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Studies on the effects of honey incorporation on quality and shelf life of aonla preserve

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Abstract: Aonla is the richest sources of Vitamin C. The raw fruit, due to its high acidic nature and astringent taste is unacceptable to the consumers. Honey is a natural high energy sweetener with many medicinal values. Keeping in view the nutritional and therapeutic values of aonla fruit and honey, aonla preserve was prepared by incorporating 7.5 and 15% of honey into them. The quality of the products was evaluated based on the physicochemical (moisture, ash, pH, TSS, browning index and Vitamin C content), it was observed that the physicochemical characteristics of the aonla preserve improved upon incorporation of honey. There was improved retention of moisture content, increased ash content, reduced browning and reduced loss of Vitamin C due to incorporation of honey. The Vitamin C content in the aonla preserve (15% honey incorporation) was 133.56 mg/100 g at the end of storage. The aonla preserve sample incorporated with 15% honey was nutritionally better as compared to samples incorporated with 7.5% honey due to its better Vitamin C retention, higher mineral content, tenderness and tangy taste. The yeast and the mould detected were few to count in the control sample. Shelf life of the product was 90 days.

Subjects: Bioscience; Food Science & Technology; Health and Social Care

Keywords: medicinal use; astringent taste; nutritional and therapeutic values; browning index

1. Introduction

Aonla (*Emblica officinalis*) is one of the oldest Indian fruits and is considered as "Wonder fruit for health" because of its unique qualities. The indigenous fruit has extensive adaptability to grow in diverse climatic and soil conditions. It is known by different names like *Amla, Amalakki*, *Nelli*, Indian

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PUBLIC INTEREST STATEMENT

Aonla is one of the oldest Indian fruits and is considered as "Wonder fruit for health". The raw fruit due to its high acidic value and astringent taste is unacceptable to consumers. The aonla preserve was prepared by incorporating honey as sweetener along with sugar, a natural energy and medicinal values. With the view of the commercial viability and marketability of product, 7.5 and 15% of honey were incorporated. The study was based on important parameters of product like pH, TSS, moisture and Vitamin C. It was found that the physicochemical properties of aonla were improved by honey incorporation.

gooseberry, etc. *Aonla* has been cultivated in India since time immemorial (Singh, Singh, & Singh, 2009). Besides India, naturally growing *aonla* trees are also found in different parts of the world, viz. Sri Lanka, Cuba, Puerto Rico, China, Thailand and Japan. The area under *aonla* (Indian gooseberry) has been expanding rapidly in the last couple of years. From just around 3,000 hectares in the early 80s, the area had stretched to 50,000 ha in 2002 (Singh, 2003). The estimated fruit production is 84,411 thousand metric tonnes of which *aonla* constitutes 1,273 thousand metric tonnes according to the second advanced estimates of the Directorate of Economics and Statistics, Ministry of Agriculture, Government of India (2013). In view of its therapeutic properties, there is a great demand for the *aonla* fruit. This hardy plant is suited for being raised in wasteland, be it arid, semi-arid, salt affected, coastal or ravine areas. It is a rich source of vitamin C and its content of ascorbic acid is next to only that of Barbados cherry (*Malpighia glabra* L.). It is one of the three constituents of the famous ayurvedic preparation, triphala, which is prescribed in many digestive disorders (Chopra, Chopra, Handa, & Kapur, 1958).

Aonla is presently an underutilized fruit, but has enormous potential in the world market. It is almost entirely unknown in the world market and needs to be popularized. *Aonla* is being exported under the category of Ayurvedic and Unani herbs. Its medicinal and nutritional properties and culinary uses need to be highlighted. The fresh fruits are generally not consumed as it is highly acidic and astringent; therefore, it is not a popular table fruit. There is always demand from the consumers all over the world for new products which may be nutritious and also delicately flavoured. Attempts have been made to process *aonla* fruits into a variety of products since it has got great potential in processed forms (Nayak, Bhatt, Shukla, & Tandon, 2011). Hence, attention has been focused on the preparation of different value-added products from *aonla*.

