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Jyoti Prabha Bishnoi
Amity institute of Biotechnology, Amity University Rajasthan, Jaipur, Rajasthan, India

Rakesh Gehlot
Centre of Food Science and Technology, CCS Haryana Agricultural University, Hisar, Haryana, India

S Siddiqui
Centre of Food Science and Technology, CCS Haryana Agricultural University, Hisar, Haryana, India

Correspondence
Jyoti Prabha Bishnoi
Amity institute of Biotechnology, Amity University Rajasthan, Jaipur, Rajasthan, India

Development of low calorie aonla ladoo using *Stevia rebaudiana*

Jyoti Prabha Bishnoi, Rakesh Gehlot and S Siddiqui

Abstract
Stevia (*Stevia rebaudiana*) is a small shrub, belonging to family Compositae. It is also known as sweet leaf or sugar leaf. Stevia has gained importance as a high potency natural sweetener. Stevia leaves contain complex mixture of diterpene glycosides, including stevioside, rebaudiosides, steviolbioside and dulcoside A which are responsible for sweetness. Stevioside are 250 to 300 times sweeter than sugar. Hence attempt was made to utilize stevia leaf powder for preparation of low calorie aonla ladoo. Low calorie aonla ladoo were prepared by replacing 0, 25, 50 and100% sugar with natural sweetener (stevia) powder. On the basis of sensory analysis, low calorie aonla ladoo replacing 50% sugar by stevia was found most acceptable. The freshly prepared low calorie aonla ladoo containing stevia as sugar replacer was analyzed for various chemical characteristics. The moisture content (48.3%), water activity (0.87), total soluble solids (42.6%), total sugars (32.3%), reducing sugars (18.3%), titratable acidity (2.29%), ascorbic acid (271 mg/100 g), pectin (0.49%), total phenols (1.84 mg/g), antioxidant activity (59.4%) and non-enzymatic browning (0.08) were recorded for low calorie aonla ladoo containing stevia. Thus, present study was first in its kind to determine overall acceptability, chemical composition and effect of storage period on chemical constituents of low calorie aonla ladoo obtained from aonla fruits cv. Chakaiya and stevia leaf powder.

Keywords: stevia, *Stevioside*, low calorie, chakaiya, ladoo, sensory analysis

Introduction
Aonla fruit is highly nutritious and known as *Kaya Kalp* according to *Hindu* mythology as it restores health and vitality. Aonla is a rare example of an edible material that is rich in tannins as well as ascorbic acid (Pathak, 2002) [38]. The vitamin C content in aonla varies from 200 to 950 mg/100 g depending upon the variety and size of fruit (Anonymous, 1988; Barthakur and Arnold, 1991) [4, 7], which is said to be second highest among all fruits and is next to Barbados Cherry (Ganachari *et al.*, 2010) [16]. The edible fruit tissues of aonla contain about three times more protein and 160 times more vitamin C than apple (Barthakur and Arnold, 1991) [7]. The main constituents of aonla are tannins, polyphenolic compound 1, 3, 6-trigalloylglucose, terehebin, corilagin, phyllantnic, beta siotostural, linolic acid, ellagic acid and lupeol. Tannins containing gallic acid, elagic acid, and glucose retard the oxidation of vitamin C and renders its value as antiscorbutic in the fresh fruit as well as in dried products. Aonla is also a source of carbohydrates, carotene, thiamine, riboflavin, and minerals like iron, calcium and phosphorus. It also contains a considerable higher concentration of minerals and amino acids than apple. It is valued for its antiscorbutic, diuretic, laxative, antibiotic, acidic, hypoglycaemic, hypolipidemic and cooling properties (Mishra *et al.*, 2010) [31]. Aonla fruit is considered useful in treating haemorrhage, diarrhoea, chronic dysentery, diabetes, jaundice, ophthalmic disorders, dyspepsia, cough, skin diseases, leprosy and greyness of hair (Ganachari *et al.*, 2010) [16]. Aonla, though an underutilized fruit, has enormous potential in the World market. The fresh fruits are not popular as a table fruit due to its high acidity and astringent taste (kumar and Nath, 1993) [26]. Further, aonla, being perishable, loses its nutritional value in absence of proper post-harvest technology; therefore, it is not popular as a table fruit, but has a great potential in processed forms. Several value added products have been developed from aonla such as pickles, preserve (murrabba), candy, sauce, chutney, jam, jelly, spread and ladoo. Aonla ladoo is prepared from aonla shreds. It is ball-shaped, exceedingly sweet, highly flavourful and easily masticated product.

