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Research Article

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Determination of Trace Element, Microbial Load, Vitamin C and Percentage of Protein for Shelf Life and Quality Determination of Prepared Amla Candy

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Abstract As part of shelf life analysis of vitamin C enriched amla candy, which was prepared form fruits *Phyllanthus emblica*, the present work was under taken to analysis the proximate microbial load, protein, iron and some other heavy metal content of the product over a six months period. The candy was prepared by applying osmotic dehydration method. Iron was observed 0.34 mg/100g; heavy metals like Hg, As, Pb, and Cd were below the limit of detection at AAS. The candy and raw amla were found to contain protein 4.29%& 4.53%; ascorbic acid (550.20- 535.83) mg/100g & (593.22- 553.74) mg/100g respectively. From the microbial point of view, screening results of microbial load test confirmed the safety of prepared amla candy over six months period. The present study results revealed that the prepared amla candy is a good source of vitamin (Ascorbic Acid) and mineral (Fe) having shelf life over six months.

Keywords Ascorbic acid, Iron, Heavy metals, Microbial load test, Protein

Introduction

A lack of vitamin C in the diet causes the deficiency disease scurvy [1]. This potentially fatal disease can be prevented with as little as 10 mg vitamin C per day [1]. Amla is an edible fruit of a little bowery tree of the Euphorbiaceous family that grows naturally and planted several areas of the country. Although vitamin-C content of Amla is second to that of Barbados cherry [3-5], it contains considerable high amount of amino acids and mineral [6] as well as poly-phenolic antioxidants like- ellagitannins, [7] like emblicanin A (37%), emblicanin B (33%), punigluconin (12%) and pedunculagin (14%) [8]. It contains punicafolin and phyllanemblinin A, phyllanemblin different polyphenols, like flavonoids, kaempferol, ellagic acid, and acid [9].
By nature, the fruit is putrescible and procurable from the month of August to January. Once harvest, its storage life in ambient conditions is barely 5–6 days. Application of suitable storage system and harvesting or processing methods can curtail the post-harvesting losses to 30% and make the fruit procurable for extended period of time. The few post-harvest technologies that exist are difficult and unaffordable to very little farmers at the farm level. Besides, its astringent test makes customers hesitant to eat it in raw form. In order to pursue some properties like: chemical properties, organoleptic properties, inhibitory activity as well as lack of provider of amla in whole year, high acidity and astringent taste, it's needed to convert Amla into processed products. There are Varieties of merchandise like murabbas (whole fruit preserve), pickle, candy, squash, square measure ready [10] etc. To form amla a fruit of mass population, product that square measure partaking, tasty and can be consumed as food things ought to be developed, but at the same time the product should retain its nutrient and therapeutic values. Processed Amla products are the exuberant sources of vitamin C, phenol, dietary fiber, antioxidant [1]. Amla and amla derived product has some health promoting effects because of the contained

phytochemicals that promote liver health, reduce sterol levels and cancer risks, control blood glucose level, prevent constipation, decreases inflammation, keep hair and skin healthy, promote biological process healthy, support psychological feature to operate etc. Murabba (whole fruit preserve) is recognized as wholesome food item and is sometimes counseled by physicians for consumption throughout summer months. Amla candy, syrup, squash and jam are also the product of processed Amla fruit. Freeze dried powders has vast potential for export to the overseas market [12]. It's been reported that amla fruits contain the mineral elements: phosphorus, magnesium, potassium, sodium, copper, and Mn [13-14]. It also contains moisture, crude fibre, dietary fibre, [14-15]. Amla fruit ash contains chromium 2.5 ppm, Zinc 4.0 ppm and copper 3.0 ppm [16]. Product of processed Amla fruit has DPPH free radical scavenging activity [17]. Considering all the result of amla fruits and amla fruits processed products, an in depth analysis program was under taken to develop a better process of valuable vitamin C enriched candy. The method doesn't involve any sophisticated instrumentation. Production and quality determination of prepared amla fruit candy for Bangladeshi people, particularly for the kids and diabetic patients, was considered because of the following reasons: (a) as tests of Alma fruits are astringent, the kids are reluctant to eat the fruit directly; so that children can have continuous supply of vitamin C and one of the minerals, Fe, in one of their food supplement of choice e.g. candy, and (b) as it conjointly contains a mineral Cr that reportedly regulates blood sugar and helps the body to insulin secretion, diabetic patient can have health benefit by taking this candy for management of blood glucose levels.

