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# Influence of process factors on antioxidant and chemical properties of blended aonla (*Emblica officinalis* Gaertn.) juice and its quality retention during storage

**VS Meena, VS Yadav, V Eyarkai Nambi, PC Sharma, Kirti Jalgaonkar and RK Gupta**

**Abstract**
A study was conducted to find out the optimum blend for aonla juice and to study the influence of processing methods on the nutritional quality during storage. Aonla juice based blends were prepared with juices of pomegranate, aloevera, kinnow, ber and ginger. Prepared blends were filled in glass bottles, pasteurized (at 85°C for 15 minutes and microwave heating) and stored in two (room and refrigerated) conditions for six months. During storage, changes in qualitative characteristics such as TSS, acidity, pH, ascorbic acid, total sugars, non-enzymatic browning and sensory attributes were examined in every 15 days. Blending proportions and pasteurization methods were found highly significant ($P<0.05$) over the quality parameter. Among the combinations, aonla (60%) with aloe vera (20%) and pomegranate (20%) resulted higher quality retention during storage. Non-enzymatic browning was observed less while applying KMS (500 ppm). This proportion was found very stable, yielded higher sensory score and microbiologically safe till the six month of storage.
**Practical Application:** Despite of its nutritional and medicinal properties, Aonla, commonly known as Indian Gooseberry (*Emblica officinalis* L.) is not being used regularly in food cuisine due to its stringent and bitter taste. In order to exploit the functional and nutritional benefits of aonla, this study was conducted to find out the optimum blend with other juices and to study the influence of processing methods on the nutritional retention quality during storage. This report propose a final juice proportion (aonla juice: 60%; aloe vera juice: 20% and pomegranate juice: 20%) which was found very stable, yielded higher sensory score and microbiologically safe till the six month of storage. These findings would benefit the processors to develop new aonla based beverages and create new avenues in the in the beverage markets, increase the consumers' acceptability and adds choice of beverages in the consumer domain.

**Keywords:** Aonla based RTS, Juice blends, nutritional retention, mixed juice, Vitamin C

## 1. Introduction

Fruits are rich source of nutrients, essential minerals and vitamins. Aonla commonly known as Indian Gooseberry (*Emblica officinalis.* Gaertn syn. *Phyllanthus emblica* L.) finds a special place in Indian subcontinent or the subcontinent is a southern region of Asia as it has tremendous medicinal and ayurveda values. According to FDA, daily recommended vitamin C intake is 60 mg. The vitamin C content in aonla varies from 300-500 mg /100 g depending upon the variety and size of the fruit (Ray *et al.* 2013) [22], hence aonla would be the best source for daily vitamin C intake. Aonla is being exported under the category of ayurvedic and unani herbs due to its medicinal and nutritional properties. The fresh fruits are generally not consumed as it is highly acidic and astringent, thus the attention of processors have been focused to develop various products from Aonla such as candies, preserves and beverages etc.

Especially, beverages prepared from fruits had attracted the consumers in recent years due to public perception/awareness about the health and nutritional aspects. Blending of various fruit juices with aonla juice may create more avenues in the beverage markets, increase the consumers' acceptability and adds choice of beverages in the consumer domain. As reported by many authors, blending of two or more fruit juices for the preparation of ready-to-serve beverage appears to be a convenient and economic alternative for utilization of aonla (Sandhu and Sidhu 1992, Saxena *et al.* 1996, Attri *et al.* 1998, Langthasa 1999) [24, 25, 3, 15].

In the scientific research domain, almost nil or minimum reports about the nutritional and other quality changes occurs during the storage of juice blends with aonla juice as base. In the view of above, a study was aimed to develop aonla based blended RTS beverages with various juices and to study the quality retention of various parameters over the period of 6 months.

## Materials and Methods

**Juice selection and preparation for blending:** Based on the sensory compatibility study, the juices of pomegranate, kinnow, ber, ginger and aloe vera have been selected for the preparation of blend. The mature fruits wise aonla (cv. *Chakaiya*), aloe vera (*Aloe babendesis)*, kinnow, ber (cv. *Gola*), ginger and pomegranate (cv. *Mridula*) were obtained from ICAR- CIPHET, Abohar (Punjab) Orchard. Fruits were first washed by tap water to remove adhering dust and reduce the surface micro flora.

Aonla fruits were deseeded and shredded using CIPHET aonla processing unit, then the juice was extracted from aonla shreds by pressing in a hydraulic press. Gels from aleo vera leaves were manually separated. Using 500 w table top juicer (HL1631-500, Philips India) juice were prepared from aloe gels, deseeded ber, peeled ginger and pomegranate arils. Manually juice extractor was used to extract juice from kinnow.

The juices, then blended as per the proportion given in Table 1 and filled in pre sterilized 200 ml glass bottles by adding preservatives glass bottles. The bottles were sealed using crown cork and subjected to pasteurization (Conventional 85°C for 15 minute and microwave 70°C for one minute). After pasteurization, the bottles were stored in two lots at refrigerated (4 ± 1°C) and ambient condition (26±6°C) respectively for a period of six months.