Food industry is currently witnessing a situation in which the labeling claims of products, like sugar free, reduced calorie, no calorie etc. are attracting health-conscious consumers. Thus sweeteners are attractive alternatives to sugar because these add virtually no or low calories to your diet. These also help in controlling weight and insulin levels. Stevia (*Stevia rebaudiana*) is a small shrub native to subtropical and tropical South America and Central America. It is popularly known as sweet leaf, sugar leaf, honey leaf and candy leaf.

Stevia is a member of Compositae family (Brandle & Telmer, 2007) [9]. Stevia leaves contain complex mixture of diterpene glycosides, including stevioside, rebaudiosides, steviolbioside and dulcoside A (Kennelly, 2002; Starrat *et al*., 2002) [24, 49], which are responsible for sweetness. Stevioside are 250 to 300 times sweeter than sugar. Stevia was used in the preparation of tea, beverage and food in Japan, China, Russia and USA since a long time. Thus stevia a natural sweetener without contributing calories and possessing sweetness several hundred times than sugar has become a boon for the calorie conscious individuals. Moreover, increasing consumer interest in natural food ingredients means that products like stevia sweeteners will be subject to increasing demand. Therefore, stevia has gained importance as a high potency natural sweetener. The present study was thus taken up to determine chemical composition and overall acceptability of low calorie aonla ladoo obtained by replacing sugar with stevia powder.

Material and Methods

Mature aonla fruit cv. Chakaiya was procured from local orchards, Hisar. Stevia powder was obtained from Green Valley Stevia Biotech. Pvt. Ltd., Nawashahr (Punjab). The fruits were washed thoroughly with clean running water to remove the dirt and other foreign material. After washing operation, aonla fruits were also subjected to pretreatments. The pretreated aonla fruits were cooled at room temperature for 30 minutes after following the best pretreatment (blanching in 2% brine solution + 2% alum solution + 0.2% KMS solution). Aonla segments were then separated from its stone and grated using fruit processor (Plate No.1). Aonla ladoo (control) were prepared by using 1 kg aonla pulp, 750 g sugar and 1 g cardamom powder (small) as per the procedure shown in flow sheet (Fig. 1). On basis of sensory evaluation, optimum quantity of stevia powder was standardized for the preparation of low calorie aonla ladoo. For processing aonla ladoo, the mixture was cooked till desired consistency was obtained. The product was then cooled, rolled into ladoo and packed in polypropylene (PP) boxes. The freshly prepared low calorie aonla ladoo containing stevia powder was analyzed for various chemical characteristics. The moisture content of low calorie aonla ladoo was determined by Dean and Stark method. The water activity of processed products was recorded by water activity meter (Labswift aw, Novasina, Switzerland). The instrument was calibrated with water activity meter calibration humidity salts (11, 58 and 84%). Total soluble solids (TSS) were estimated at ambient temperature by hand refractometer (0-32%) and the values were expressed as percent TSS. Ascorbic acid was analyzed according to AOAC (1990) [1] method. Non- enzymatic browning (NEB) was determined according to the method of Ranganna (2008) [41]. Total phenols were analyzed as per the methods given by Amorium *et al.* (1997) [2], while organoleptic evaluation by using 9 point hedonic scale. Sugars were estimated by the method of Hulme and Narain (1931) [18]. Total phenols (expressed as tannins) were estimated by the Amorium *et al*. (1997) [2]. Antioxidant activity was measured using stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical as per the method described by Shimada *et al*. (1992).

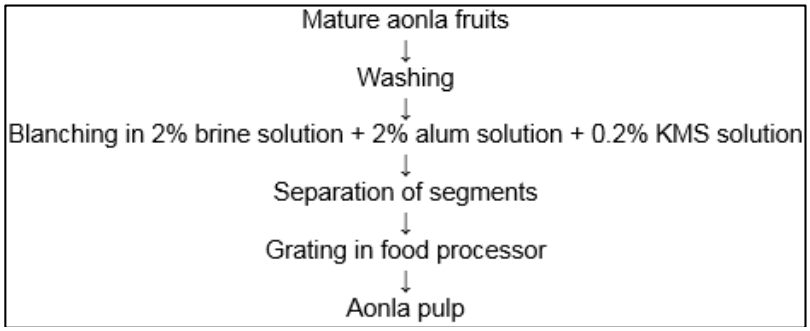

Fig 1: Flow sheet for processing of aonla pulp

Plate 1: Flowchart for processing of aonla pulp cv. Chakaiya

Results & Discussion

Organoleptic quality of low calorie aonla ladoo: The data pertaining to effects of incorporation of various levels of supplements (stevia) on sensory attributes of aonla ladoo has been shown in Table 1. Aonla ladoo were evaluated for sensory attributes *viz.*, colour and appearance, taste, texture, mouth feel and overall acceptability. Mean score for colour and appearance, taste, texture, mouth feel and overall acceptability of control aonla ladoo were 8.2, 8.1, 8.0, 8.0 and 8.0, respectively. No significant change in mean score of various sensory attributes was noticed in low calorie aonla ladoo havingreplacement of 25% & 50% sugar by stevia powder. However, the mean score for sensory attributes *viz.*, colour and appearance taste, texture, mouth feel and overall acceptability of low calorie aonla ladoo having replacement of 100% sugar by stevia powder was significantly lower than control. Thus, formulation having replacement of 50% sugar by stevia powder was selected for preparation of low calorie aonla ladoo.

