "the wand of heaven" and "heaven's blessing" [2]. *Aloe vera* leaf has two parts known as the outer green rind having vascular bundles and the second part is inner colorless parenchyma containing the aloe gel. Providing details of Inner part of *Aloe vera* leaf is mostly confusing because of different name are used for inner part such as mucilaginous jelly, mucilage tissue, inner pulp, mucilaginous gel, leaf parenchyma tissue and inner gel. The term 'parenchyma tissue' and 'pulp' described as the flashy inner part of the leaf which include organelles and cell walls and on the other hand 'mucilage' and 'gel' defined as the viscous clean liquid in the parenchyma cells [3,4].

Between the inner and outer layer of leaf there is a middle layer which has yellow sap having glycosides and anthraquinones. 99% water found in the innermost layer rest is made of amino acids, lipids, sterols etc. Solid material consist of 0.5-1% having compounds minerals, polysaccharides, fat-soluble vitamins, water-soluble vitamins, enzymes, organic acids and phenolic compounds. Vitamins are found in the *Aloe vera* gel such as vitamin B1 (thiamin), vitamin B6, vitamin B2 (riboflavin), niacin, carotenoids, ascorbic acid, folic acid and tocopherols. *Aloe vera* has the majority of antioxidant activity. Vitamin B12 has also been found in the gel in minute quantity [5,6]. There are six different types of enzymes found in the *Aloe vera* gel which include: amylase, catalase, bradykinase, cellulose, oxidase and carboxypeptidase. It is assumed that this

composition of *Aloe vera* pulp contribute to the adverse effect of therapeutic and pharmacological activities which has been observed in the *Aloe vera* products. *Aloe vera* provides many health benefits to human body. Orally ingested *Aloe vera* gel has been beneficial for kidney problems, ulcerous and gastrointestinal problems [7].

Scientific studies shows that there are many evidences which shows that in both human and animal studies which suggest in increase benefits of orally ingested *Aloe vera* for increasing the force of cardiac contraction, decreasing the tri-glyceride level as well as the cholesterol level, and release long-lasting blood glucose control properties [8]. In the past 15 years there have also been reports on the antidiabetic activity of *Aloe* extracts. *Aloe vera* gel shows effect of lowering the blood glucose level in many clinical and preclinical (animal) studies. On the daily basis consumption of 10-20 ml per day of *Aloe vera* gel reduced total cholesterol to 15%, triglycerides to 30% and hyperlipidemia in patients by lowering the low-density lipoprotein cholesterol to 18%. [9].

2. Materials and Methods

2.1 Methods

The gel was used to prepare drink with different treatment 10, 20, 30, 40g/L. Prepared drink was filled in polyethylene terephthalate bottles and analyzed after 0, 15, 30, 45 and 60 days of storage. The *Aloe vera* drink was subjected to pH, Titratable Acidity, TSS, Ascorbic Acid, Antioxidant and Total Phenols.

2.2 Procurement and preparation of raw material

Aloe vera along with other ingredients was procured from local market of Lahore, Punjab-Pakistan for preparation of *Aloe vera* drink in the food science lab of University Institute of Diet and Nutritional Sciences, Faculty of Allied Health Sciences, University of Lahore.

Aloe vera Leaves were cleaned manually to remove the impurities followed by washing. *Aloe vera* leaves were peeled to get the gel. Leaves impurities were removed through washing of the gel.

2.3 Preparation of *Aloe vera* Drink

Aloe vera gel was homogenized in a blender. The homogenized gel was kept at 4°C overnight and then filtered

through muslin cloth. The clear filtrate was kept at 20°C. Blended *Aloe vera* gel @ 10, 20, 30, and 40 g/1000ml along with other ingredients such as malic acid (0.6 g), Citric acid (1 g), ascorbic acid (1 g), stevia (0.80 g) and guar gum (1 g) was added in 1000 ml of water to prepare drink.

2.4 Analysis of the *Aloe vera* drink

The prepared drink was packed in glass bottles and analyzed for the following parameters at 15 days interval for the period of 60 days.