**Chemical analysis**: Ascorbic acid was determined by the direct colorimetric method using 2, 6- dichlorophenol-indophenols as decolorizing agent by ascorbic acid in sample extract and in slandered ascorbic acid solution (AOAC 2005) [2]. Acidity was determined by dissolving a known weight of sample in distilled water and titration against 0.01 N NaOH using phenolphthalein as indicator (Ranganna 1986) [20]. Digital pH meter was used for pH determination. Total sugar was estimated by using anthrone (Dubois *et al.* 1951). Reducing and non- reducing sucrose and non-enzymatic browning (NEB) of juices was determined by method as detailed by Ranganna (1986) [20]. The TSS (°Brix) was determined by measurement of refractive index with digital refractometer (Atago, japan).

**Non-enzymatic browning:** Non-enzymatic browning (NEB) is one of the most important chemical reactions responsible for quality and color changes during the heating or prolonged storage of fruit products. The NEB of blended juices measured in term of optical density at 440 nm.

**Sensory evaluation:** The blended beverages were evaluated for sensory qualities on the basis of colour, appearance, taste and aroma, palatability and overall acceptability by a panel of 10 judges on a 9-point Hedonic scale (Amerine *et al.* 1965) [1].

**Data analysis:** The data obtained was subjected to statistical analysis using Factorial RBD (Randomized Block Design) and the means were compared by using LSD (Least Significant Difference) test. The statistical analysis as well as the graphical representations was prepared using Microsoft-Excel.

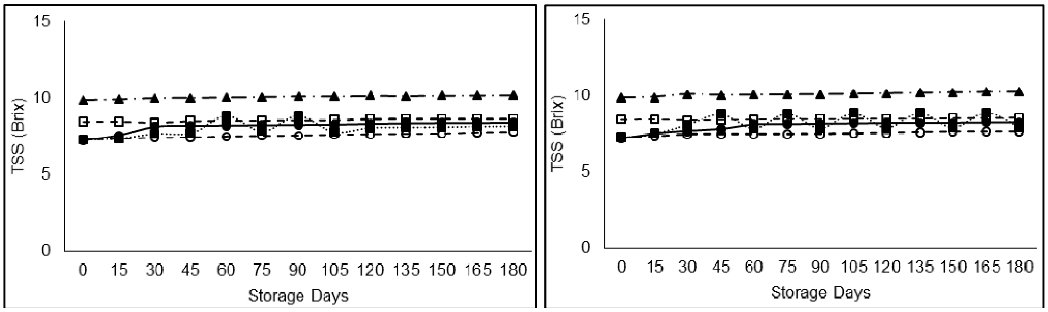
## Result and Discussion

The chemical properties of various juices at the time of extraction were measured and given in Table 1. The results indicated that the moisture content of aonla, ber, Kinnow, pomegranate, ginger and aloe vera ranged between 78 to 96 percent. As expected aloe vera exhibited highest the moisture content (96 %), while ginger had the lowest moisture content (78 %). TSS and total sugar ranged between 1.8 to 15.0 °B and 0.65 to 10.6% 6 respectively. Titrable acidity and pH in all fruits, ginger and aloe vera found between 0.3-1.6 percent and 3.2- 5.6 respectively. Among all fruit selected for study the aonla fruit exhibited highest ascorbic acid (350 mg/ 100 ml). The initial and final values (after 6 months of storage) of quality parameters of juice blends were tabulated along with the statistical results.

**Changes in TSS and acidity of juice blends during storage:** The changes in TSS, acidity and pH during the storage in two different conditions are shown in Fig 1-3. Maximum TSS was recorded under $A_4$ juice blend (9.84°B) which comprise of aonla juice (60%), aloe vera extract (20%) and ginger extract (20%) followed by $A_3$ (8.41°B) combination containing of aonla, kinnow and ginger juice in ratio of (80:10:10). Likewise, the maximum titrable acidity was found in $A_1$ (1.80) and minimum under $A_5$ (0.64) juice blends proportions. The TSS increased with gradual passage of storage time (Table 3), which might be due to hydrolysis of polysaccharides into monosaccharide and oligosaccharides. The minimum increase (7.26 to 7.65 ºBrix) in TSS was recorded in T5 treatment, while maximum increase recorded in A4 which was statistically superior to other treatments. Similar results were also reported by Deka and Sethi (2001) [7] in juice blends and Deka *et al.* (2001) [7] found an increasing trend in total soluble solids during storage at ambient and low temperature in lime-aonla and mango-pineapple spiced RTS beverages. However, the rate of increase was more at ambient temperature as compared to refrigerated storage. Another possible reason for the rapid increment in soluble solid contents may be due to hydrolysis of sucrose to invert sugars. The present finding were in accord with the results reported by Deka and Sethi (2001) [7] Deka and Sethi (2001) [8] in aonla juice blends and Deka *et al.* (2001) [8] in lime-aonla and mango-pineapple spiced RTS beverages.

