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Akanksha Shrivastava
Ph.D., Scholar, Department of Fruit Science, Faculty of Horticulture, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

SG Bharad
Professor and Head, Department of Fruit Science, Faculty of Horticulture, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

DM Panchbhai
Associate Dean, College of Agriculture Nagpur, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Corresponding Author:
Akanksha Shrivastava
Ph.D., Scholar, Department of Fruit Science, Faculty of Horticulture, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Influence of preservation conditions and storage duration on the bioactive compounds of pulp from *Annona* species

Akanksha Shrivastava, SG Bharad and DM Panchbhai

Abstract
The most important factors for a healthy diet are nutrients which must be present in sufficient quantity and quality to provide nutritional and caloric balance in a balanced combination. Annona species are highly rich in bioactive compounds but the hurdles such as short season availability, chilling injury during storage as whole fruit, off flavour and colour development during conventional processing methods can setback their high nutritional and pharmacological potential. Hence, an investigation has been conducted for two consecutive years 2017-18 and 2018-19, where the *Annona* species *viz.*, *Annona squamosa* L. and *Annona atemoya* pulp was extracted, as minimally processed pulp has properties quite similar to the fresh fruits; however, this also has side-effect as being more susceptible to spoilage and deterioration in quality than the other processed products. With the intent of increasing the shelf life of such minimally processed pulp with minimum deterioration in quality, both species pulp was stored under freezing conditions at -20 ^{0}C for eight months and evaluated for assessing the influence of low temperature and duration of storage on bioactive compounds of both species individually. The storage of *Annona* species pulp in the frozen form given positive results in terms of maintaining the quality of pulp with minimum losses concerning to physico-chemical and sensory characteristics. The *Annona atemoya* species responded comparatively better to the storage conditions than *Annona squamosa* L. species. Although, the ascorbic acid and antioxidant activity varied significantly; however, as the storage progressed, the variation rate reduced, leading to minimize losses in the quality.

Keywords: *Annona squamosa* L., *Annona atemoya*, antioxidant activity, ascorbic acid content, frozen pulp

1. Introduction
Importance of fruits in human diet plays a vital role in the nutrition of rural and urban mass. The fruits aptly ascribed as 'protective foods' due to the presence of several components as vitamins, flavonoids, anthocyanins, carotenoids, minerals and other phenolics compounds which contribute to their chemo preventive potential (Mahattanatawee *et al.*, 2006) [16] and (Serrano *et al.*,2007) [26]. The consumption of antioxidant-rich fruits and vegetables has protective effects against degenerative diseases by deactivating free radicals which otherwise cause malfunctioning or death of cells in the body (Rafieian-Kopaei *et al.*, 2013) [23]. Ascorbic acid also called as a synergistic antioxidant due to its oxygen scavenging action and eliminating the supply of oxygen for auto-oxidation reactions which in turn increases the antioxidant activity of phenolic components.

India has admirable richness concerning to the diversity in fruit crops cultivation; however, scarcity of irrigation water is the core hindrance for further development. Hence, dryland fruit crops are getting attention specially the *Annona* genus fruits, as these have tremendous potential of giving a high yield of appropriate quality even with limited resources and expenses. Furthermore, *Annona* fruits contain a considerably higher polyphenolic compound as determined in some fruits including members of the genus *Annona*; *e.g. Annona muricata* L. (Hassimotto *et al.*, 2005) [11] with 0.26 mg QUE/ g total flavonoid, 75 mg/100g total antioxidant activity (Lako *et al.*, 2007) [14], *Annona squamosa* L. (Yan *et al.*, 2006) [35] 97.99% (Nandhakumar and Indumathi, 2013) [22] and *Annona cherimola* L. (DPPH activity with IC_{50} 72.2 µg/ml) (Loizzo *et al.*, 2011) [15].