2.5 PH, Titratable Acidity and Total soluble solids

PH, Titratable Acidity and Total soluble solids of *Aloe vera* Drink were determined by the method of AOAC (2007) [10]. PH was determined by portable pH meter (ST3100, OHAUS Corporation, USA). Titratable Acidity was measured by the Sodium Hydroxide 0.1N and phenolphthalein indicator and was showed as Malic Acid percentage [10]. Total soluble solids were measured by hand refractometer (DR301-95, KRUSS, Germany) and were showed as Brix [10].

2.6 Ascorbic Acid

Ascorbic Acid of *Aloe vera* drink was measured by the method of Dashman [11] with minor changes using reagent Folin-Ciocalteu. 20mm Sample was taken in 100mL Volumetric Flask into 2mL Tetrachloroacetic acid solution (10%) and make volume of 100mL by adding distilled water. After pouring sample into conical flask mixed gently for 1 minute and gave stay time for 1 minute and filtered by Whatman filter paper. 1 mm of sample was taken in a test tube followed by the mixing of distilled water 3 mL and Folin-Ciocalteu 0.4mL and for 10 minutes it was incubated at room temperature. Absorbance was noted by spectrophotometer (U-2900UV/VIS, HITACHI, Japan) at 760 nm. Values were measured as mg/100gFW.

2.7 Antioxidant activity

Aloe vera leaves were cut into small pieces place in glass plate and give two days stay time for lyophilized in vacuo and then after grounding get the gel powder. 80% ethanol v/v was added in 1 g of lyophilized *Aloe vera* powder in round flask, ethanol used was 50mm, then sonification done for 30 minutes and filtered and with 10 mL of ethanol it was washed twice. The filtrate was combined onto a flask that was weighed previously with a constant weight and concentrated to dryness at 30 °C by rotary evaporation in vacuo. Containing dried materials flask was weighed and weight the difference of empty and sample flask noted as the mass of solid in the *Aloe vera* extracts. Dehydrated *Aloe vera* extracts were dissolved in 75% ethanol (v/v) with a final concentration of 100 mg of solid L-1 and stored in a

refrigerator for further use.

2.7.1 Free Radical Scavenging Activity (DPPH assay)

In a research analysis, stable free radical DPPH was used to determine the free radical scavenging activity of *Aloe vera* extract. In this experiment, the *Aloe vera* extract was considered as an agent who tends to donate hydrogen or in other words has radical scavenging ability. DPPH radical scavenging activity was measured [12]. To determine the extent of scavenging activity, one mL of DPPH was added into 4 mL volume of *Aloe vera* drink. After addition, this mixture was incubated for 30 minutes at room temperature. The rate of absorbance was also observed at 517 nm, for this purpose Spectrophotometer (U-2900UV/VIS, HITACHI, Japan) was used. This experiment is repeated for 3 times to reduce the extent of any type of error. After data collection, Percent inhibition was also calculated. The formula used for this purpose is as mentioned bellow;

$$\text{Percentage reduction of absorbance (\%)} = [(AB - AA) / AB] \times 100$$

$$AB = \text{rate of absorbance of blank sample (t = 0 min)}$$

$$AA = \text{total absorbance of tested extract solution (t = 30 min)}$$

2.8 Total Phenolic Compounds

Spectrophotometer was used to determine the Total Phenolic compounds by the method of Folin-Ciocalteu method Singleton [13]. The Folin-Ciocalteu reagent (125µL) was mixed with drink (125 µL) along with distilled water 500 µL and give stay time of five minutes at 22 °C. 7% of Sodium Bicarbonate 4.5 mL was added to mixture. Absorbance was measured via Spectrophotometer (U-2900UV/VIS, HITACHI, Japan) against control at 765 nm. Value was calculated and showed as gallic acid equivalent.

3. Results and Discussion

3.1 PH

Highest mean value of pH was 3.09 having the concentration of *Aloe vera* gel 40 g in the drink. The lowest pH 3.05 was noted in drink concentration of 10g *Aloe vera* Gel. Range of pH was 3.05 to 3.14 during storage period of 60 days. At 0 day pH was noted 3.05 then at 15 days interval it was 3.09, 3.11, and 3.14 in drink respectively during the storage. It was observed in a study that juices which decrease the acidity have increased in pH. Due to more organic acid, juices have low pH. Increase in pH at 120 days storage study in guava drink with *Aloe vera* at ambient temperature [14].