**Table 1:** Chemical properties of juices used for experimentation.

| Juice | Moisture (%) | TSS (°Brix) | Total Sugar (%) | pH | TA (%) | Ascorbic Acid (mg/100g) |
|---|---|---|---|---|---|---|
| Aonla cv. *Chakaiya* | 81±2.0 | 8.9±0.2 | 6.4±0.1 | 3.2 | 1.6 ±0.2 | 350.0±5.0 |
| Ber cv. *Gola* | 81±2.0 | 14±0.1 | 10.2±2.0 | 4.0 | 0.4± 0.1 | 35.5±1.0 |
| Kinnow | 82±2.0 | 13±0.1 | 4.4±1.0 | 4.0 | 0.7± 0.2 | 25.6±1.0 |
| Aloe vera gel extract | 96±1.0 | 1.8±0.1 | 0.65±0.2 | 5.6 | 1.0± 0.2 | 11.23±1.0 |
| Pomegranate cv. *Mridula* | 81±2.0 | 15±1 | 10.60±1.0 | 3.4 | 0.3± 0.2 | 15.5±1.0 |
| Ginger | 78±1.0 | 2.4±0.2 | 1.2±0.5 | 4.0 | 0.6± 0.2 | 2.60±1.0 |

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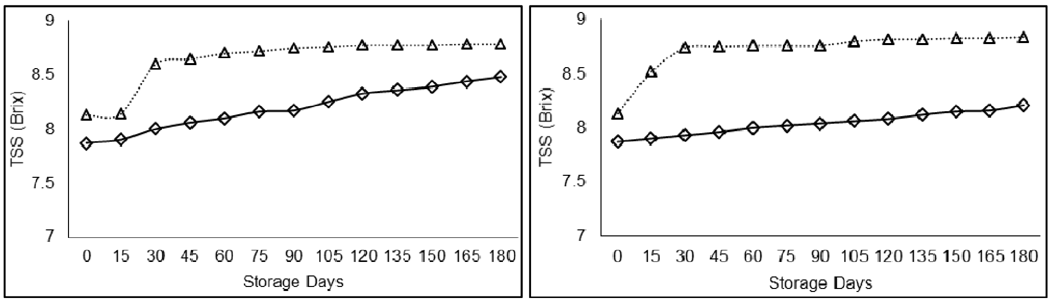


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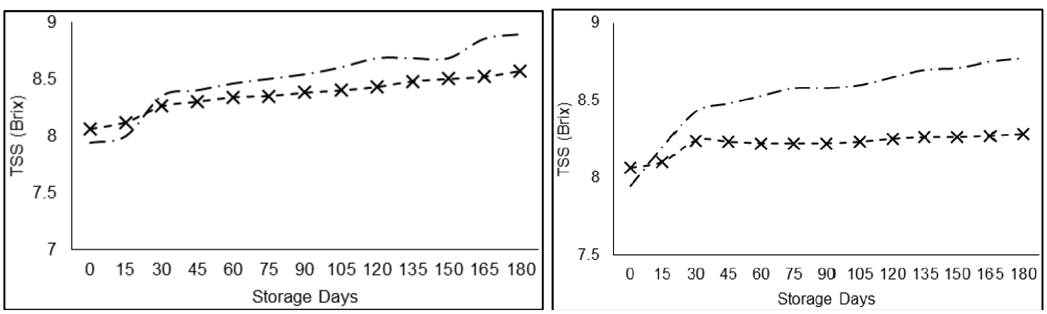


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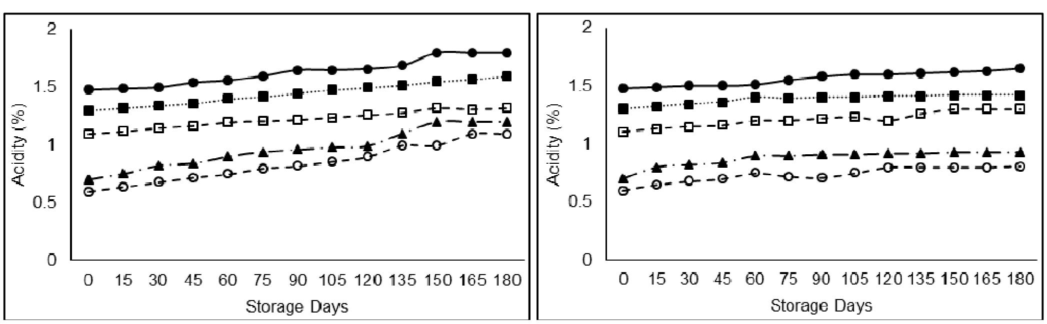
**Fig 1**: Changes in TSS in two different storage conditions with respect to a. different juice concentrations ( A1 A2 A3 A4 A5 ), b. different processing methods ( T1 T2 ) and c. different preservatives ( P1 P2 )

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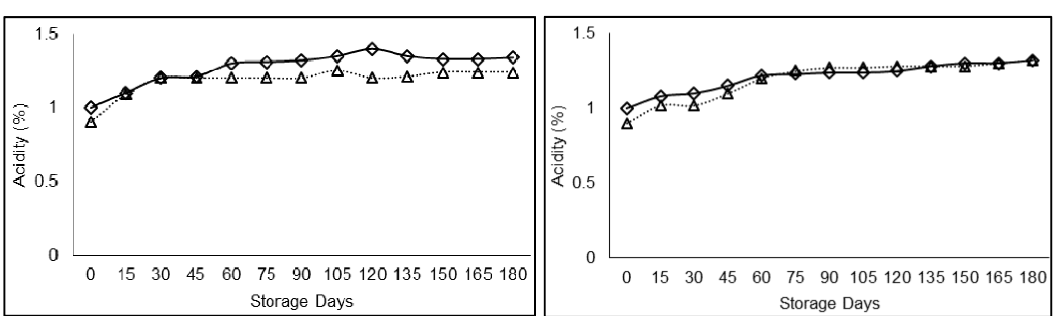