Among the two *Annona* species examined during the course of experimentation, the *Annona squamosa* L. plant has already been recognized to have nutraceutical and industrial pharmaceutical potentials *viz.*, anti-tumour activity (Ranjan and Sahai, 2009) [25], antifertility activity (Fabricant and Farnsworth, 2001) [8] in seeds, antiulcer activity (Yadav *et al.*, 2011)

[33], anti-diabetic (Mujeeb *et al.*, 2009) [21]; as it possesses potent bioactive principles in all its parts such as alkaloids annoinaine, higenamine, romerine, nereorydine, limonine, rutin and good source of vitamin A and C. (Sharma *et al.*, 2013) [27]. The *Annona atemoya* is a natural hybrid between cherimoya and sugar apple (*Annona cherimola* Mill x *Annona squamosa* L.), though it gained the position of species commercially. It is richer in fibre, a vital property that improves intestinal function, control cholesterol and higher contents of vitamin C, which strengthens the immune system and improves iron absorption (Egydio-Brandao *et al.* 2016) [7]. However, as these fruit crops have the drawback of high perishability, seasonal glut, short season availability and postharvest losses, there is a need of concurrent preservation technology to overcome these barriers and meeting the demands during the off-season. The ultimate vital necessity is the retention of antioxidant content and its correlated parameters in different forms, as this helps in more preservation by shielding the nutritional characteristics from oxidative degradation. The preservation in form of pulp by freezing is advantageous for these *Annona* species as compared to whole fruit or the conventional thermal preservation methods, as this can counter back problem of chilling injury, off-flavour and off-colour development. The freezing process restricts the enzymatic activity and slows down the rate of various deteriorative reactions. Consumers are also increasingly demanding and opting for longer shelf-life products with maintained sensory and nutritional quality. Thus, the aim of this study was to evaluate the influence of preservation conditions (freezing at -20 ^{0}C) for eight months on the bioactive compounds of pulp from both *Annona* species specifically.

2. Materials and Methods

The present storage experiment was laid out in Factorial Randomized Block Design (FRBD) with two factors, *Annona* species *viz. Annona squamosa* L. (S_1) and *Annona atemoya* (S_2) and storage duration of eight months (P_0 to P_8). The second-grade fruits of the *Annona* species having a small size, not much appealing as per skin appearance, although with good quality pulp were procured from the orchard of Fruit Science department, Faculty of Horticulture, Dr. PDKV Akola, Maharashtra during the peak season, *i.e.* November. The extracted pulp of both species after addition of 0.1 per cent potassium metabisulphite (KMS) preservative, immediately packed in the airtight plastic containers as per the treatment details and stored at -20 ^{0}C for eight months.

2.1 Extraction of pulp and pre-treatment

As the *Annona* species seeds are toxic, precautions were taken that seeds should not break during extraction operation. Hence, the PDKV de-seeding & pulper machine was used in which the de-seeding mainly carried out by rubbing action of the brush. The selected fruits were split into two halves, scooped out the pulp with a stainless-steel spoon and then seeds separated from the pulp under restricted hygienic conditions in the machine. Later, after addition of 0.1 per cent potassium metabisulphite (KMS) preservative, the refined pulp of both *Annona* species was immediately packed in the airtight plastic containers as per the treatment details.

2.2 Storage of pulp

After packing in individual boxes of 150g each, the pulp kept in the deep freezer (Make: Blue Star, A/S Vest frost Denmark) with specifications as 386 gross volume, 362 Net volumes, 220-240 Volt, 50 Hz frequency and 160 Watts power requirements. The temperature in the deep freezer already brought to -20 ^{0}C in advance, and same maintained for the storage duration of eight months.

