

Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com



E-ISSN: 2278-4136 P-ISSN: 2349-8234 www.phytojournal.com JPP 2020; 9(6): 601-604 Received: 27-08-2020 Accepted: 09-10-2020

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Improving the juice yield of Aonla juice cv. NA-7 and seedling Aonla using enzymatic juice extraction method

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Abstract

Method for juice extraction and storage of aonla juice cv. NA-7 and *Desi* (Seedling) aonla was optimized. Out of four different combinations, enzymatic pretreatment of grated aonla mass by adding 1000ppm pectinase enzyme at 50°C for 4 hours followed by juice extraction in screw type juice extractor resulted in a significant improvement in juice yield i.e. 68.1% in cv. NA-7 and 65% in Desi (seedling) aonla against 50.1% in NA-7 and 48.1% in Desi (seedling) aonla fruits. The extracted juice exhibited 9.9-10.13°B TSS, 1.21-1.24% titratable acidity, 543.3-545mg/100ml ascorbic acid, 3.19-3.29mgGAE/100ml total phenols, 0.97-0.99% tannins with 56.3-58.6% antioxidant activity. Juice of Desi (seedling) aonla fruits on the other hand contained comparatively higher level of TSS 12-12.06°B, Ascorbic acid 586-588.9mg/100ml, 3.39-3.48mg GAE/100ml total phenols, 1.09-1.19% tannins with 62.3-64.9% antioxidant activity.

Keywords: Enzymes, juice extraction, juice yield, pectin, pectinase.

Introduction

Aonla (Phyllanthus emblica) or Indian gooseberry is indigenous to Indian subcontinent. It is mostly cultivated in the states of Uttar Pradesh, Maharashtra, Guirat, Rajasthan, Andhra Pradesh, Karnataka, Tamilnadu and Himachal Pradesh etc. Total area under Aonla in India was approximately 93 thousand ha. with the production of 1077 thousand MT during 2018 (NHB, 2018)^[8]. Banarasi, Chakaiya, Kanchan, NA-7 and NA-10 are some of the commercially cultivated varieties of aonla in India. Aonla has prominent antibiotic, antiulcerogenic, diuretic, laxative, antitumor, cardiotonic and antiviral properties (Jose and kutton, 2000)^[5]. Fruits are rich in ascorbic acid and polyphenols and thereby possessing the ability to scavenge free radicals. It has been reported that a single aonla fruit contains almost 20 times more ascorbic acid than two oranges. Aonla is good source of phytochemicals such as tannins, polyphenols, emblicol, phyllemblin etc. (Baliga and Dsouza, 2011)^[1]. Aonla juice is one of the important processed products which is known to retain maximum amount of ascorbic acid after processing. It has good potentiality for blended and spiced beverages along with direct consumption as health drink. The traditional method of juice extraction is through the use of mechanical presses viz., traditional rack and cloth press, and the belt press. However the juice yield in these methods is very less. Enzymatic treatment prior to mechanical extraction gives significant increase in juice recovery (Joshi et al., 1991) [4]. Enzymes break down the tissues of cell wall and release the juice. The enzyme treatment not only ensures highest possible juice recovery but also ensures good quality of end product. Currently pectinases, cellulases and hemicellulases are collectively called as macerating enzymes and are used in better pressing, extraction and clarification of juices. Amylases are also used in fruit juice production as it breaks down the starch.

Material and methods

Physico- chemical characteristics

Aonla fruits and the extracted juice of both the varieties viz; cv. NA-7 and Desi (Seedling) aonla was evaluated for physico-chemical attributes by following standard analytical methods as described below

Fruit size

The fruit size of randomly selected ten fresh fruits of both the varieties was determined with the help of a vernier caliper and expressed in mm as mean \pm SD.

Fruit weight

Ten Aonla fruits from each variety were randomly selected and weighed with electronic weighing balance. The mean fruit weight was expressed in gm as mean \pm SD.

Stone size and weight

Stone size and weight was assessed using vernier caliper and electronic weighing balance respectively.

Juice yield

Juice yield was expressed in percentage.