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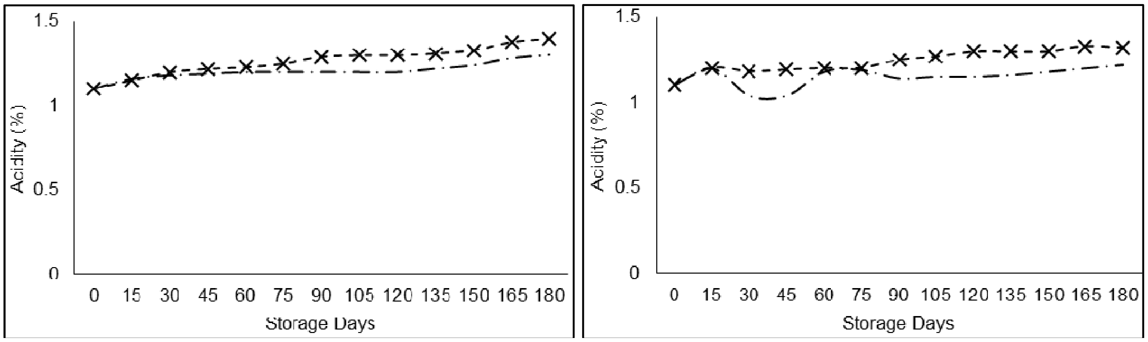


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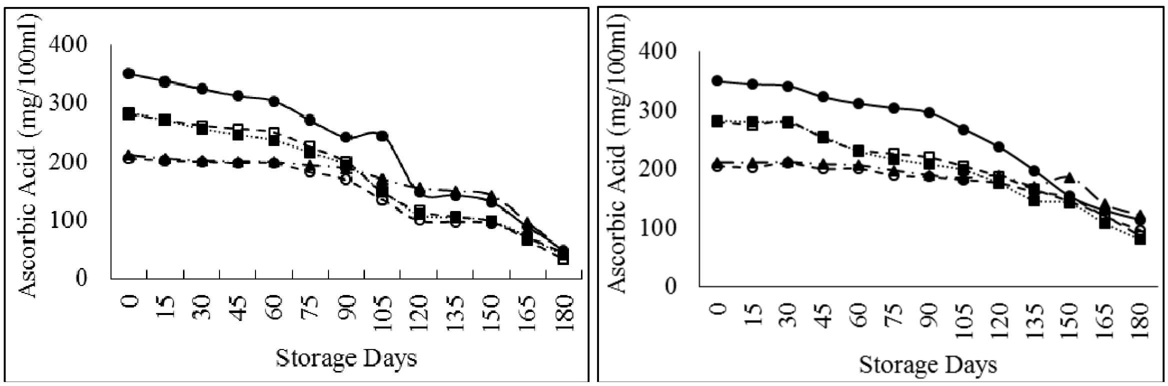
**Fig 2**. Changes in acidity in two different storage conditions with respect to a. Different juice concentrations ( A1 A2 A3 A4 A5 ), b. Different processing methods ( T1 T2 ) and c. Different preservatives ( P1 P2 )

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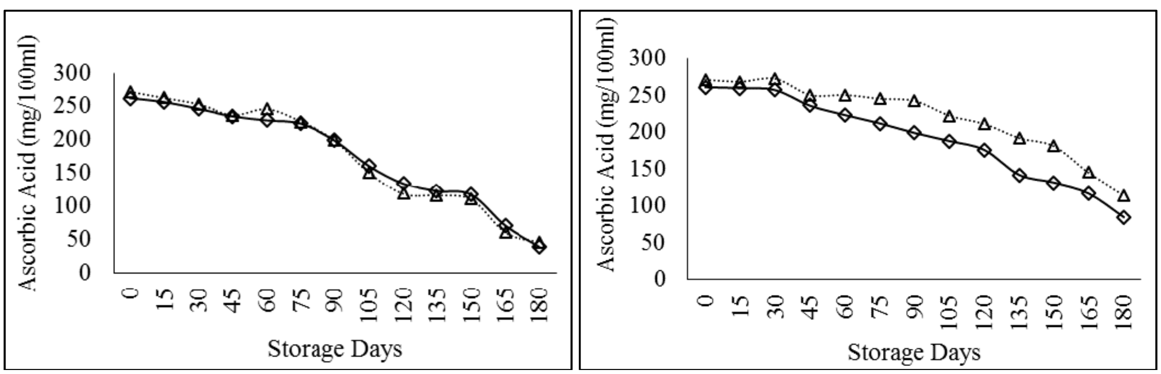