2.3 Ascorbic acid determination

The ascorbic acid content estimated by the method described by Ranganna (1979) [24]. A known volume of aliquot was taken and transferred to 100ml volumetric flask and volume made up with 3 per cent metaphosphoric acid. From this, 10ml aliquot was taken into 100ml beaker and titrated against 2, 6-dichlorophenol indophenols dye solution until the solid faint pink colour appeared for 10 seconds. The ascorbic acid content of the pulp is calculated by using the following formula and expressed as mg/ 100g.

$$\text{Ascorbic acid (mg/100g)} = \frac{\text{Titre x Dye equivalent x Volume made up}}{\text{Aliquot ta ken for estimation x weight of sample}} x100$$

2.4 Antioxidant Activity

The antioxidant activity in pulp as estimated by Diphenyl-p-picrylhydrazyl (DPPH) method described by Manzocco *et al.* (1998) [18]. The 2ml of DPPH solution (0.5ml) added to 0.2ml methanol-diluted sample. After 30 minutes, the absorbance was measured at 517nm (Alam *et al.*, 2013) [1] in Double beam UV-VIS Spectrophotometer (Make: Montras Scientific Model No. UV PLUS). The principle behind the DPPH method is the electron transfer which produces a violet solution in methanol as the molar absorption of DPPH reduces by the pairing of odd electrons with hydrogen from the antioxidants. The colour intensity is proportional to the degree of inhibition, and hence, the percentage of the DPPH radical scavenging was calculated and recorded as % DPPH scavenging activity using the equation as given below:

$$\% \text{ DPPH scavenging activity} = ([A_{br} - A_{ar}])/Abr) \times 100$$

Where A_{br} is the absorbance before reaction and A_{ar} is the absorbance after the reaction has taken place.

2.5 Statistical analysis

The data collected on various observations, during the investigation were statistically analyzed by Factorial Randomized Block Design as suggested by using the Statistical Software Package (OPSTAT), CCS HAU, Hisar as suggested by Sheoran *et al.*, (1998) [28]. Critical difference for examining treatment means for their significance recorded at 5% and 1% level of significance.

3. Results and Discussion

3.1 Variation in ascorbic acid content of *Annona* species pulp

Table 1 clarified the influence freezing and storage period on the ascorbic acid content of the *Annona* species pulp under the low-temperature storage. The *Annona atemoya* species (S_2) logged its significantly higher ascorbic acid content in both consecutive years (2017-18;2018-19) as well as in pooled mean at 0.01 level of significance. The general trend of decline in ascorbic acid was observed in ascorbic acid content of both species pulp under the low-temperature storage. As

per the pooled mean data, the ascorbic acid content *Annona squamosa* pulp dropped from 31.46 mg/100g (S_1P_0) to 28.21 mg/100g (S_1P_8) with altogether decline of 10.33% (by 3.25 factor). Although, the pulp maintained on par ascorbic acid content as the fresh pulp up to the two months of storage (S_1P_2), *i.e.,* 30.94 mg/100g at 1 per cent level of significance (C.D.= 0.566). The ascorbic acid of *Annona atemoya* pulp in the initial stage of storage was 34.16 mg/100g (S_2P_0) which reduced to 32.46 mg/100g in the last month of storage (S_2P_8) as a result of a total percentage drop of 4.97% (1.7 factor), *i.e.* lower than *Annona squamosa* L. pulp by 1.55 factor or in other words 5.36 per cent. The rate of variation diminished with the following months of storage; moreover, the pulp retained its initial ascorbic acid content as at par values up to the completion of the third month of storage (S_2P_3), *i.e.,* 33.62 mg/100g with a critical difference of 0.566 ($p< 0.01$).

