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Ascorbic acid loss, microbial spoilage and sensory changes in aonla juice during storage

S.K. Jain* and D.S. Khurdiya
Division of Post- Harvest Technology, IARI, New Delhi- 110 012

Abstract

Being acidic and astringent in taste and perishable in nature the potential of aonla for preparation of vitamin C rich beverages has been explored on. In present endeavor attempt was made to study the effect of pasteurization, sulphitation in combination with different storage conditions so as to draw inference as to how long the quality of aonla juice stands intact. Sulphitation (350 ppm SO_2) of aonla juice coupled with storage at low temperature minimized the loss of vitamin 'C' and produce best sensory quality even after six months of storage. Pasteurization and sulphitation both inhibited the yeast growth throughout the storage period and thus helped extend the storability of aonla juice.

Key words: *Ascorbic acid, aonla, juice, pasteurization, storage, sulphitation*

Introduction

The aonla (*Emblica officinalis* Gaertn.) also known as Indian Gooseberry is one of the oldest minor fruits of India. It is well known for its nutritional quality being rich in vitamins, minerals and tannins. It is the richest source of vitamin 'C' among the fruits except Barbados cherry. Its vitamin 'C' content varies from 200-900 mg 100 g^{-1}, depending upon variety and size of the fruit (Barthakur and Arnold, 1991).

Owing to high acidity and astringency, aonla fruit is not popular as a desert or table fruit. Value addition seems viable option to avert this constraint. Being amenable to help extend the period of availability and offering variety, the technique is effective in ensuring economic utilization of fruit also. It is the value addition which ensures its utilization as processed products viz. preserve (murabba), candies, jam etc.; dehydrated products viz. aonla shreds, supari, tit-bits, salt (churan) and various kinds of pickles. Besides aonla fruit have its uses across ayurvedic preparations such as *Chyavanprasha, Triphala, Ashoka-Arishtha, Arogya- Vardhini,* etc. There has been a considerable increase in the consumption of processed products especially fruits and vegetables beverages in the world during last few years (Anon., 1998). Various workers (Singh and Kumar, 1995; Nath, 1999; Jain and Khurdiya, 2004; Jain, *et al.*, 2006) have reported utility of aonla fruits in preparation of vitamin 'C' rich beverages. Since, aonla is seasonal in nature it is important to store the juice all round the year for use as raw material for processing into various types of beverages. But during processing and subsequent storage, the aonla juice suffers from loss of vitamin 'C', microbial spoilage and changes in sensory quality. In line to this problem, the present investigation was conducted to study the effect of sulphitation, pasteurization and storage conditions on the vitamin 'C' content, microbial load and sensory quality of the aonla juice during storage.

***Corresponding author's E- mail:**
jain_shashi_kumar@yahoo.co.in

Materials and methods

Sample preparation and treatments

For the study, aonla fruits of Chakaiya variety were procured from Azadpur Fruit Mandi, New Delhi. Juice was extracted by crushing and pressing the blanched fruits (after seed removal) and water in 1:1 ratio (Jain and Khurdiya, 2002). The whole juice was divided into four lots and following treatments were given before filling and sealing in 200 ml glass bottles.–

1. Untreated (Control)
2. Pasteurization at 90^0C for 1 minute and filling hot in the pre-sterilized, hot glass bottles (Jain and Khurdiya, 2003).
3. Treatment with 350 ppm sulphur dioxide.
4. Pasteurization at 90^0C for 1 minute; cooled to 60^0C and added with 350 ppm sulphur dioxide before sealing in glass bottles.

For each treatment, total number of bottles were divided into three lots and stored under following storage conditions for a period of six months: -

1. Room temperature (25 ± 1^0C) in ambient light
2. Room temperature(25 ± 1^0C) in dark condition
3. Cool storage (4 ± 1^0C)

Vitamin 'C' analysis

The vitamin 'C' (ascorbic acid) content of the samples was determined by 2, 6-dichlorophenol indophenol visual titration method. The interference due to SO_2 was eliminated by adding 40 per cent formaldehyde and HCl before titration (Ranganna, 1997).