Among natural sweeteners honey is nutritionally a high energy carbohydrate food considered to be the best source of heat and energy, giving over 3,200 calories/kg. About 95% of the honey dry matter is composed of carbohydrates, mainly fructose and glucose. About 5–10% of the total carbohydrates are oligosaccharides, in total about 25 different di- and trisaccharides. Besides, honey contains small amounts of proteins, enzymes, amino acids, minerals, trace elements, vitamins, aroma compounds and polyphenols. Honey has been shown to possess antimicrobial, antiviral, antiparasitory, anti-inflammatory, antioxidant, antimutagenic and antitumor effects. At present the annual world honey production is about 1.2 million tons, which is less than 1% of the total sugar production.

Honey can be utilized in the processing of various fruits by replacing the white sugar or jaggery completely or by adding to the product in parts. Its application with various selected fruits that have medicinal properties to produce designer foods having added therapeutic needs some basic research regarding the standardization of recipes, characterization of product and shelf life study. Hence, the studies were carried out with the objectives of development, quality evaluation and storage stability of *aonla* preserve incorporated with honey (partly) as sweetener (Bogdanov et al., 2003; White, 1975).

2. Materials and methods

2.1. Method for preparation of aonla preserve

Mature *Aonlas* free from blemishes and bruising was procured from Agricultural Faculty garden and packed in with low density polyethylene (100 µm) and kept at 5°C. Sugar and packaging materials were arranged from the local market. The entire experimental studies were conducted in the Department of Post Harvest Engineering and Technology, Faculty of Agricultural Sciences, A.M.U., Aligarh.

The mature *aonla* of medium size were selected from the lot procured. Then, the stems were trimmed and the damaged *aonlas* were discarded. Proper washing was done to remove the adhering dirt and then proper puncturing was done on the *aonlas* using a pricking tool. Next, the *aonlas* bitterness was removed by submerging in limewater (50 g/l, for each kg of fruit), and taken out after 4 h.

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Hot water was used to remove the adhering CaO from the *aonlas*. Next, the *aonlas* were steeped in 2% salt solution for one day. Proper washing was done to remove the salt on the next day. Then, blanching of *aonlas* was done for 5 min at a temperature of 5°C below the boiling point of water (Srivastava & Kumar, 1994a; 1994b). The *aonlas* were divided into three parts for honey incorporation (the three parts for incorporation were 0, 7.5 and 15% of honey per kg of aonla). The *aonlas* were removed and then spread in layers with equal quantity of sugar (done in three separate large jars) and left for 2–3 days. Moisture migrated from the *aonlas* and formed solution with the sugar in jars (Figure 1).

2.2. Physicochemical properties

Moisture content (kg/100 kg of fruit bar) and acidity (kg/100 kg of fruit bar) were determined by methods as described in Ranganna (1994). The pH of the fruit bar samples were measured by digital pH meter (Khera model, India). TSS (°Brix) and Vitamin C (mg/100 g) were determined by methods as described in Srivastava (1994). Non-enzymatic browning determined by method given in Balouch Buckle, and Edwards (1973) were using spectrophotometer (Tanco, India). The browning index is defined as the logarithmic ratio of intensity of incident light to that of emergent light. The per cent transmittance may be correlated to optical density by using an expression 2log T_1, where T_1 is the transmittance.

2.3. Microbiological characteristics

2.3.1. Preparation of sample (serial dilution)

One gram of sample was taken and transferred to the test tube with 9 ml of NSS. It was marked as 10^{-1} test tube and others as 10^{-2}, 10^{-3}, ..., 10^{-6}. The test tube containing the sample was homogenized with the help of cyclomixer (Cappuccino & Sherman, 1992). One millilitre of sample suspended in saline solution from 10^{-1} test tube was transferred to test tube marked as 10^{-2} with the help of micropipette and homogenized. The tip was then discarded. One millilitre sample from 10^{-2} marked tube was transferred to 10^{-3} with a sterilized 1 ml of micropipette. Similarly, the sample was transferred up to the test tube marked as 10^{-6}.