Ascorbic acid ($C_6H_8O_6$) considered as an essential naturally occurring hydrophilic micronutrient which is naturally found in fruits and vegetables, mainly the fruits from the *Annona*ceae family are rich in ascorbic acid which plays a crucial role in preserving the nutritional and sensorial qualities, thereby increasing the shelf life by its synergistic antioxidant and oxygen scavenging characters (Daiuto *et al.*, 2011) [5]. The *Annona atemoya* pulp had higher ascorbic acid content than the *Annona squamosa* L. pulp, which possibly the effect of genetic variability, location and ecological factors during cultivation. The ascorbic acid content of these species is in line with that of the (Anuragi *et al.*, 2016) [2], who reported that per cent content of ascorbic acid in fruit pulp varies from 9.22 to 60 mg/100 g across the indigenous custard apple genotypes. Ascorbic acid degradation depends on different factors, such as time-temperature conditions, type of fruit, species, pretreatments, type of package, preservation process (Skrede, 1996) [30]. In comparison to a little higher temperature, a slight decline recorded in the ascorbic acid content of frozen pulp under -20 ^{0}C temperature storage, which might be due to a lower temperature and high RH, which slowed down the rate of oxidation. This inference is confirmed by comparing with the previous storage studies of custard apple pulp by Sravanthi *et al.* (2014) [31] at a temperature of 5 ^{0}C and Kumhar *et al.* (2014) [12] at two different temperatures (5 ^{0}C and 0 ^{0}C). A similar relationship between the ascorbic acid losses with the temperature rise has been noticed in previous storage studies of mango pulp by Manisha *et al.* (2017) [17] at 4 ^{0}C and -20 ^{0}C and in plum pulp by Kundu *et al.* (2015) [13].

With the advancement of the storage period, the ascorbic acid declined which could be ascribed to the oxidative destruction of ascorbic acid to dehydroascorbic acid, which then further degraded to 2,3-diketo-gluconic acid in the presence of molecular oxygen by ascorbic acid oxidase enzymes (Mapson, 1970) [19]. The decline in ascorbic acid as is in consonance with the other storage studies of frozen pulp including Yamashita *et al.* (2003) [34] of acerola pulp with 3.00% reduction in the ascorbic acid content at –12 and –18 °C for four months and Silva *et al.* (2008) [29] of cagaita pulp at –18 °C for four months, where a gradual reduction of approximately 30% in the first month and 50% in the third month was recorded as compared with the initial concentration. The frozen pulp of *Annona atemoya* species retained more ascorbic acid after the completion of storage period as compared to the *Annona squamosa* L. species pulp. The possible cause for this difference in both species could be related to the drip losses occurred while thawing for which moisture content act as a carrier agent (Cano *et al.*, 1993) [4]. As *Annona squamosa* L. pulp had higher moisture content hence, higher drip losses concluded based on ideas stated by Tagubase *et al.* (2016) [32] in frozen durian pulp storage.

Table 1: Influence of freezing and storage duration on ascorbic acid content of *Annona* species pulp

Sp. Months	First year (2017-18)			Second year (2018-19)			Pooled mean		
	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean
P_0	32.24	34.53	33.39	30.68	33.79	32.24	31.46	34.16	32.81
P_1	31.98	34.32	33.15	30.30	33.59	31.95	31.14	33.96	32.55
P_2	31.70	34.14	32.92	30.17	33.43	31.80	30.94	33.79	32.36
P_3	31.44	33.96	32.70	29.89	33.27	31.58	30.67	33.62	32.14
P_4	31.17	33.80	32.49	29.66	33.14	31.40	30.42	33.47	31.94
P_5	30.94	33.66	32.30	29.41	33.02	31.22	30.18	33.34	31.76
P_6	30.72	33.51	32.12	29.17	32.92	31.05	29.95	33.22	31.58
P_7	30.51	33.37	31.94	28.93	32.82	30.88	29.72	33.10	31.41
P_8	28.86	32.75	30.81	27.55	32.16	29.86	28.21	32.46	30.33
Mean	31.06	33.78		29.53	33.13		30.30	33.45	
	S	P	S×P	S	P	S×P	S	P	S×P
F-test	**	**	*	**	**	NS	**	**	**
SE (m)±	0.075	0.159	0.224	0.080	0.170	0.241	0.049	0.104	0.147
CD@5%	0.215	0.456	0.645	0.231	0.490	-	0.141	0.298	0.422
CD@1%	0.288	0.612	0.865	0.310	0.657	-	0.189	0.400	0.566

S_1 = *Annona squamosa* L.; S_2= *Annona atemoya* P_0 – P_8 = Storage duration (Initial to eight months of storage at -20^{0}C); ↑= increasing with storage duration, ↓= decreasing with storage duration, ΔP= per cent change in variable with progressing period

3.2 Variation in the antioxidant activity of *Annona* species pulp: The *Annona squamosa* L. pulp (S_1) was found to be significantly superior to *Annona atemoya* pulp (S_2) in the antioxidant activity irrespective of the storage period.