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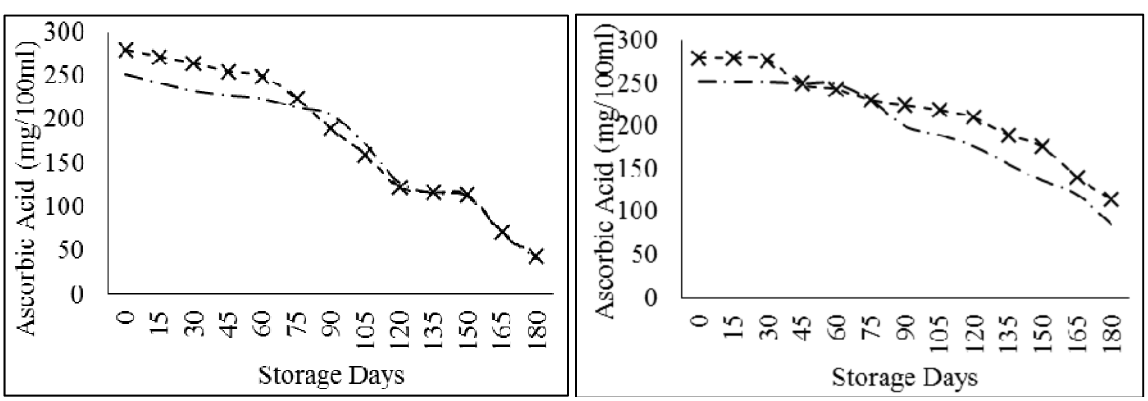


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**Fig 3**. Changes in ascorbic acid in two different storage conditions with respect to a. different juice concentrations ( A1 A2 A3 A4 A5 ), b. different processing methods ( T1 T2 ) and c. different preservatives ( P1 P2 ).

The acidity among different blends ranged from 0.64 to 1.6 %, with maximum acidity in $A_1$ and minimum in $A_5$. There was a significant increase in titratable acidity content during storage. During storage period of 6 months, there was gradual increase in acidity of beverages from 1.48 to 1.80 % at ambient compare to 1.48 to 1.65 % at refrigerated storage.
Increment of acidity of blended juice during storage may be due to disassociation of weakly acids. Similar trends were reported by Ilamaran and Amutha (2007) [12], an increment in acidity values of banana and sapota beverage stored at ambient conditions (35-36°C) and low temperature (3-5°C). The findings are in close akin with Yadav *et al.* (2013) [27] on aloe vera RTS, Pareek *et al.* (2015) [18] on kinnow and Byanna and Gowda (2013) [6] on blended sweet orange beverage.

**Changes in ascorbic acid of juice blends during storage**

The data presented in Table 3 reveal that the ascorbic acid content of juice blends was significantly affected by different fruit juice blending proportions, processing and preservative application. The same changes could be observed in Fig.3. It is clear from the fig.3 that maximum ascorbic acid content recorded in $A_1$ (349.9 mg /100 ml). From these observed results it could be inferred that reduced proportion of aonla juice in blends reduced ascorbic acid content (Table 3). When the proportion of aonla juice decreased in juice blends, ascorbic acid content get decreased viz. $A_1$ (349.6) to $A_5$ (205.8) which consist of aonla juice (60%), aloe vera extract (20%) and ginger extract (20%)). When the aonla juice proportion decreased from 100 to 60 percent it resulted to ascorbic acid decrease up to 31.62 percent.
The data presented in Fig 3 revealed that the ascorbic acid content of juice was significantly affected by juice blending ratio, processing temperature and preservative during storage. The ascorbic acid content decreased in all the treatments as the storage period advanced. The rate of decrease was higher up to fourth month of storage (Fig 3). Juice blend combination $A_4$ was found more ascorbic acid retentive compared to others. The treatment $T_2$ retained significantly higher ascorbic acid content as compared to $T_1$ under refrigerated condition. A comparatively lower loss of ascorbic acid was observed in juice samples preserved with KMS.
Among the beverages prepared with pomegranate and ginger juice ($A_4$ and $A_5$) were better ascorbic acid retention compared to others in both ambient and refrigerated conditions. This occurs due to that pomegranate and ginger juice might have reduced the oxidation process and also effective in reduction of non-enzymatic browning. Similar results were also reported by Bhardwaj and Mukherjee (2011) [5 on kinnow, Yadav *et al.* (2014) [29] on carrot and Deka and Sethi (2001) [8] on spiced blended juice.
The ascorbic acid content of the juice during storage reduced with the advancement of storage period because the ascorbic acid is most labile vitamin in all and very sensitive to oxidation. Losses in ascorbic acid depend upon various factors such as oxygen, heat, light and storage (Robertson and Samaniego 1990) [23]. Juice blend combination $A_4$ was found more ascorbic acid retentive compared to others. Similar finding also reported by Paul and Ghosh (2012) [19] revealed that the degradation of ascorbic acid increased with the increase in the temperature but the degradation was slower in addition of pomegranate juice. This was probably due to the presence of various phytochemicals in pomegranate juice and their synergistic effect with other juices. These losses of ascorbic acid occur enzymatically or non-enzymatically.
Degradation of ascorbic acid proceeds in both conditions in presence and absence of oxygen (Johnson *et al.* 1995) [14]. Heat treatment of juice at 70°C and 85°C reduced the ascorbic acid, irrespective of holding times. Heat processing and the presence of air at the headspace of glass bottles during storage is one of the most important reasons for its degradation (Huelin 1953) [10]. In the present investigation, the maximum loss in ascorbic acid was higher when processing temperature was higher (85°C). This might be due to more oxidation of ascorbic acid at higher temperature. Similar findings were also observed by Ranote and Bains (1982) [21] in Kinnow juice. Microwave processing resulted higher ascorbic acid retentive as compared to conventional processing up to the end of storage. Similar finding also reported by Igual *et al.* (2010) [11] observed that microwave pasteurization of grape fruit juice remain stable up to two months at refrigerated and frozen storage.
There was significant decrease in ascorbic acid (36 to 34.3 mg/100gm) processed by microwave pasteurization. This might be due to the higher concentration of KMS reduced the oxidation of ascorbic acid during storage for long time. The retention of ascorbic acid was more at lower storage temperature as compared to storage temperature of at ambient storage. Pareek, Paliwal, and Mukherjee (2015) [18] also observed similar results on mandarin juice during storage. Beltran *et al.* (2009) [4] also reported same results that 74% loss of ascorbic acid in stored orange juices up to six months of storage. Similar results also noticed by various workers including Bhardwaj and Mukherjee (2011) [5] on kinnow, Yadav *et al.* (2014) [29] on carrot and fruit juices blend and Panesar *et al.* (2000) [17] in kinnow juice.
The maximum retention of ascorbic acid content in refrigerated storage might be attributed to low temperature and high relative humidity in storage, which inhibited the rate of oxidation and metabolic activities. These finding are in concurrence of with finding of Igual *et al.* (2010) [11] on grape fruit, Viberg *et al.* (1999) on strawberries, Bhardwaj and Mukherjee (2011) [5] on kinnow, Yadav *et al.* (2014) [29] on carrot, Ranote and Bains (1982) [21] on kinnow fruit.