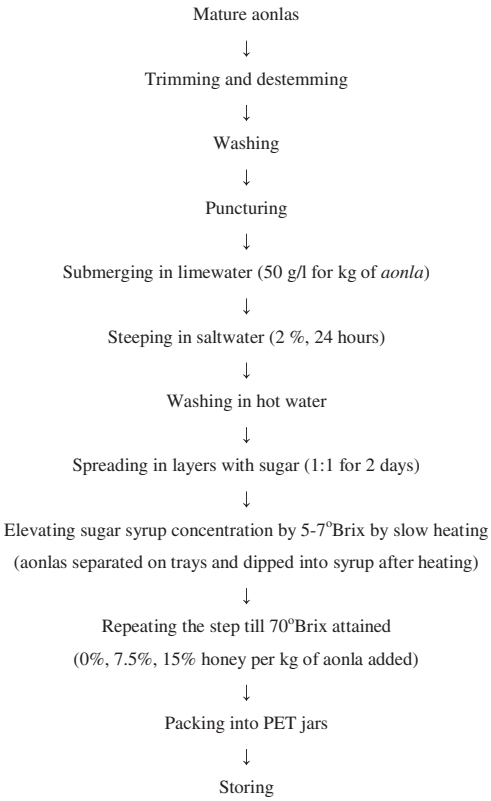

Figure 1. Process flow chart for preparation of aonla preserve.

0.5 ml of the sample suspended in saline solution from 10^{-1} was taken with micropipette and transferred to petridish marked as10^{-1} of PDA media. The microbial tip was discarded and another sterilized tip was used to transfer sample from 10^{-3} saline solution to 10^{-3} PDA plates. Precautions are taken in inoculation so that contamination could not take place. Similarly, all the samples suspended in saline solutions were transferred to respective petridishes of PDA media. For each, two replications were taken. A control of PDA media was also kept without inoculation. The inoculated petridishes were incubated in BOD incubator for 3–5 days at 25°C. After incubation, the average count of colonies present in petriplates were multiplied by dilution factor and expressed as cfu (colony forming unit)/ml of sample.

2.3.2. Statistical analysis

Data obtained from experimental observation was subjected to analysis of variance (Two ways ANOVA). All statistical analyses were performed using SPSS Version 10.0 for Windows (SPSS Inc., Chicago, IL, USA) as described by field (2005).

3. Result discussion

3.1. Physicochemical characteristics of the aonla preserve incorporated with honey partly as sweetener

3.1.1. Effect on moisture content

In fresh sample, the moisture content of aonla preserve was found to decrease a little with honey incorporation. However, during ambient storage of 90 days the trend was found to reverse and the honey incorporated samples of aonla preserve had higher moisture contents. The control 7.5 and 15% honey incorporated samples had, respectively, 44, 50.32 and 53%. Similar findings were reported by Durrani and Verma (2011).

The overall per cent moisture content decreased significantly ($p < 0.05$) as the storage period increased for all the three samples. The moisture content of fresh samples were 69.33, 68 and 67.54% which decreased to 44, 50.32 and 53% during 90 days storage period for *aonla* preserve incorporated with 0, 7.5 and 15% honey, respectively. A decreasing trend of moisture content was also reported by Daisy and Gehlot (2006) in *aonla* preserves. The decrease in moisture content can be contributed to the transfer of sugar from syrup to the *aonla* and migration of moisture from fruit into the syrup. That the highest decrement in moisture was in *aonla* preserve with 0% honey incorporation followed by 7.5 and 15% honey incorporation. Hygroscopic nature of honey may be the reason for the minimum decrement in samples containing higher honey per cent.

3.1.2. Effect on ash content

The ash value is a measure of the amount of added minerals. Natural ash content is due to the minerals like calcium, phosphorous and iron. Ash content of food stuffs represents inorganic residue remaining after destruction of organic matter (Rao, Van Buren, & Cooley, 1993). They are not destroyed by heating and have low volatility when compared to other food components.

The average experimental values for the ash content of the *aonla* preserve samples were found to be in the range of 0.142–0.350%. They have been presented in Table 1. A higher amount of ash was found to be present in the *aonla* preserve sample containing higher amount of honey incorporation. The reason for the higher amount of ash content in 15% honey incorporated samples than in the samples incorporated with 7.5 and 0% honey may be due to the contribution of the ash content by honey itself.