Table 2: Influence of freezing and storage duration on the antioxidant activity of *Annona* species pulp

Sp. Months	First year (2017-18)			Second year (2018-19)			Pooled mean		
	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean
P_0	95.13 (9.75)	78.15 (8.84)	86.43 (9.30)	92.29 (9.61)	73.79 (8.59)	82.78 (9.10)	93.70 (9.68)	75.95 (8.72)	84.59 (9.20)
P_1	92.35 (9.61)	75.00 (8.66)	83.45(9.14)	92.03 (9.59)	73.22 (8.56)	82.36 (9.08)	92.19 (9.60)	74.10 (8.61)	82.90 (9.11)
P_2	96.37 (9.82)	78.32 (8.85)	87.11(9.33)	92.67 (9.63)	78.32 (8.85)	85.35 (9.24)	94.51 (9.72)	78.32 (8.85)	86.23 (9.29)

P_3	85.13 (9.23)	75.63 (8.70)	80.31 (8.96)	89.87 (9.48)	72.53 (8.52)	80.97 (9.00)	87.48 (9.35)	74.07 (8.61)	80.64 (8.98)
P_4	85.25 (9.23)	78.80 (8.88)	81.99 (9.06)	79.74 (8.93)	78.80 (8.88)	79.27 (8.90)	82.48 (9.08)	78.80 (8.88)	80.63 (8.98)
P_5	81.00 (9.00)	75.69 (8.70)	78.32 (8.85)	78.50 (8.86)	76.45 (8.74)	77.47 (8.80)	79.74 (8.93)	76.07 (8.72)	77.90 (8.83)
P_6	82.02 (9.06)	73.50 (8.57)	77.70 (8.82)	77.79 (8.82)	76.10 (8.72)	76.94 (8.77)	79.89 (8.94)	74.79 (8.65)	77.32 (8.79)
P_7	83.17 (9.12)	80.28 (8.96)	81.72 (9.04)	82.69 (9.09)	78.50 (8.86)	80.58 (8.98)	82.93 (9.11)	79.39 (8.91)	81.15 (9.01)
P_8	81.18 (9.01)	75.11 (8.67)	78.12 (8.84)	77.09 (8.78)	72.53 (8.52)	74.79 (8.65)	79.12 (8.90)	73.85 (8.59)	76.46 (8.64)
Mean	86.75 (9.31)	76.71 (8.76)		84.62 (9.20)	75.56 (8.69)		85.68 (9.26)	76.14 (8.73)	
	S	P	S×P	S	P	S×P	S	P	S×P
F-test	**	**	**	**	**	**	**	**	**
SE (m)±	0.015	0.032	0.045	0.026	0.054	0.077	0.013	0.028	0.040
CD@5%	0.043	0.091	0.128	0.073	0.156	0.220	0.038	0.081	0.115
CD@1%	0.057	0.122	0.172	0.099	0.209	0.296	0.051	0.109	0.154

S_1 = *Annona squamosa* L.; S_2= *Annona atemoya* $P_0 – P_8$ = Storage duration (Initial to eight months of storage at -20^{0}C); ↑= increasing with storage duration, ↓= decreasing with storage duration, ΔP= per cent change in variable with progressing period; (* Figures in parentheses indicate square root transformed values)