Table 1: Effect of treatments and storage on pH of *Aloe vera* drink

pH	Storage Intervals	Treatments				
		ALD-10	ALD-20	ALD-30	ALD-40	Means
	0	3.08	3.13	3.11	3.14	3.05±0.05 ^A
	15	3.08	3.08	3.08	3.11	3.09±0.06 ^B
	30	3.04	3.06	3.04	3.09	3.11±0.06 ^C
	45	3.04	3.06	3.04	3.09	3.11±0.06 ^C
	60	3.01	3.01	3.00	3.05	3.14±0.07 ^D
	Means	3.05±0.02 ^D	3.07±0.04 ^C	3.05±0.03 ^D	3.09±0.03 ^B	

3.2 Titratable Acidity

Highest mean value of Titratable Acidity was 0.52% having

the concentration of *Aloe vera* gel 40 g in the drink. The lowest titratable acidity (0.24%) was noted in drink

concentration of 10g *Aloe vera* gel. Range of titratable acidity was 0.33% to 0.38% during storage period of 60 days. At 0 day titratable acidity was noted 0.33% then at 15 days interval it was 0.34, 0.36 and 0.38% in drink respectively during the storage. Hydrolysis of polysaccharides is the main reason for change in acidity, conversion of non-reducing sugars into reducing sugars also

responsible for this [15].

The loss of acidity might be attributed to the chemical interaction between the organic constituents of juice induced by temperature and the action of enzymes. A study observed the decrease in titratable acidity at 120 days storage study in guava drink with *Aloe vera* at ambient temperature [16].

Table 2: Effect of treatments and storage on Titratable Acidity (%) of *Aloe vera* drink

	Storage Intervals	Treatments				
		ALD-10	ALD-20	ALD-30	ALD-40	Means
Titratable acidity	0	0.26	0.33	0.36	0.46	0.33±.11 ^B
	15	0.22	0.33	0.34	0.50	0.34±0.09 ^B
	30	0.24	0.37	0.34	0.54	0.36±0.10 ^{AB}
	45	0.24	0.37	0.34	0.54	0.36±0.10 ^{AB}
	60	0.36	0.38	0.38	0.55	0.38±0.09 ^A
	Means	0.24±0.04 ^D	0.35±0.03 ^B	0.35±0.05 ^{BC}	0.52±0.04 ^A	

3.3 Total soluble solids

Highest mean value of TSS was 2.04% having the concentration of *Aloe vera* gel 40 g in the drink. The lowest TSS (2.01%) was noted in drink concentration of 10g *Aloe vera* gel. Range of TSS was 2.01% to 2.05% during storage

period of 60 days. At 0 day TSS was noted 2.01% then at 15 days interval it was 2.03, 2.04, and 2.05% in drink respectively during the storage. During storage study of drink it is analyzed that for better juice quality increase in a minimum quantity of TSS value is desirable for drink [17].

Table 3: Effect of treatments and storage on TSS of *Aloe vera* drink.

	Storage Intervals	Treatments				
		ALD-10	ALD-20	ALD-30	ALD-40	Means
Total soluble solids	0	2.06	2.03	2.06	2.00	2.01±0.63 ^A
	15	2.00	2.03	1.96	2.07	2.03±0.81 ^A
	30	1.93	2.11	2.03	1.96	2.04±0.98 ^A
	45	2.07	1.93	2.00	2.04	2.04±0.81 ^A
	60	1.96	2.06	2.10	2.10	2.05±0.84 ^A
	Means	2.01±0.89 ^A	2.03±0.84 ^A	2.02±0.82 ^A	2.04±0.84 ^A	

3.4 Ascorbic Acid

Highest mean value of Ascorbic Acid was 0.84% having the concentration of *Aloe vera* gel 40 g in the drink. The lowest ascorbic acid (0.73%) was noted in drink concentration of 10g *Aloe vera* Gel. Range of Ascorbic Acid was 0.73% to 0.84% during storage period of 60 days. At 0 day Ascorbic Acid was noted 0.84%

then at 15 days interval it was 0.92, 0.82, 0.69 and 0.52% in drink respectively during the storage.

In storage study of a drink it was suggested that Vitamin C is sensitive to heat, oxygen and light which goes to oxidized in non-enzymatic and enzymatic catalysts [18].

