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Evaluation of Nutraceutical Applications of *Annona squamosa* L.based Food Products

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Abstract
Annona Squamosa L. (Custard apple) is an underutilized tropical fruit crop growing feral on Deccan Plateau and some parts of central India. The fruit is known for its sweet taste, distinct flavour, and pleasant, aromatic creamy pulp. The fresh fruit pulp contains high nutritional value as it is rich in protein, carbohydrate, sugars, vitamins and minerals. In present study, an attempt has been taken to aim the processing of ripe fruits to add value to these fruits, jelly have been developed. The results for the chemical composition analysis of custard apple pulp, juice and jelly were respectively: moisture (74.6, 76.2 and 31.42 $g.100g^{-1}$), total soluble solids (26, 23 and 65 ^{0}B), total sugar (20.9, 12.45 and 49.83 $g.100g^{-1}$), ascorbic acid (32.5 16.96 and 14.8 $mg.100g^{-1)}$ with a titrable acidity (0.29, 0.49 and 0.46 $g.100g^{-1}$). The mineral content for custard apple pulp, juice and jelly were respectively: calcium (642.5, 581.5 and 582.0 $mg.kg^{-1}$), potassium (4280.0, 5455.0 and 4119.0 $mg.kg^{-1}$), sodium (627.5, 467.5 and 190.0 $mg.kg^{-1}$), magnesium (545, 650 and 412 $mg.kg^{-1}$), iron (28.0, 22.5 and 26.5 $mg.kg^{-1}$) etc. The jelly were subjected to Quantitative Descriptive Analysis. The attributes like flavor, consistency, appearance and overall acceptability of the jelly were evaluated by 12 trained panellists on a 9-point hedonic scale. Based on sensory evaluation, custard apple jelly presented a good overall acceptability. The flavour was the most appreciated attribute, which had an average score of 8.05.

Keywords: *Annona squamosa* L., nutraceutical, minerals, ICP OES

Introduction
The genus Annona, of the family Annonaceae, comprised of 60 or more species, typically of tropical American origin. The four well recognized bearers of edible produce(Mortan, 1946) are true custard apple or bullock's heart (*A. reticulata Linn.*), the sugar apple or sweetsop (*A. squamosa Linn.*), the cherimoya (*A. cherimola Linn.*), and the sour sop (*A. muricata Linn.*) (Mortan, 1966). The sugar apple is a native of the Caribbean area, and the East Indies (Prasanna *et al.*, 2000). These fruits are called as Buddha's head after its shape in India, and are commonly known as custard apple, sugar apple, sweet sop or Atemoya in other part of the world. Original habitat of the *A. squamosa* L. is considered to be in West-Indian-islands, but it is cultivated as well in and around tropical area today (Iwasa, 2001). In India Custard apple (*Annona squamosa* L.) is also popularly called as sitaphal seureuba, sugar apple, gishta, sweet apple, zimtapfel, sharifa, sitappalm, matomoko, aati, atis, and anoda. *Annona squamosa Linn* is a tropical branched shrub cultivated throughout India for its fruits and different parts viz. seed, leaf, root, bark for the treatment of various diseases (Padhi *et al.*, 2011). The custard apple (*Annona squamosa L.*) is produced as 20,497 MT from 4,990 ha of area in Maharashtra (Sontakke, 2003). It is exported in huge extent from India to UAE, Bangladesh, Saudi Arabia, and Kuwait (Chadha, 1995). Its cultivation in India has been estimated to be 30,000 hectares with an annual production of 228 thousand MT in the year 2014-15 while for the year 2015-2016 cultivated in 35,000 hectares area with production of 271 thousand MT (NHB report, 2015-16).