In the year 2017-18, the antioxidant content expressed in the form of per cent DPPH scavenging activity was 86.75% DPPH scavenging in *Annona squamosa* L. pulp (S_1), which was significantly higher than *Annona atemoya* pulp (S_2) with 76.71% DPPH scavenging at 0.01 level of significance. The *Annona squamosa* L. pulp antioxidant activity was higher by 13.08% (by a factor of 10.04). Similarly, the 2018-19 and pooled mean analysis displayed the fact that *Annona squamosa* L. (S_1) was higher antioxidant activity (84.62% and 85.68% DPPH scavenging activity) than *Annona atemoya* pulp (S_2) (75.56% and 76.14% DPPH scavenging activity) with 11.99%, *i.e.,* by 9.06 factor and 12.52% (a factor of 9.54).

The pooled mean analysis provided similar observations regarding the variations occurred in the antioxidant activity of pulp from both species during the storage session of eight months at -20^{0}C temperature. The antioxidant activity of *Annona squamosa* L. pulp was 93.70% DPPH scavenging activity in freshly frozen sate (S_1P_0) which reduced to the final value of 79.12% DPPH scavenging activity (S_1P_8). While on the other hand, the antioxidant activity in the pulp of *Annona atemoya* was 75.95% DPPH scavenging activity (S_2P_0) which shifted to 73.85% DPPH scavenging activity (S_2P_8). Remarkably, the percentage decline in antioxidant activity of this species (15.56%, *i.e.,* by 14.58 factor) was much more than that noticed in the *Annona atemoya* pulp (2.76%, *i.e.,* by a factor of 2.1). Similar declining trend observed in frozen cambuci pulp by Genovese *et al.* (2008) [9] and in frozen sapota pulp by Monteiro *et al.* (2018) [20].

The overall fluctuations and rate of variations were lower under storage at -20 ^{0}C than comparatively higher temperature or ambient temperature as confirmed by comparing with the studies of Kumhar *et al.* (2014) [12] for *Annona* species pulp stored at different temperatures (ambient, 5 ^{0}C and 0 ^{0}C) and by the reports of Arampath and Dekkar (2019) [3] from the study regarding the effect of storage temperature on antioxidant activity of mango and pineapple pulp. The lower variation rate at -20 ^{0}C likely due to the decline in the PPO enzyme activity. Furthermore, the preservative potassium metabisulphite (KMS) added to the pulp also aid in declining the enzyme activity, though its effectiveness decreases with time as it functions by releasing sulphurous acid and ions. The inclination as observed in few months perhaps due to the process of conversion of soluble tannins into insoluble tannins and effect of variations in pH and acidity changes since ionizable hydrogens present in the sample, which reacted with DPPH resulting in increased discolouration of the radical as also noticed by Harris & Brannan (2009) [10] in pawpaw pulp and Damiani *et al.* (2013) [6] in frozen marolo pulp. The sudden rise in variation rate during last months, possibly due to the increase noticed in pH, which hinders the KMS effectiveness as maximum activity of KMS is at pH below 4. The effectiveness of KMS in maintaining the antioxidant activity and decline in its concentration are also reported by Manisha *et al.* (2017) [17] in mango pulp and Sravanthi *et al.* (2014) [31] in custard apple pulp.

4. Conclusion

The storage of Annona species pulp in the frozen form at -20 0C has been successful in maintaining the quality of pulp. As the storage at -20^{0}C temperature progressed, the ascorbic acid content showed a declining trend antioxidant activity did not follow any specific trend but decreased on the whole. However, the rate of variation for these parameters diminished variation as the storage progressed towards its end, which results into minimum losses in quality up to the end of storage. Among the species, the variation rate was relatively lower in *Annona atemoya* species pulp than *Annona squamosa* L. species pulp. Hence, *Annona atemoya* species emerged out nearly equivalent to the *Annona squamosa* L. species by the end of the storage concerning the biochemical properties. Hence, the pulp of both species can be stored for a long duration. Owing to the minimum loss of quality and sensory characteristics as observed during the storage in the present study, *Annona* species pulp can be stored for a longer duration in a frozen state.

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