Materials and Methods

*Determination of vitamin C :*The presence of vitamin C were observed in processed amla fruits candy by a simple UV- Spectrophotometric (Model-Genesys 20) method where oxidation of ascorbic acid to dehydroascorbic acid by bromine water takes place in presence of acetic acid [18].

*Sample preparation:*10 gm of sample of blended candy were mixed in a beaker with 50 ml acetic acid and mixed it homogenously and then it was taken into a 100 ml volumetric flask and shaken it for homogenous dispersion. After a while filtration were performed and clear filtrate was taken for determination of Vitamin C.

Then bromine water solution was added to the filtrate until the oxidation completed which was observed by the appearance of color. After that thio-urea solution was added to clear the solution, total vitamin C was measured by a complex reaction between Vitamin C and 2, 4- DNP solution and the absorbance was spectrophotometrically measured by UV-spectrophotometer at wavelength 520 nm.

Determination of Total Nitrogen and Calculation of protein content: For nitrogen estimation Kjeldahl apparatus (Model- P SELECTA) was used. 2 g sample, 20ml conc. H_2SO_4 and digestion catalyst ware taken in Kjeldahl flask and digested. After digestion flasks were allowed to stand until it become normal and titration was performed. Percentage of nitrogen and percentage of protein were calculated according to the Eq-1 and Eq-2 respectively.

$$\%\text{Nitrogen} = \frac{1.4\text{x } (V_1 - V_2)\text{xN}}{\text{P}} \quad \text{... Eq-1}$$

$$\%\text{Protein} = \%\text{Nitrigen x F} \quad \text{... Eq-2}$$

P=weight of sample in gram

F= Conversion Factor, 6.25 [19]

Determination of trace and heavy metals: The atomic absorption measurements were performed using Thermo fisher-3300 AA atomic absorption spectrophotometer with hollow cathode lamp (HCL) light source. For analysis of all the metals, oxyacetylene flame was used. For the determination of arsenic and mercury, hydride generator was used and mercury was determined using cold vapor analysis. The standard instrumental configuration and experimental condition maintained for the analysis of Pb, Cd, Fe, Hg, and As are given in table-1.

Table 1: Instrumental condition for trace and heavy metal analysis by AAS

Element	**Pb**	**Cd**	**Fe**	**Hg**	**As**
AAS Specification					
Wavelength	217.1	228.8	214.3	253.7	193.7
Current (mA)	9	4.0	9	3	12
Flame	AA	AA	AA	AA	AA

Fuel(L/min)	2.9	4.99	2.99	7.66	2.4
Working range ppm	2-10	0.2-1	2-10	0.2-1	0.04-0.1
Read Time (Sec.)	3	3	3	3	3
Wash time (Sec.)	10	10	10	10	10

Sample preparation: Accurately weighed sample, 3.0 g, was placed in a flask and treated with 3 mL of concentrated HNO_3 for 4-5 hours. A mixture of HNO_3 and $HClO_4$ in a ratio of 2:1 (3 mL per gram of sample) was added. The mixture was heated at 120-130ºC for 5-6 hours, until fumes stops and resulting solution is clear. Then 10 mL of Milli-Q water was added and boiled again for 10-15 min and volume was reduced to the half, cooled to room temperature and filtered using Whatman filter paper no. 42. The entire filtrate was mixed and made the volume upto 50 mL with Milli-Q water. Blank was also prepared for every sample in the same way. Each sample was aspirate twice and the experiment was repeated for five times.

Microbiological analysis: By using sterile mortar and pestle size of amla candy was converted to a semi-solid form to have it dispensable. 1 g of each sample was placed in 10 ml of water and then serial dilution was performed. Dilution of 10-3, 10-5 and 10-7 were used for bacterial. The total microbial load was determined using nutrient agar medium prepared according to the guidelines of IFST, BCSIR. Serial dilution was done using physiological salt solution containing NaCl and $NaHPO_4$ (1.45 g, 10 g, and 6.25 per 2.5 L) as diluents. The aim was to maintain the microorganisms in their physiological state to prevent plasmolysis resulting from osmosis. One hundred mL of the diluent was measured into bottles used for serial dilution containing 11 g of each sample. The mixture was shaken using a horizontal shaker (Model SM, Einrichtungen, Germany) for 30 minutes. Further dilutions were made, and dilution 10^1 and 10^3 were plated in duplicates. Total viable bacterial counts were determined from nutrient agar plates of the serial dilutions, using Automatic Colony Counting System Based on Image Processing [20].