**Changes in total sugars of juice blends during storage**

It is obvious from the data in the Table 2 that total sugar content of juice blends increased with advancement of storage during period of experimentation. A significant difference was observed among the juice blending treatments. Among the juice blending treatments, the maximum total sugars (9.73 and 8.29 percent) were recorded in treatment $A_4$ and $A_3$ respectively. The highest rate of increase was also recorded in same treatments $A_4$ (9.23 to 9.73) and $A_3$ (7.81 to 8.29 %) compared to all other treatments.
Total sugar content was found lowest in $A_5$ (5.83) having aonla, aloe vera and ginger in ratio of (60:20:20) respectively. As the proportion of pomegranate increased it got increased as in case of $A_4$ (9.22) combination. The interaction and combined effect of juice blending proportion and processing method and preservative application was found to be non-significant during entire period of storage. It is clear from the Table 2 that the total sugars content was higher under $T_1$ treatment as compared to $T_2$ treatment during entire period of storage. However, on $6^{th}$ month the maximum total sugars content (7.62) was recorded under $T_1$ treatment. It is also clear from the Table 2 that the total sugars content increased in an erratic manner under $P_1$ and $P_2$ treatment. There was enhancement of sugar concentration during storage due to hydrolysis of starch in monosaccharide or polysaccharides.

Similar trend of increase in sugars have been reported by Waskar and Khurdiya (1987) [27] in phalsa syrup and Jadhav *et al.* (2006) [13] in kokum syrup and Yadav *et al.* (2014) [29] on carrot juice blends.

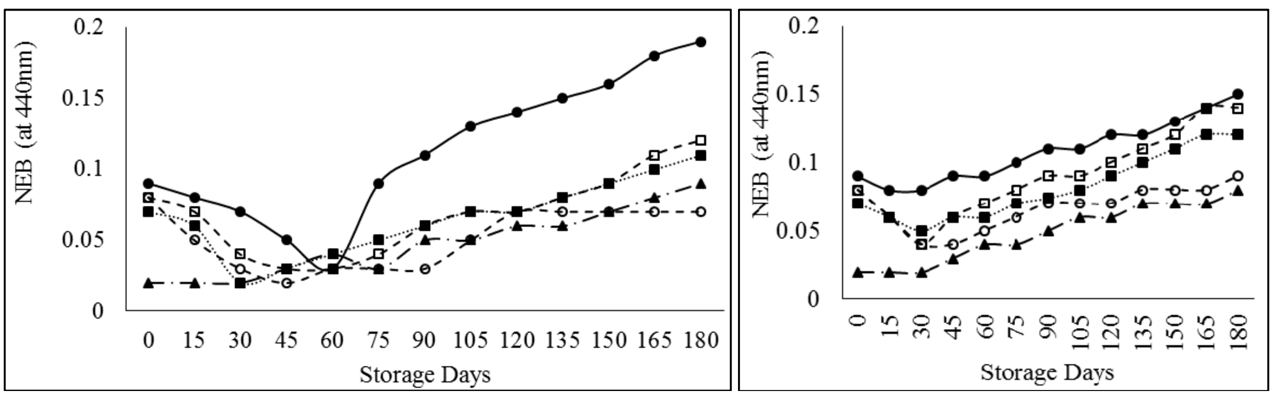
### Change in NEB during storage

It is clear from the data presented in Fig 4. that the non-enzymatic browning of fruit juice was significantly affected by juice blending proportions. Non-enzymatic browning was recorded in the range of 0.02 to 0.19 with the maximum of in $A_1$ (0.19)