Microbial spoilage

The stored aonla juice was evaluated qualitatively and quantitatively for microbial load to determine the effect of various treatments and storage conditions. Five media viz. Potato Dextrose Agar (PDA), Plate Count Agar (PCA), Nutrient Agar (NA), Malt Extract Agar (MEA) and Dextrose Trypton Agar (DTA) were prepared. The media were sterilized in autoclave at 1.1 kg cm^{-2} pressure (121°C) for 20 minutes and poured into pre-sterilized petri- dishes. Each sample bottle was thoroughly shaken before opening under aseptic condition (laminar-air-flow cabinet). A loop full of sample was streaked on the medium in a Petri dish. Streaked plates were inverted and incubated in a BOD incubator at 25 ± 1°C and observations were recorded after every 24 hours for 7 days.

For the determination of microbial population of the juice, initially as well as after fermentation, spotting method was used. Serial dilutions from 10^{-1} to 10^{-6} were prepared from the original juice samples, using sterilized distilled water. A drop of known volume was spotted on the solid Plate Count Agar (PCA) medium with the help of a Pasteur pipette. The plates were left undisturbed for 1 hr for the spot to be absorbed in the medium. The plates were then inverted and incubated in a BOD at 25±1^{0}C. The colonies were counted on a colony counter after 72 hr of incubation. The results were expressed in cfu/ml of juice.

Sensory quality evaluation

The juice from various treatments and storage conditions were used for preparation of RTS beverage containing 10 per cent juice, 10° Brix TSS and 0.225 per cent acidity and evaluated for colour, flavour and taste on a 9 point hedonic scale rating (Amerine *et al.*, 1965) by a panel of seven expert judges. A score of 5.5 and above was considered as acceptable.

Statistical analysis

The entire experiment was laid out in complete randomized block design with three replications. Data collected for the experiment during storage of aonla juice was subjected to statistical analysis by the analysis of variance technique as suggested by Panse and Sukhatme (1989). Wherever variance value (f value) was found significant, the critical difference value at 5 % level of probability was compared for making the comparison between the different treatments.

Results and discussion

The stored juice was analyzed for ascorbic acid and sensory attributes at monthly interval up to a period of six months. Microbial load was enumerated in fresh juice and after fermentation which was evident by gas formation, development of fermented smell, bulging of crown cap of bottle and formation of white film on the surface of the juice.

Ascorbic acid

Irrespective of the treatments and storage conditions a continuous decrease in ascorbic acid content of the aonla juice was observed as the storage period progressed. After six months of storage, maximum vitamin 'C' content (232.7 mg/ 100 ml) was observed in SO_2 treated juice stored at low temperature, followed by 195.5 mg/ 100 ml in pasteurized + SO_2 treated and 189.3 mg/ 100 ml in pasteurized aonla juice stored at low temperature. Similar findings have also been reported in lemon juice (Bansal and Dhawan, 1993). The higher loss of ascorbic acid at room temperature than cool store might be due to higher rate of degradation of ascorbic acid at higher storage temperature (Mehta and Rathore, 1976 and Deka, 2000). Exclusion of light by keeping the aonla juice in dark condition retained more ascorbic acid as compared to light condition at ambient temperature. This might be due to light sensitivity of vitamin 'C'. These findings are in conformity with the findings of Deka (2000).

Microbial quality of aonla juice

In fresh aonla juice, there were no evidences of mould or bacterial contamination but growth of yeasts was noticed (Table 1). However, treatments viz., pasteurization, SO_2

Table 1. Occurrence of microorganisms in aonla juice as affected by different preservation methods on different media

Treatment	Media				
	PDA	PCA	NA	MEA	DTA
Untreated	+ (yeast)	+ (yeast)	+ (yeast)	+ (yeast)	+ (yeast)
Pasteurization	–	–	–	–	–
SO_2 (350 ppm)	–	–	–	–	–
Pasteurization + SO_2	–	–	–	–	–

PDA - Potato Dextrose Agar
PCA - Plate Count Agar
NA - Nutrient Agar
MEA - Malt Extract Agar
DTA - Dextrose Trypton Agar
(+): Presence of viable microorganisms
(–): Absence of viable microorganisms

treatment and pasteurization + SO_2 treatment completely inhibited the growth and did not show any evidence of fermentation of juice by yeast throughout the storage period, irrespective of storage conditions. Similarly, complete inhibition of yeasts by pasteurization and SO_2 treatment has been reported by Kalra and Revathi (1981) in guava pulp.