The fruit contains moisture (73.5%), proteins (1.6%), carbohydrates (23.9%), minerals (0.9%), vitamin A and C, fats (0.3%) (Gopalan *et al.*, 1991). It gives about 40% pulp with TSS 26.4^{0} brix, pH 5.5 and tannins 0.5%, and may play an important role as nutraceutical (Nanjundaswamy, 1990). Custard apple is a nutritionally balanced food which constitutes protein, fiber, minerals, vitamins, energy and very little fat (Babu, 2014). It is an excellent store of vitamin C, vitamin B2, vitamin B6, vitamin A, a good source of dietary fibre, magnesium and potassium. Vitamins A is essential for maintaining the skin, eyes, hairs and body tissues healthy while, Vitamin C acts as an anti-oxidant that fight free radicals in our body. Magnesium and potassium helps in cleaning our systems by removing acids from joints and prevents arthriritis.

They also maintain the water balance in our body, improves fortitude and ability to improve oxygen supply and body-building amino acids (Davidson, 1975).

Roots, leaves, fruits, seeds and bark of custard apple has several applications (Suresh, 2008; Pandey, 2012). The fruits are rich source of calorific value, minerals and vitamins. Athletes can use it for their high energy content. The seeds have major insecticidal properties and could be used for removing head lice (Suresh *et al*., 2005; Ranjan *et al*., 2009; Kumar *et al*., 2010). The seeds are also utilized as a pesticide in the field of agriculture and horticulture. In folk medicines, fruits are effectively used as anti-depressant and antioxidant. Experimentally, it was proven that the fruits increases hemoglobin content of RBC i.e. haematinic in nature, provides cooling effect and could act as sedative, tonic, and expectorant (Tiangda *et al*., 2000).

Nutritional composition:

A. squamosa L. is one of the delicious fruit much relished by people due to its pleasant flavor, mild aroma, soft, sugary, juicy and granular pulp. The fruit is nutritionally more valuable consisting of about 40% pulp with carbohydrates, proteins, minerals like calcium, phosphorus, magnesium and iron etc. The calorific value of custard apple ranges between 860 to 1140 kcal per kg as compared with 741 kcal per kg of mango (Patel, 2009). The content of ascorbic acid in custard apple was reported to be in the range of 34 mg to 44 mg.100g-1 (Alwazeer, 2003). The content of pectin is almost insignificant in all the varieties of Annona (Prasanna, 2000). The pulp is rich in dietary fibre and easy to digest and consumed by children and people of all ages (Chavan, 2006). The presence of various biochemical in the custard apple have important applications in biological functions such as helping to protect bones and teeth, providing strong muscles and improving overall general health.

The custard apple fruit has limited shelf life of 3-4 days after ripening. Due to its high respiration rate it soften vary rapidly and becomes difficult to consume fresh. To prevent from such losses and to retain its nutritional quality, it can be processed into different products like juice, jam, jelly, powder, wine, candy. In this way development of new products or food formulations through incorporation of such valuable fruit becomes an alternative to ensure nutritional security and health benefits. This paper presents the study on formulation of custard apple based products like jelly and determination of its biochemical composition and mineral content in order to verify its nutritional benefits.

Materials and Methods:

The fruits of custard apple were procured from the local market of the Ranchi, Jharkhand. The fully ripened fruits were selected for extraction of pulp. The fruits were washed with tap water and the treated with 200 ppm of sodium hypochlorite for 15 minutes followed by thorough washing to remove traces of chemicals. The fruits were cut manually and scooped with stainless steel spoon for separation of seeds. The peeled fruits were passed through pulper for separation of seeds and pulp. Extracted pulp were mixed with 1000 ppm of potassium meta bisulphite (KMS) and stored for further processing.

Juice extraction from custard apple pulp:

Custard apple juice was extracted by enzymatic treatment with pectinase. The extracted pulp was treated with 0.75 % (w/w) pectinase enzyme and incubated at a temperature of 40 ^{0}C for three hours. The enzyme treated samples were heated to 80 ^{0}C for 30 seconds to stop enzyme activity. The samples were centrifuged at 3000 rpm for 15 minutes for removal of suspended material and juice clarification followed by filtration of juice through cheese cloth to collect the clarified juice. Pasteurization of clarified juice was done by heating juice upto 85 ^{0}C for 20 minutes in a pre sterilized bottles followed by cooling to room temperature.