Chemical characteristics of aonla juice Total Soluble Solids

Total soluble solids were determined by using hand refractometer (range $0-32^{\circ}B$). Total soluble solids of sample were measured by placing a drop of the juice on the prism of the refractometer and reading was expressed in the term of degree brix (Ranganna, 2014)^[9].

Titratable Acidity

The titratable acidity of aonla juice was determined by the standard method as explained by Ranganna (2014)^[9]. 10ml of sample was diluted with 100ml distilled water. Further, 10ml of this aliquot was titrated against 0.1N NaOH solution to a pink end point using phenophthalein as an indicator. The titratable acidity as citric acid was calculated by the following expression:

$$Titratable \ acidity \ (\%) = \frac{\text{Titre value \times normality of alkali \times Vol.made \times Equivalent weight of acid}}{\text{Volume of sample taken \times Volume of aliquot taken \times 100}} \times 100$$

Ascorbic acid

The ascorbic acid content was determined by the standard method of Ranganna (2014)^[9]. 10ml of sample (juice) was mixed with 3% metaphosphoric acid to make 100ml volume followed by filtration through filter paper. 5ml of the aliquot

was taken and titrated with standard dye (2, 6dichlorophenol- indophenol dye) to a faint pink colour end point. Ascorbic acid (mg/100ml) in the sample was calculated using following expression:-

Ascorbic acid (mg/100ml) =
$$\frac{\text{Titre value } \times \text{ Dye factor } \times \text{ volume made}}{\text{Weight of sample taken for estimation } \times \text{ Vol. of aliquot taken}} \times 100$$

Total phenol

Total phenol was estimated by the method of Swain and Hillis (1959) ^[12]. To 10ml of sample, 80% methanol (5ml) was added and placed over water bath shaker for half an hour and after cooling, the mixture was filtered/ centrifuged to collect the clear supernatant. The volume of supernatant was made to 10ml with 80% methanol, to which 0.5ml of Folin ciocalteu

reagent was added and shaken thoroughly. After 5 minutes, 1ml of saturated solution of sodium carbonate was added to the mixture and allowed to stand for 1 hour. The absorbance of the developed blue colour was read at 760nm against blank. The concentration of total phenol was determined from standard curve prepared by using Gallic acid (10-50 μ g) and expressed as μ g GAE/ml.

$$Total Phenol (mg GAE/ml) = \frac{Standard concentration \times Total Volume \times Sample OD}{Standard OD \times Aliquot taken \times Volume of extract used}$$

Aonla Juice Extraction

Aonla juice was extracted by crushing the fruits in fruit grater/mill (M/S Bajaj machineries Pvt. Ltd. Delhi) and then passing the grated mass through a screw type juice extractor heavy duty (M/S Bajaj machinery Pvt. Ltd. Delhi) with or without enzymatic pre-treatments. Different concentrations of ezymes viz; Pectinase and Amylase were used in both the varieties. The fruits were destoned prior to crushing. The crushed material was then passed through screw type juice extractor and juice was recovered. In enzymatic juice extraction the appropriate quantity of enzyme was added to the crushed mass followed by its incubation at 50 °C temperature for 4 hours (Sharma *et al.*, 2014)^[11] to extract the juice. After juice extraction, the juice was filtered and pasteurized at 90 °C for 10 seconds and then packed in juice bottles.

Results and discussions Physical Characteristics of fresh Aonla

The various physico-chemical characteristics of Aonla fruit cultivar '*NA-7*' and "*Seedling Aonla*" assessed in the present study are presented in Table 4.1.