Table 1: Organoleptic quality of low calorie aonla ladoo

Treatments		Characters				
		Colour & appearance	Taste	Texture	Mouth feel	Overall Acceptability
Sugar replaced with stevia (%)	0	8.2	8.1	8.0	8.0	8.0
	25	7.2	7.0	6.9	6.8	7.0
	50	6.8	6.5	6.3	6.1	6.5
	75	6.0	5.8	5.7	5.3	5.6
	100	5.8	5.3	5.0	4.8	5.2
	CD at 5% 0.32 0.49 0.43 0.39 0.32					

Chemical constituents of low calorie aonla ladoo: Changes in Moisture %,Water activity, total soluble solids (TSS), Total sugar, Reducing Sugar, Titratable acidity, ascorbic acid, total phenols, non-enzymatic browning, Antioxidant activity and pectin in low calorie aonla ladoo during three months storage period in present study have been presented in Tables 2. The moisture content of low calorie aonla ladoo having replacement of 50% sugar by stevia powder was found decreased significantly during three months storage. This may be due to evaporation of moisture from the samples at room temperature during storage. Singh *et al.* (2010) [45] reported a decline in moisture content of intermediate moisture baby corn during storage. Singh *et al.* (2012) [44] also reported decrease in moisture content in aonla supari during 135 days of storage period. Water activity of low calorie aonla ladoo ranged from 0.87 to 0.77 during three months of storage. The progressive decrease in a_w of low calorie aonla ladoo during storage might be due to loss of moisture content from the samples. Ayub and Alam (2002) [5] reported decline in a_w of dehydrated sweetened guava slices. Singh *et al.* (2012) [44] also reported decline in water activity of aonla supari during 135 days storage period.