At ambient temperature of storage ascorbic acid level was decreased in a drink guava and *Aloe vera* blend [19].

Table 4: Effect of treatments and storage on Ascorbic Acid (mg/100mL) of *Aloe vera* drink

	Storage Intervals	Treatments				
		ALD-10	ALD-20	ALD-30	ALD-40	Means
Ascorbic acids	0	1.00	1.10	0.83	1.10	1.01±0.16 ^A
	15	0.83	1.00	0.86	1.00	0.92±0.09 ^A
	30	0.73	0.83	0.83	0.86	0.82±0.07 ^B
	45	0.63	0.73	0.70	0.66	0.69±0.07 ^C
	60	0.46	0.53	0.46	0.60	0.52±0.07 ^D
	Means	0.73±0.19 ^B	0.74±0.21 ^A	0.84±0.19 ^B	0.84±0.20 ^A	

3.5 Antioxidant

Highest mean value of Antioxidant was 387.74 having the concentration of *Aloe vera* gel 40 g in the drink. The lowest Antioxidant 96.92 was noted in drink concentration of 10g *Aloe vera* gel. Range of Antioxidant was 251.88 to 125.92 during storage period of 60 days. At 0 day Antioxidant was noted 251.88 then at 15 days interval it was 226.50, 201.147, 163.53 and 125.76 in drink respectively during the storage.

The capacity of antioxidant of vegetables and fruits, that is helpful for human health, is mainly related with TPC and anthocyanin.

Without main losses of monomeric anthocyanins in the *Aloe vera* drink, during storage FRAP values were not higher, it was suggested that it might be formation of compounds which are polymeric in form, and monomeric anthocyanins during storage were able for the loss of antioxidant activity [20].

Table 5: Effect of treatments and storage on Antioxidant (GAE/L) of *Aloe vera* drink

	Storage Intervals	Treatments				
		ALD-10	ALD-20	ALD-30	ALD-40	Means
Antioxidant	0	126.07	251.67	377.67	504.02	251.88±1.39 ^A
	15	113.30	226.65	339.64	453.01	226.50±1.76 ^B
	30	100.81	201.56	302.47	402.55	201.48±1.73 ^C
	45	81.67	162.79	245.65	327.55	163.53±1.90 ^D
	60	62.77	125.73	188.73	251.59	125.76±9.09 ^E
	Means	96.92±3.30 ^D	193.66±4.47 ^C	290.83±9.47 ^B	387.74±2.77 ^A	

3.6 Total Phenols

Highest mean value of Total phenolic content was 1.42% having the concentration of *Aloe vera* gel 40 g in the drink. The lowest total phenol (0.34%) was noted in drink concentration of 10g *Aloe vera* gel. Range of Total phenolic content was 0.92% to 0.45% during storage period of 60

days. At 0 day Total phenol was noted 0.92% then at 15 days interval it was 0.82, 0.73, 0.59 and 0.45% in drink respectively during the storage. It was suggested during a study of a drink that the transformation of monomeric anthocyanins into polymeric compounds was the main reason in the loss of total phenols in the drink^[21].

Table 6: Effect of treatments and storage on Total Phenols (GAE/L) of *Aloe vera* drink

	Storage Intervals	Treatments				
		ALD-10	ALD-20	ALD-30	ALD-40	Means
Total Phenols	0	0.45	0.92	1.37	1.87	0.92±0.68 ^A
	15	0.41	0.81	1.23	1.66	0.82±0.60 ^B
	30	0.37	0.72	1.10	1.47	0.73±0.53 ^C
	45	0.28	0.59	0.88	1.20	0.59±0.43 ^D
	60	0.21	0.44	0.68	0.92	0.45±0.33 ^E
	Means	0.34±0.09 ^D	0.70±0.17 ^C	1.05±0.25 ^B	1.42±0.34 ^A	

4. Conclusions

The *Aloe vera* drink has high content of vitamin C, antioxidant activity and total phenol. However, these compounds were lost during 2 months of storage at 28°C. As the supplementation level of *Aloe vera* gel increase from 10 g/L to 40 g/L pH (1.31%), Titratable Acidity (116%), Ascorbic Acid (15%), TSS (1.4%) was increased.