 Table 1: Physical chacteristics of Aonla cv. NA-7 and Desi (Seedling Aonla) fruits

Varieties	NA-7	Desi (Seedling aonla)		
Parameters	Mean±SD	Mean±SD		
Fruit length(mm)	30.37±0.49	19.79±0.46		
Fruit dia.(mm)	33.71±1.20	23.75±0.54		
Fruit weight(g)	19.76±1.06	6.96±0.28		
Stone length (mm)	14.52±0.55	10.77±0.42		
Stone dia. (mm)	12.77±0.29	9.42±0.40		
Stone weight(g)	1.64±0.05 (8.3%)	1.02±0.05 (14.7%)		
Pulp weight(g)	18.12±0.05 (91.7%)	5.94±0.05 (85.3%)		

Juice Yield (%)

It is evident from Table 4.2 that Aonla juice yield in cold pressed juice or control was found to be 50.1% and 48.1% in

Aonla cv. NA-7 and Desi respectively while enzymatic pretreatment exhibited a juice yield of 68.3% and 65.2% in NA-7 and Desi respectively. The highest juice yield (%) was

found in aonla crushed mass treated with Pectinase 1000ppm. Sharma *et al.*, (2014) ^[11] studied the effect of enzymes on juice yield and reported juice yield in range of 62-92%. He reported 63% juice yield in pineapple after enzymatic treatment and 86% juice recovery in Bael fruits. The enzyme treatment of plum, peach, pear and apricot have shown clearly that the juice yield increased upto 78% in plum, 63% in peach, 72% in pear and 80% in apricot, respectively (Joshi *et*

al., 2011). Hence use of pectinase enzyme treatment of crushed aonla fruits for 4 hours at $50\pm1^{\circ}$ C was considered the most appropriate for improving the juice yield. Further with respect to to concentration of pectinase there was no significant difference in juice yield as such. So 1000ppm pectinase was considered best in improving juice yield in both the varieties i.e. NA-7 and Desi (seedling) aonla.

Table 2: Effect of enzymatic treatments of juice extraction on juice yield (%) of cv NA-7 and Desi (Seedling insitu) aonla fruits

	Parameters				
		NA-7	Desi	Maaa	
Enzymes		Aonla	(seedling) aonla	Mean	
	Control	50.1	48.1	49.1	
Destinaça (nom)	1000	68.3	65.2	66.8	
Pectinase (ppin)	2000	68.4	65.3	66.8	
	3000	68.5	65.4	66.9	
Amylase (ppm)	1000	50.3	48.6	49.5	
	2000	50.3	47.7	49.0	
	3000	50.3	48.8	49.6	
	500+500	51.8	49.7	50.8	
Pectinase + Amylase(ppm)	1000+1000	65.8	62.2	64.0	
	1500+1500	65.8	63.0	64.4	
	Mean	59.9	56.4		
	CD0.05				
Treatments (A)	0.62				
Varieties (B)	0.28				
A X B	0.88				

Total Soluble Solids (TSS)

It is evident from Table 4.3 that Total soluble solids of the Aonla fruit juice in all the treatments ranged between 9.9 to 10.13° Brix and 12.0 to 12.06° Brix in *NA-7* and *Seedling Aonla*, respectively. Minimum total soluble solids (TSS) was found in cold pressed juice without enzyme treatment whereas maximum was found in enzymatically treated juice in both the varieties. Total soluble solids in Aonla juice has been reported within the range of 7°Brix to 10°brix as reported by Thakur *et al.*, 2018 ^[13]. Earlier Kumar (2015) ^[6] reported the TSS from 10 to 14.43°brix in different varieties of Aonla. Singh *et al.* (2012) studied TSS of Aonla cultivars and reported that cv. *Desi* had maximum TSS (13.70B) followed by *Banarasi* (12.20B), and *Chakaiya* (8.10B).

Titratable Acidity

Scrutiny of data in Table 4.3 indicates that the titratable acidity of all the treatments of Aonla juice ranged between 1.21- 1.24% (as citric acid) and 1.23 to 1.25% (as citric acid) in NA-7 and Desi (seedling) aonla respectively. Minimum acidity was found in Aonla juice without enzymatic treatment while Aonla juice obtained after Pectinase (1000ppm) treatment showed higher acidity. This may be due to the action of pectolytic enzyme on cell wall constituents consisting of protopectin and pectin and thereby increasing acidity of the product as reported by Sharma *et al.*, 2005. In conformity with our results Mehta (1995) ^[7] found 1.5% acidity in fresh aonla fruits.