With increase in the storage days there was a slight increase in the ash content. The ash content at 0 day was 0.142% in *aonla* preserve sample without honey incorporation. This increased to 0.159% during the 90 days of storage. Similarly the ash content increased from 0.212 to 0.247% and from 0.323 to 0.350% in samples incorporated with 7.5 and 15% honey, respectively, during the 90 days

Table 1. Ash content of aonla preserve samples during the storage period

Sample code	Ash content (%)/storage period (number of days)						
	0 days	15 days	30 days	45 days	60 days	75 days	90 days
CP	0.142±0.00	0.144±0.00	0.149±0.00	0.151±0.00	0.154±0.00	0.157±0.00	0.159±0.00
H_1P	0.212±0.00	0.219±0.01	0.235±0.00	0.236±0.00	0.240±0.00	0.244±0.00	0.247±0.00
H_2P	0.323±0.01	0.329±0.00	0.336±0.00	0.339±0.00	0.342±0.00	0.346±0.00	0.350±0.00

CP = 0% honey, H_1P = 7.5% honey and H_2P = 15% honey.

Values are means of three replicate ± SD.

of storage. However, there should not be any change in ash content during storage but the change maybe due to the decrease in moisture content with respect to the fresh samples. The ANOVA results also indicated that the ambient storage significantly ($p < 0.05$) increased the ash content of all the samples. Hence, the combined effect of treatment and storage significantly ($p < 0.05$) increased the ash content of treated *aonla* preserve.

3.1.3. Effect on pH

Fruit products are being effectively preserved at low pH. The pH estimation was done in order to find out whether a low pH was maintained throughout the study which could be an effective means of preservation. Table 2 presents the observed values of pH. The pH value was in the range of 2.5–3.09 for the *aonla* preserve samples. The lower pH in the honey incorporated samples may be attributed to lower pH of honey itself.

The increasing trend of pH with storage period can be due to the leaching losses of acids into the syrup. Singh, Shivhare, Singh, and Bawa (1999) and Tripathi, Singh, and Singh (1998) have also reported the loss of acidity in *aonla* preserve during storage. Singh et al. (1999) had also reported that acidity of *aonla* preserve decreased with time at any given concentration of sugar solution. It was reported by them that the rate constant for acidity increased appreciably when temperature was increased from 30 to 40°C but remained particularly unchanged from 40 to 50°C i.e. the rate of loss of acidity increased with temperature to its maximum at 40°C and remained constant. The pH change was from 2.58 to 3.09 in *aonla* preserve without honey incorporation during 90 days of storage. The initial pH of 2.52 changed to 2.93 after 90 days storage in *aonla* preserve incorporated with 7.5% honey. The pH range for *aonla* preserve incorporated with 15% honey was 2.5–2.9 during the storage period of 90 days. The ANOVA results also indicated that the ambient storage significantly ($p < 0.05$) increased the pH of all the samples. Hence, the combined effect of treatment and storage significantly ($p < 0.05$) increased the pH of *aonla* preserve.

3.1.4. Effect on TSS

Table 3 shows the result for TSS of *aonla* preserve samples during storage period of 90 days. It is clear from the table that the TSS which was observed in the range of 49.83–77.17 was highest for the sample without the honey incorporation and lowest for the sample with 15% of honey incorporation. The low moisture content in the preserve sample without honey incorporation may be the reason for higher TSS value. In the fresh condition, the values of TSS were almost similar although the sample with 15% honey incorporation had a slightly higher value, the reason for this is unknown.

Table 2. The pH of aonla preserve samples during the storage period

Sample code	pH/storage period (days)						
	0 days	15 days	30 days	45 days	60 days	75 days	90 days
CP	2.58±0.00	2.67±0.00	2.73±0.01	2.79±0.01	2.94±0.00	3.00±0.00	3.09±0.01
H_1P	2.52±0.00	2.59±0.00	2.64±0.00	2.70±0.00	2.76±0.00	2.82±0.00	2.93±0.01
H_2P	2.50±0.01	2.57±0.00	2.64±0.00	2.69±0.00	2.76±0.00	2.80±0.01	2.90±0.00

CP = 0% honey, H_1P = 7.5% honey and H_2P = 15% honey.

Values are means of three replicate ± SD.