**Table 2:** Effect of blending ratio, processing temperature and preservatives on total, reducing and non reducing sugars of blended aonla juice during storage

| Treatments | Ambient storage | | | | | | Refrigerated storage | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TS (%) | | RS (%) | | NRS (%) | | TS (%) | | RS (%) | | NRS (%) | |
| | I | F | I | F | I | F | I | F | I | F | I | F |
| $A_1$ | 5.83 | 6.43 | 4.06 | 4.34 | 2.46 | 2.1 | 5.83 | 6.65 | 4.06 | 4.3 | 2.46 | 2.14 |
| $A_2$ | 6.33 | 6.93 | 4.16 | 4.5 | 2.78 | 2.4 | 6.33 | 7.19 | 4.16 | 4.4 | 2.78 | 2.36 |
| $A_3$ | 7.81 | 8.29 | 5.30 | 5.65 | 2.85 | 2.4 | 7.81 | 8.53 | 5.30 | 5.6 | 2.85 | 2.4 |
| $A_4$ | 9.23 | 9.73 | 6.60 | 7.20 | 2.7 | 2.3 | 9.23 | 9.92 | 6.60 | 7.3 | 2.7 | 2.38 |
| $A_5$ | 5.83 | 6.41 | 3.25 | 3.63 | 2.25 | 1.9 | 5.83 | 6.89 | 3.25 | 3.63 | 2.25 | 2.08 |
| SE± | 0.44 | 0.26 | 0.56 | 0.30 | 0.22 | 2.1 | 0.44 | 0.26 | 0.56 | 0.32 | 0.22 | NS |
| CD | 0.88 | 0.53 | 1.14 | 0.60 | 0.44 | 0.35 | 0.88 | 0.51 | 1.14 | 0.65 | 0.44 | NS |
| $T_1$ | 7.07 | 7.62 | 4.49 | 5.15 | 2.72 | 2.72 | 7.07 | 8.11 | 4.49 | 5.11 | 2.72 | 2.35 |
| $T_2$ | 6.95 | 7.49 | 4.3 | 4.98 | 2.45 | 2.45 | 6.95 | 7.56 | 4.3 | 4.91 | 2.45 | 2.2 |
| SE± | NS | NS | 0.26 | NS | NS | 0.11 | NS | NS | 0.26 | NS | NS | NS |
| CD | NS | NS | 0.53 | NS | NS | 0.22 | NS | NS | 0.53 | NS | NS | NS |
| $P_1$ | 7.24 | 7.7 | 4.80 | 5.06 | 2.56 | 2.74 | 7.24 | 8.09 | 4.80 | 4.96 | 2.56 | 2.22 |
| $P_2$ | 6.77 | 7.41 | 4.59 | 5.07 | 2.61 | 2.75 | 6.77 | 7.58 | 4.59 | 5.06 | 2.61 | 2.33 |
| SE± | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| CD | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |

**Note**: * Significant at 5% level, ** Significant at 1% level, NS: non significant, SE: standard error, CD: critical difference, TS; Total Sugar, RS; Reducing Sugar, NRS; Non reducing sugar, I: Initial data at the time of filling and F: after 6 months storage

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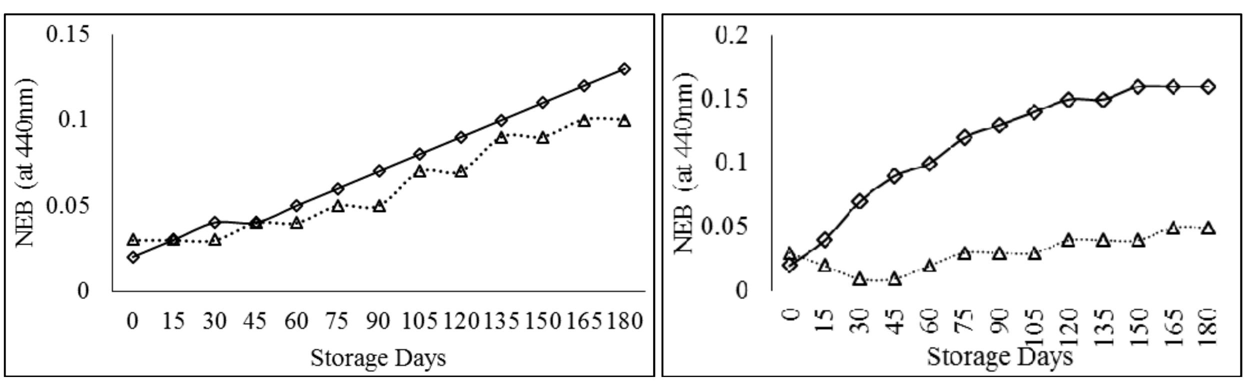