 Table 3: Effect of enzymatic treatments of juice extraction on TSS (°Brix) and titratable acidity (%)of Aonla juice of cv. NA-7 and Desi (Seedling) aonla fruits

	Parameters	TSS (°Brix)			Titratable acidity (%)		
Enzymes		NA-7	Desi	Mean	NA-7	Desi	Mean
×	Control	9.9	12.00	10.98	1.21	1.24	1.23
	1000	10.13	12.05	11.10	1.24	1.26	1.25
Pectinase(ppm)	2000	10.13	12.06	11.15	1.23	1.26	1.25
	3000	10.10	12.06	11.10	1.23	1.25	1.24
Amylase(ppm)	1000	10.03	12.03	11.03	1.21	1.24	1.23
	2000	10.03	12.03	11.03	1.21	1.24	1.23
	3000	10.03	12.03	11.03	1.21	1.24	1.23
Pectinase+ Amylase(ppm)	500+500	10.00	12.04	11.03	1.22	1.25	1.23
	1000+1000	10.03	12.05	11.08	1.23	1.26	1.24
	1500+1500	10.06	12.06	11.11	1.23	1.26	1.24
	Mean	10.05	12.04		1.22	1.25	
CD _{0.05} Treatments (A) Varieties (B) A X B			0.076 0.034 NS			0.010 NS NS	

Ascorbic acid

A perusal of data in Table 4.4 and Figure 4.2 reveal that the Ascorbic acid content in all the treatments ranged between 543.3 to 545.5 mg/100ml and 584.7 to588.9 mg/100ml in NA-7 and Desi Aonla respectively. As generally expected the Desi (seedling) aonla fruit juice showed slightly higher level of ascorbic acid than that of *NA-7* fruit juice. Desi Aonla was having higher Ascorbic acid content of 588.9mg/100ml by cold juice extract method. Dhaliwal *et al.* (2012) ^[3] reported ascorbic acid content in cv. Balwant 541.98 mg/100 g and in cv. Neelam 505.69mg/100 g.

Total Phenol

A perusal of data in Table 4.4 reveal that total phenol content of Aonla juice ranged between 3.19-3.29 mgGAE/100ml and 3.39-3.48 mgGAE/100ml in *NA-7* and *Seedling* Aonla respectively. Minimum phenols were found in cold juice extract method without enzymatic treatment and maximum in juice treated with Pectinase @3%. Further higher proportion of total phenols were found in juice extracted from desi (Seedling) aonla. Bhattacherjee *et al.* (2011) ^[2] reported similar results in aonla juice of cv. *Chakaiya*.

Table 4: Effect of enzymatic treatments of juice extraction on Ascorbic acid and total phenols of Aonla juice of cv. NA-7 and Desi (Seedling)
aonla fruits

	Parameters	Ascorbic acid (mg/100ml)			Total phenols (mgGAE/100ml)			
Enzymes		NA-7	Desi	Mean	NA-7	Desi	Mean	
Pectinase (ppm)	Control	545.4	588.9	567.2	3.19	3.39	3.29	
	1000	544.0	586.9	565.4	3.26	3.43	3.35	
	2000	544.0	586.8	565.3	3.28	3.46	3.37	
	3000	543.9	586.9	565.4	3.29	3.48	3.39	
Amylase (ppm)	1000	543.3	586.7	565.0	3.21	3.40	3.31	
	2000	543.6	586.8	565.6	3.23	3.39	3.31	
	3000	543.9	586.8	565.3	3.24	3.39	3.32	
Pectinase+ Amylase (ppm)	500+500	543.9	586.5	565.2	3.26	3.39	3.33	
	1000 + 1000	544.0	586.7	565.4	3.29	3.44	3.36	
	1500+1500	543.9	586.0	564.9	3.28	3.46	3.37	
	Mean	543.9	586.1	565.5	3.26	3.42		
CD _{0.0} Treatment Varieties A X F	5 s (A) (B) 3		1.436 0.642 NS		0.070 0.031 NS		0.070 0.031 NS	

Acknowledgement

I whole heartedly dedicate this research work to my respected guide Dr. P.C. Sharma.

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