Table 3. TSS of aonla preserve samples during the storage period

Sample code	TSS (°Brix)/storage period (days)						
	0 days	15 days	30 days	45 days	60 days	75 days	90 days
CP	49.83±0.29	59.00±0.87	69.67±0.58	71.00±0.00	72.83±0.29	74.17±0.29	77.17±1.44
H_1P	51.33±1.15	58.00±0.50	67.00±0.00	68.67±0.58	69.75±0.66	70.33±0.29	70.92±0.14
H_2P	52.33±1.15	58.83±0.29	65.33±0.29	68.67±0.58	70.00±0.00	71.33±0.29	73.00±0.00

CP = 0% honey, H_1P = 7.5% honey and H_2P = 15% honey.
Values are means of three replicate ± SD.

The overall effect of storage of 90 days on TSS was that TSS increased considerably. The decreasing moisture content may be the reason for the increasing TSS. A similar trend was reported by Kumar and Singh. In a study conducted by Durrani and Verma (2011) on Honey amla murabba a decreasing trend in the TSS was observed. The values of TSS of fresh *aonla* preserve incorporated with 15, 7.5 and 0% honey were 52.33, 51.33 and 49.83. At 90 days period, the TSS increased to 73, 70.92 and 77.17. The reason for overlapping of the graph for the *aonla* preserve samples is unknown. It may be attributed to uncertainty (error in experimentation). The lower percentage of increase in TSS in honey incorporated *aonla* samples may be due to the slower rate of diffusion of syrup because of the high viscosity of honey.

3.1.5. Effect on vitamin C

The average experimental values for the Vitamin C content of the *aonla* preserve samples have been presented in Table 4. A higher amount of Vitamin C was found to be retained in the *aonla* preserve sample containing higher amount of honey incorporation. The antioxidant property of honey may be the reason for preventing the dissolved oxygen in the water to cause the oxidation of Vitamin C.

It was observed that with increase in the storage days there was a markedly higher decrease in the Vitamin C content (Figure 2). In the fresh condition, the Vitamin C content was 170.46, 179.35 and 180.43 mg/100 g for *aonla* preserve samples incorporated with 0, 7.5 and 15% honey by weight of the *aonla.* At the end of 90 days, the values had declined to 115.44, 129.79 and 133.56 mg/100 g for the three respective samples. The leaching of Vitamin C into the syrup maybe the reason for this decline. Nayak et al. (2011) have also reported that with increase in storage period of *aonla* fruits under ambient condition the ascorbic acid content decreased significantly. They mentioned oxidation of ascorbic acid to dehydroascorbic acid as the main reason for the loss during storage. Figure 2 depicts the general trend of change in ascorbic acid content in the *aonla* preserve sample during the 90 days storage period. There was a general decrease in trend in the change of ascorbic acid content. The lower percentage decrease in ascorbic acid content in honey incorporated samples can be attributed to the antioxidant property of honey. The ANOVA results also indicated that the ambient storage significantly ($p < 0.05$) decreased the Vitamin C content of all the samples. Hence, the combined effect of treatment and storage significantly ($p < 0.05$) decreased the Vitamin C content of treated *aonla* preserve.

Table 4. Vitamin C content of aonla preserve samples during the storage period

Sample code	Vitamin C content (mg/100 g)/storage period (days)						
	0 days	15 days	30 days	45 days	60 days	75 days	90 days
CP	170.46±0.36	161.40±0.73	149.55±0.68	140.70±0.37	130.39±0.35	122.70±0.63	115.44±1.33
H_1P	179.35±0.84	170.01±1.52	160.59±0.41	151.57±0.23	141.04±0.81	134.21±0.69	129.79±0.99
H_2P	180.43±1.18	172.36±0.27	164.4±0.17	156.09±0.59	145.68±0.53	140.47±0.35	133.56±0.29

CP = 0% honey, H_1P = 7.5% honey and H_2P = 15% honey.
Values are means of three replicate ± SD.