Results and Discussion

The prepared Amla Candy, and Raw Amla were evaluated for Vitamin-C, proximate microbial load and results are depicted in the table-2 and the table-3 Which indicates the microbial safety and efficacy of the product. The observed results of trace element, heavy metals and % proteins are shown in the table-4 and the table-5.

Table 2: Analysis of vitamin C of Raw Amla & prepared Amla Candy

Sl. No.	**Name of Specimen**	**Amount of Vitamin-C mg/100 g 2,4-Dichloroindophenol dye method**	
		Initial	**Six months Later**
	Raw Amla	593.22	553.74
	Amla Candy	550.2	535.83

Table 3: Analysis the proximate microbial load

Sl. No.	**Test**	**Results**	
		Initial	**Six months Later**
1.	Total viable count, cfu/g	<10*	<10*
2.	Coliforms, MPN/g	<0.3**	<0.3**
3.	*Escherichia coli* MPN/g	<0.3**	<0.3**

*<10 indicates absence of test organism in 1gm sample, ** As per MPN Chart, The most probable number <0.3 indicates absence of test organism in 1 gm sample

Table 4: Iron and some other heavy metal (Cadmium, Mercury, Lead and Arsenic) content

Sample	**Elements**				
	Trace element	**Heavy metals**			
Amla Candy (Date)	Fe	Cd	Hg	Pb	As
Amla Candy (Date)	3.4 ppm	<DL	<DL	<DL	<DL

<DL – Below Detection Limit

Table 5: Total Nitrogen and Calculation of protein content (% of Dry Matter)

Sample	HCl Consumption on Titration in ml (V1)	HCl Consumption on Blank Titration in ml (V0)	Normality of HCl (N)	% Nitrogen	% Protein
Raw Amla	4.42	0.28	0.25	0.72	4.53
Amla Candy	4.21	0.28	0.25	0.69	4.29

P= Weight of Sample in gram (2g)

Like many other mammalian species, Human lacks the L-gulonolactone oxidase (GULO) enzyme which is required in the last enzymatic step of synthesis of vitamin C from glucose [21]. So, their food supplement should contain necessary amount of vitamin C for maintenance of good health. Due to the shortage of vitamin C not only scurvy, but also symptoms including malaise, gum disease, poor wound healing, and shortness of breath and bone pain [22] are cause to happen in people of different age groups. Recommended Dietary Allowances (RDA) of vitamin C, as suggested by the Institute of Medicine, is 90 mg for males and 75 for females [23]. Currently, non-communicable diseases are thought to have been the main challenge to the medical researchers [24] which can be mitigated to some extent by ingesting fresh and diversified vegetables. But, due to the urbanization a large amount of population are deprived of the fresh availability of fruits and vegetables where the content of vitamin is reduced. To overcome the drawback, food supplement *prepared* Amla candy can play significant role as in ingestion of 2 g prepared Amla *candy* can provide approximately 12 mg vitamin C (Table-2). Because of higher growth rate, children and adolescent; because menstrual bleeding, women have a tendency of iron deficiency. Presence of iron in both the raw Amla and Amla Candy @ 0.34 mg/100g (Table-4) justifies the beneficial effect of human consumption of the raw fruits as well as prepared Amla candy. Besides, the absence of some deleterious heavy metal (Table-4) and test organism (Table-3), and nutritional value in terms of % Nitrogen and %Protein (Table-5) in the prepared Amla Candy indicates the socioeconomic aspect of viability of consumer food industrial set up.

Conclusion

Phyllanthus emblica is one of the major natural sources of Vitamin-C as well as poly-phenolic anti-oxidants and mineral like Iron. Appropriate preservation retains Vitamin-C and iron with very little diminish which indicate Prepared Amla Candie's potential to be an alternative of the OTC of vitamin-C and Iron tablets' for preventive application for protection of human health.

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