With the advancement of storage period there was a gradual increase in the population of yeasts in untreated aonla juice (Table 2). But the rate of yeast growth was slower in cool stored aonla juice as compared to room temperature. Initially, the yeast population was $3.57x10^2$ cfu/ml which got increased to $20.5x10^3$ and $21.5x10^3$ cfu/ml at second month in light and dark condition of room temperature, respectively. This coincided with the fermentation of the sample. At this stage, the yeast population in cool stored juice was $1.5x10^3$ cfu/ml, which was still acceptable. But yeast count got increased to $16.7x10^3$ cfu/ml at third month and caused fermentation of juice. Fermentation was also evident by bulging of crown cap of the bottles, gas formation and frothing. These findings are in consonance with the findings of Kalra and Revathi (1981) who reported higher yeast count in guava pulp stored at ambient temperature as compared to low temperature, after 45 days of storage.

Table 2. Yeast population (colony forming units/ml) in untreated aonla juice stored under different storage conditions

Storage condition	Storage period (months)		
	0	2	3
Room temperature (light)	$3.57x10^2$	$20.5x10^3$	*
Room temperature(dark)	$3.57x10^2$	$21.5x10^3$	*
Cool store	$3.57x10^2$	$1.5x10^3$	$16.7x10^3$

(*) = Observation not recorded since sample discarded due to complete spoilage at 2nd month

The yeasts which caused fermentation in untreated juice, stored at both the light and dark conditions of room temperature and cool store were identified as *Saccharomyces cereviceae* and *Candida tropicalis*, respectively.

Sensory quality of RTS beverage

The data presented in Table 3 indicate that initially, the RTS beverage prepared from untreated and pasteurized aonla juice had higher sensory quality as compared to that from SO_2 treated and pasteurized + SO_2 treated aonla juice. This may be due to smell of sulphur dioxide in the KMS treated aonla juice. The sensory quality of RTS prepared from SO_2 treated aonla juice increased during first month of storage, thereafter it decreased. This increase in sensory quality during first month of storage may be due to reduction in the residual SO_2 content in the juice. However, in case of untreated and pasteurized juice, the sensory quality of RTS beverage showed continuous decline right from the start of storage study and throughout the storage period. Similar decline in sensory quality of aonla juice was

Table 3. Sensory score (out of 9) of RTS beverage prepared from aonla juice as affected by preservation methods and storage conditions

Storage condition	Treatment	Storage period (months)						
		0	1	2	3	4	5	6
RT (light)	Untreated	7.50	6.53	6.11[a]	*	*	*	*
	Pasteurization	7.37	7.08	6.65	6.26	5.78	5.18	4.42
	Sulphitation (SO_2)	6.87	7.04	6.75	6.48	6.14	5.51	4.93
	Past. + SO_2	6.87	7.04	6.77	6.46	6.13	5.53	4.98
RT (dark)	Untreated	7.50	6.14	5.57[a]	*	*	*	*
	Pasteurization	7.37	6.73	6.37	6.12	5.85	5.36	4.75
	Sulphitation (SO_2)	6.87	6.89	6.5	6.27	6.11	5.38	5.08
	Past. + SO_2	6.87	6.80	6.35	6.18	5.97	5.60	5.12
Cool store	Untreated	7.50	6.97	6.46	*	*	*	*
	Pasteurization	7.37	7.17	6.86	6.68	6.38	5.97	5.47
	Sulphitation (SO_2)	6.87	7.39	7.31	7.15	6.95	6.77	6.56
	Past. + SO_2	6.87	7.30	7.04	6.89	6.58	6.37	6.18
Mean		7.15	6.92	6.56	6.50	6.21	5.74	5.28
C. D. at 5%								
Storage condition × treatment		NS	0.13	0.07	0.14	0.13	0.18	0.14

RT = Room temperature (25±1°C)

(a) Juice spoiled due to fermentation

(*) Observations not recorded due to fermentation

reported by Tripathi *et al.* (1988).

After six months of storage, only the RTS beverage prepared from aonla juice preserved by SO_2 alone and Pasteurization + SO_2, stored in cool store was acceptable whereas, others were acceptable only up to five months. This may be due to better retention of colour due to lower NEB and better flavour retention due to SO_2 treatment, as also reported by Ranote and Bains (1982) in Kinnow mandarin juice. Similarly, better retention of sensory quality of beverages under low temperature has also been reported by Khurdiya *et al.* (1995) in ameliorated juice of teinturier grape hybrids.

There was no significant difference in sensory quality of aonla juice, stored in light and dark conditions at room temperature at fifth month of storage. These findings are in consonance with the findings of Granzer (1983) in orange juice.

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