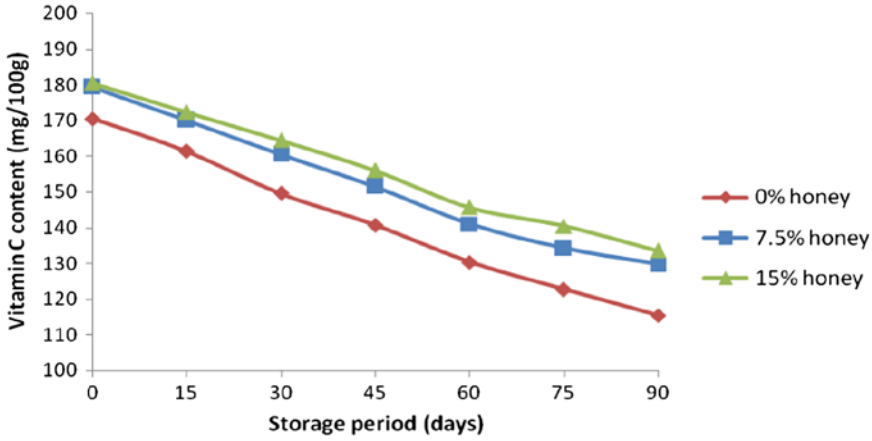

Figure 2. Change in Vitamin C content of aonla preserve samples during storage.

3.2. Microbiological characteristics of aonla preserve

Microbiological characteristics of the preserve samples were evaluated based on the yeast and mould count. The aonla preserve samples were found to be stable throughout the 90 days period of study as shown in Table 5. The yeast and mould detected were too few to be counted in the sample without honey incorporation. The high TSS maintained during the study period, the acidic pH, the environmental conditions and the hygienic packaging practice may be the reason for this. All the above reasons, together with the antimicrobial property of honey, may be the reason for non-detection of yeast and mould in honey incorporated samples. The results of microbiological study indicated that the *aonla* preserve samples were stable enough and the study conducted for 90 days established that the products were in safe and edible condition. Thus, it can be predicted that the shelf life of the product was long enough and it can be stored for around 3–4 months. In study conducted by Durrani and Verma (2011) on honey *amla murabba* the shelf life of the product was reported to be 180 days.

4. Conclusion

The study has indicated that incorporation of honey partially (7.5 and 15%) as a sweetener brought considerable change in physicochemical (moisture content, ash content, pH, TSS, browning index and Vitamin C content) and sensory characteristic of *aonla* preserve. It also indicated that incorporation of honey (7.5 and 15%) into *aonla* preserve improved its moisture content and ash content, reduced the loss of Vitamin C and rate of browning and also helped to maintain a low pH. Sensory attributes like colour, taste and texture of *aonla* preserve and candy samples improved with the incorporation of honey. The study also indicated that the *aonla* preserve sample prepared by 15% incorporation of honey retained more moisture and improved the texture by imparting more tenderness to the product. Due to this reason, *aonla* preserve and candy samples incorporated with 15% honey were more accepted by the people based on texture as compared to the samples incorporated with 7.5% honey. Increased amount of ash and lesser loss of Vitamin C than the other samples were the other features of *aonla* preserve and candy incorporated with 15% honey. The lower TSS and lower pH in the honey incorporated *aonla* preserve and *aonla* candy samples (7.5 and 15%) compared to the samples without

Table 5. Yeast and Mould count for aonla preserve samples during the storage period

Sample code	Yeast and Mould count/storage period (days)						
	0 days	15 days	30 days	45 days	60 days	75 days	90 days
CP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	T.F.T.C.
H_1P	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
H_2P	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

CP = 0% honey, H_1P = 7.5% honey and H_2P = 15% honey.

N.D. = Not Detected, T.F.T.C = Too Few To Count.

incorporation helped the product to maintain a tangy and likeable taste and yeast mould count was not detected at the end of 90 days of storage. From all these results it can be concluded that the *aonla* preserve and candy samples incorporated with 15% honey was the superior product nutritionally (better Vitamin C retention and higher mineral content) and organoleptically (tenderness and taste). The levels of honey incorporated as sweetener (partly) described are economically feasible and commercially viable. Incorporation of honey rendered a lot of qualitative improvement in the products. The samples incorporated with honey (7.5 and 15%) was more accepted by the panel members even after 90 days of storage compared to the sample prepared without incorporation of honey.

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