Total soluble solids were found to increase significantly in low calorie aonla ladoo during three months storage. This might be due to conversion of polysaccharides intosoluble sugars by hydrolysis process and reduction in moisture content of the product during storage. Similar results were reported by Ayub *et al.* (2005) [6] in sweetened guava slices, Daisy *et al.* (2007) [12] in aonla preserve, Ram *et al.* (2011) [40] in aonla bael blended RTS beverage and Choudhary *et al.* (2012) [10] in aonla nectar. There was a gradual and significant increase in total sugars of low calorie aonla ladoo with the advancement of storage period. The increase in level of sugars can be attributed to loss of moisture from the products and hydrolysis of starch and pectin into simple sugars. Similar finding were reported by Ram *et al.* (2011) [40] in aonla bael blended RTS beverage and Choudhary *et al.* (2012) [10] in aonla nectar, Gaikwad *et al.* (2013) [15] in aonla ginger RTS beverage and Patel *et al.* (2013) [36] in aonla murabba. Reducing sugars of low calorie aonla ladoo ranged from 18.3 to 21.2 during three months of storage. The increase in reducing sugars corresponds to increase in total soluble solid (TSS). Thus, it could be due to moisture loss and inversion of non-reducing into reducing sugars by hydrolysis. Rani and Bhatia (1986) [42] also showed an increase in reducing sugars during 24 weeks storage of Bagughosa preserve, which was ascribed to increased inversion of sugars. Similar results were also reported by Mir and Nath (1993) [30] in fortified mango bar, Sivakumar *et al.* (2007) [46] in guava toffee, Nagpal and Rajyalakshmi (2009) [33] in bael-citrus fruit blend and Panwar (2014) [35] in IMF aonla segments and aonla candy**.** Gradual and significant increase in titratable acidity of low calorie aonla ladoo was observed during three months storage. The increase in acidity might be due to conversion of sugar into acids (Manivasgan *et al*., 2006) [28], degradation of polyphenols and conversion of proteins to amino acids. Pectic acid has also been reported to increase the acidity in fruit products; hence, degradation of insoluble pectic substances into soluble pectate might also have contributed towards an increase in acidity of some products. These results are in accordance with those of Hussain *et al.* (2004) [20] in osmotically dehydrated banana slices, Kaikadi *et al*. (2006) [21] in ber candy, Manivasagan *et al*. (2006) [28] in karonda candy and Nayak *et al*. (2012) [34] in aonla candy. Ascorbic acid content decreased significantly inlow calorie aonlaladooduring three months storage period. This loss of ascorbic acid could be attributed to oxidation of ascorbic acid to dehydro-ascorbic acid with passage of time. Similar findings have been confirmed by reduction by Tandon *et al.* (2003) [50] in aonla candy, Muhammad *et al.* (2008) [32] in apple jam, Hussain and Shakir (2010) [19] in apricot and apple jam, Bhuiyan (2012) [8] in fresh hog plum chutney, Choudhary *et al.* (2012) [11] in aonla syrup, Souad *et al.* (2012) [47] in watermelon waste jam and Vikram *et al.* (2012) [51] in aonla herbal jam. The ascorbic acid content was found to decrease more rapidly inthe initial stages but the decrease was slow in the later stages with increase in storage period. Similar result was reported by Patel *et al.* (2013) [36] in aonla murabba during 180 days of storageperiod. A non-significant decrease in pectin content of low calorie aonla ladoo was noticed during first month of storage period. However, decrease in pectin content of low calorie aonla ladoo was found significant in later months of storage. This decrease in pectin content might be due to degradation of pectin into pectic acid during storage. Similar results were reported by Mehta *et al.* (2005) [29] in galgal peel candy and Patel *et al*. (2013) [36] in aonla murabba. A significant decrease in total phenols of low calorie aonla ladoo was recorded during three months storage. The decrease in total phenols during storage might be due to their condensation into brown pigments (Fennema, 1976) [14]. The phenolic acids are oxidized to 0- semiquinone residuals or 0-quinone molecules, which are reactive to give brown products of high molecular weight. Decrease in total phenols during storage was also reported by Kannan and Thirumaran (2001) [22] in jamun products (RTS drink, squash, syrup and jam), Kaushik *et al.* (2002) [23] in bael preserve, Deka *et al.* (2005) [13] in mango-pineapple based spiced RTS drink, Punam *et al*. (2009) [39] in bael-mango RTS drink and squash. Non-enzymatic browning increased significantly in low calorie aonla ladoo with the advancement in three months storage period. This might be due to condensation of tannins into brown pigments and inversion of non-reducing to reducing sugars, which participated in maillard browning. Browning index of ash gourd candy (Srivastava *et al*., 2006) [38] and karonda candy (Manivasagan *et al*., 2006) [48] also increased during storage. Similar increase in browning index during six months of storage was also reported in IMF aonla segments and aonla candy by Panwar (2014) [35]. Antioxidant activity decreased significantly in low calorie aonla ladoo during three months storage period. Phenolic compounds and

ascorbic acid content have been proved to be responsible for the antioxidant activity of aonla fruit (Kumar *et al.*, 2006; Sabu and Kuttan, 2002; Anila and Vijaya lakshmi, 2003) [25, 43, 3]. This loss in antioxidant activity could be attributed to oxidation or loss of ascorbic acid and phenolic compounds in low calorie aonla ladoo with the passage of time. Similar results were reported by Kumari (2014) [27] in rainy and winter season guava fruits during two weeks storage.

Table 2: Effect of storage period on chemical constituents of low calorie aonla ladoo

Low calorie aonla ladoo	Storage period (Months)				CD at 5%
	0	1	2	3	
Moisture %	48.3	45.4	40.5	36.7	0.32
Water activity	0.87	0.85	0.83	0.77	0.01
TSS	42.6	48.4	52.7	58.4	2.1
Total Sugar	32.3	32.9	33.8	34.5	0.52
Reducing Sugar	18.3	19.1	20.3	21.2	0.56
Titratable acidity	2.29	2.31	2.33	2.40	0.09
Ascorbic acid (mg/100g)	271	233	214	200	1.9
Total phenols	1.84	1.76	1.68	1.54	0.20
NEB	0.08	0.11	0.17	0.22	0.03
Antioxidant activity	59.4	49.4	40.8	33.3	0.32
Pectin %	0.49	0.48	0.47	0.45	0.01

Conclusion

It can be concluded from the present investigation that among different levels of sugar replacement by stevia powder, low calorie aonla ladoo having 50% sugar replaced by stevia was found to be the most acceptable formulations for preparation low calorie aonla ladoo. The moisture content (%), water activity (aw), ascorbic acid, pectin, total phenols &antioxidant activity decreased significantly in low calorie aonla ladoo during three months of storage. While TSS, total sugar, reducing sugars, titratable acidity and non-enzymatic browning increased significantly in low calorie aonla ladoo during three months of storage. Thus, present study was first in its kind to determine overall acceptability and chemical composition of low calorie aonla ladoo obtained from aonla fruits cv. Chakaiya and Stevia leaf powder.

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