5. References

- Talmadge J, Chavez J, Jacobs L, Munger C, Chinnah T, Chow JT. *et al.* Fractionation of *Aloe vera* L. inner gel, purification and molecular profiling of activity. *International immunopharmacology.* 2004; 204(14):1757-73.
- Sahu PK, Giri DD, Singh R, Pandey P, Gupta S, Shrivastava AK. *et al* Kumar A, Pandey KD. Therapeutic and medicinal uses of *Aloe vera*: a review. *Pharmacology & Pharmacy.* 2013; 84(08):599-61s0.
- Tizard IR, NI Y. Analytical methodology: the gel-analysis of aloe pulp and its derivatives. In *Aloes* CRC Press, 2004, 129-144.
- Boudreau MD, Beland FA. An evaluation of the biological and toxicological properties of *Aloe barbadensis* (miller), *Aloe vera*. *Journal of Environmental Science and Health Part C.* 2006; 124(1):103-54.
- Bunyapraphatsara N, Chansrakaew W, Pornchirasilp S, Peungvicha P, Chokechaijaroenporn O. Antidiabetic effect of fresh and preserved aloe gel. *Thai J Phytopharm.* 1995; 2(2):1-7.
- Choi S, Chung MH. A review on the relationship between *Aloe vera* components and their biologic effects. In *Seminars in integrative medicine.* WB Saunders. 2003; 1(1):53-62.
- Gupta MB, Nath R, Gupta GP, Bhargava KP. Antiulcer activity of some plant triterpenoids. *The Indian Journal of Medical Research.* 1981; 73:649-52.
- Yongchaiyudha S, Rungpitarangsi V, Bunyapraphatsara N, Chokechaijaroenporn O. Antidiabetic activity of *Aloe vera* L. juice. I. Clinical trial in new cases of diabetes mellitus. *Phytomedicine.* 1996; 13(3):241-3.
- Shapiro K, Gong WC. Natural products used for diabetes. *Journal of the American Pharmaceutical Association.* 2002; 142(2):217-26.
- AOAC Official Methods of Analysis. 18th Edition. Association of Official Analytical Chemists, Gaithersburg, 2007.
- Dashman T, Blocker DE, Baker N. Laboratory manual for human nutrition. 2nd ed. Harwood Academic Publishers, New York, NY, 1996.
- Vinson JA, Al Kharrat H, Andreoli L. Effect of *Aloe vera* preparations on the human bioavailability of vitamins C and E. *Phytomedicine.* 2005; 12(10):760-5.
- Boudreau MD, Beland FA. An evaluation of the biological and toxicological properties of *Aloe barbadensis* (miller), *Aloe vera*. *Journal of Environmental Science and Health Part C.* 2006; 24(1):103-54.
- He Q, Changhong L, Kojo E, Tian Z. Quality and safety assurance in the processing of *Aloe vera* gel juice. *Food control.* 2005; 16(2):95-104.
- Yen GC, Duh PD. Scavenging effect of methanolic extracts of peanut hulls on free-radical and active-oxygen species. *Journal of Agricultural and Food Chemistry.* 1994; 42(3):629-32.
- Skrede G, Larsen VB, Aaby K, Jørgensen AS, Birkeland SE S Antioxidative Singleton VL. *et al* Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In *Methods in enzymology.* 1999; (299):152-178). Academic press.

17. Kumar SS, Sreenivas KN, Shankarappa TH, Ravindra V. Standardization of recipe for value added nutraceutical beverage of guava blended with *Aloe vera* and roselle. *J. Environ. Ecol.* 2012; 30:995-1001.
18. Bhardwaj RL, Pandey S. Juice blends—a way of utilization of under-utilized fruits, vegetables, and spices: a review. *Critical reviews in food science and nutrition.* 2011; 51(6):563-70.
19. Ziena HM. Quality attributes of Bearss Seedless lime (*Citrus latifolia* Tan) juice during storage. *Food chemistry.* 2000; 71(2):167-72.
20. Iversen CK. Black currant nectar: effect of processing and storage on anthocyanin and ascorbic acid content. *Journal of Food Science.* 1999; 64(1):37-41.
21. Ziena HM. Quality attributes of Bearss Seedless lime (*Citrus latifolia* Tan) juice during storage. *Food chemistry.* 2000; 71(2):167-72.