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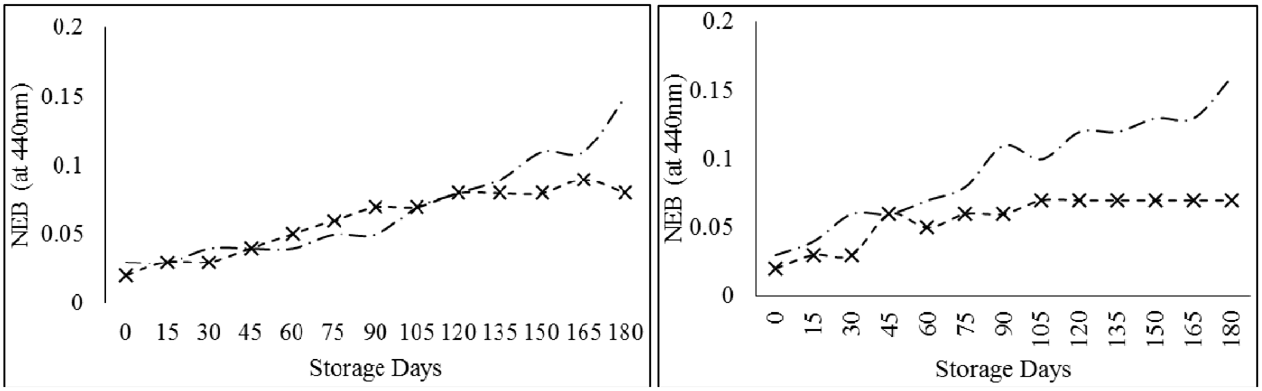



**(C)**

**Fig 4**. Changes in neb in two different storage conditions with respect to a. different juice concentrations ( A1 A2 A3 A4 A5 ), b. different processing methods ( T1 T2 ) and c. different preservatives ( P1 P2 ).

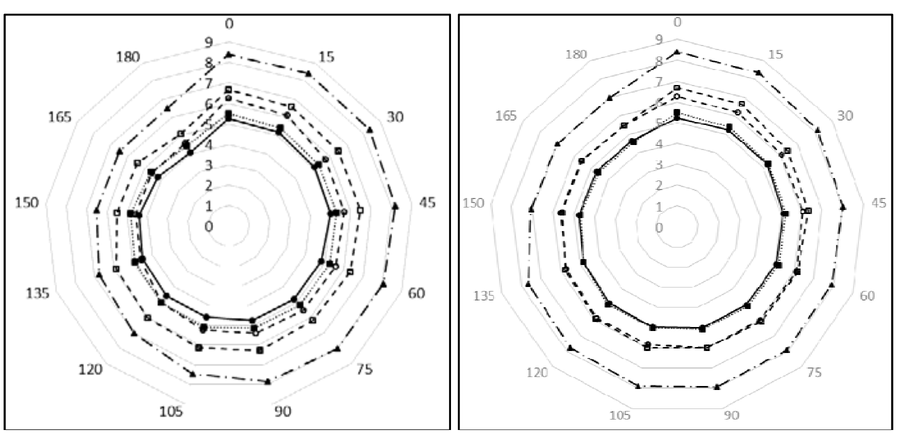
And minimum in $A_5$ (0.07), respectively at the sixth month of storage. $T_1$ treatment has more browning as compared to $T_2$ treatment (Fig.4). Further, It is clear from the data presented in Fig 4.4 that it was lower in juice preserved $P_1$ (KMS 500 ppm) compared to $P_2$ throughout the storage. The combined effect of juice blending ratio and potassium-meta-bi-sulphite was found to be non-significant during the entire period of storage. The interaction effect also found to be non-significant during the entire period of storage.

A linear increase in non-enzymatic browning was observed during six months of storage irrespective of juice blend. The increase in non-enzymatic browning during storage might be due to non-enzymatic reaction of organic acid with sugars or oxidation of phenols, which leads to the formation of brown pigments. Thermal degradation of sugars, amino acids and ascorbic acid can produce off-flavors and NEB products (Lee and Nagy 1988) [16]. Khurdiya and Anand (1981) also reported a gradual increase in browning and formulation of hydroxyl methyl furfural (5-HMF) in stored phalsa beverage.

The minimum increase in non-enzymatic browning in the juice blended with pomegranate juice (20%) and ginger juice (20%) might be due to suppression of polyphenol oxide activity by ascorbic acid. The pomegranate juice was also effective in the reduction of non-enzymatic browning due to higher sugar content. Colour, flavour and taste of the pomegranate squash remained better at low temperature than at room temperature. Similar findings were reported by Waskar and Khurdiya (1987) [27] on pomegranate.

**Change in Overall sensory quality**

The details on the change of overall sensory of blended fruit juice as affected by juice blending proportion are presented in the form of radar plot (Fig. 5). The plot indicated that the overall sensory of blended juice increased with blending other fruit juices particularly, pomegranate and aloe vera juices. Maximum score for blended juice was recorded in $A_4$ (8.42) treatment followed by $A_5$ (6.25). Processing method found non-significant. Preservative did not affected sensory quality during storage. All blends got deteriorated with the advancement of storage period from 1st to 6th month



**(A)** **(B)**

**Fig 5:** Radar plots on overall sensory scores of different juice blend during storage. ( A1 A2 A3 A4 A5 ), a. juice blends stored in room temperatures, b. juice blends stored in refrigerated temperatures.

**Conclusion**

Blending of aonla with aloe vera and pomegranate in the proportion of 60:20:20 gave better nutritional quality retention with higher sensory score. Storage of blended beverage containing above formulation remained more shelf stable when stored for 6 months. So blending of aonla juice with aloe vera and pomegranate juice can prove a boon to the growers in getting a good remunerative for their produce and to consumers in getting antioxidant rich beverage at reasonable price.

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