Preparation of jelly from custard apple juice:

Custard apple jelly was prepared from juice extracted from pulp in presence of enzyme pectinase (Yosuf and Ibrahim, 1964). The recovery of juice was very less (61%) therefore only 60 % (v/v) of juice was used for preparation of jelly. The custard apple juice was mixed with 40% (v/v) water and 50-100% (w/v) of sucrose and heated for about 3-5 min. To this mixture, varying amount of high methoxyl content of pectin was added with continuous stirring to avoid lump formation. The pH of sample was adjusted with citric acid. Preservative like potassium meta bisulphite (KMS) 40 ppm was added when TSS reaches 62 ºB. The mixture was boiled to reduce the amount of water content in the sample for lowering water activity (a_w) resulting final TSS of jelly to 65 ºB. The jelly was quickly filled into jar containenrs which were already sterilized with boiling water for 30 minutes.

Proximate analysis of custard apple pulp, juice and jelly:

Various physico-chemical properties of pulp, juice and jelly were analyzed and recorded in triplicates. Total soluble solids (TSS) of custard apple pulp, juice and jelly was determined by ERMA hand refractometer and expressed as ºB. Reducing sugar and total sugar of theses samples were estimated by Lane and Eyon method (AOAC, 1990). Titrable acidity was measured by titration with 0.1N NaOH as described by Ranganna, 1986. pH of all samples were recorded by Zenieth digital pH meter.

Mineral analysis:

0.2 g of all samples of pulp, juice and jelly of custard apple was weighed in a conical flask followed by addition of nitric acid and hydrogen peroxide. The samples were digested in closed-vessel microwave digestion systems (Multiwave 3000 SOLV) at 210 °C temperature and 40-bar pressure for 60 min as described by Amateifio and Mosase, 2006. Mineral content of custard apple based products were determined using inductively coupled plasma atomic absorption spectrometry (ICP-AAS). The content of Ca, Mg, Fe and Zn in sample was determined from calibration curve by plotting (absorbance peak area) against concentration. The calibration curve is prepared by using concentrations of standard solutions within working range.

Sensory evaluation of custard apple jelly:

The judges had good abilities to find out the organoleptic taste and sensory evaluation of custard apple products like jam and jelly. The Sensory Attributes like Colour, Flavour, Aroma, Texture and Overall acceptability of the entire products were evaluated following nine point hedonic scale (9 = like extremely, 8 =like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, 1 = dislike extremely) (Monteiro, 1984).

Result and Discussion

In present study, selected fruit samples of *Annona squamosa*

L. were utilised for pulp extraction, the extracted pulp was then processed for juice with the help of enzymatic methods. Thus obtained juice may be utilized for formulation of beverages or may be processed into jelly to conserve its nutritional values. The pulp, juice and jelly of custard apple were analysed for its nutritional parameters and results were presented in table 1.

Table 1: Nutritional parameters of custard apple pulp, juice and jelly

S. No.	Constituents	Pulp	Juice	Jelly
1.	Moisture (g.100g^{-1})	74.6	76.2	31.42
2.	Total Soluble Solids	26	23	65
3.	pH	5.48	5.02	3.52
4.	Titrable Acidity (g.100g^{-1})	0.29	0.49	0.46
5.	Total sugar (g.100g^{-1})	20.9	12.45	49.83
6.	Vitamin C (mg.100g^{-1})	32.5	16.96	14.8

The moisture content of pulp, juice and jelly was found 74.6, 76.2 and 31.42 (g.100g^{-1}) whereas the TSS (total soluble solids) was recorded 26, 23 and 65 ^{0}B respectively. The high TSS was a good indicator of sweetness. The pH of the pulp was recorded as 5.48 while it was 5.02 and 3.52 for juice and jelly. A low amount of pH in the range of 3 - 5 provides good keeping quality. The total sugar was found to 20.9, 12.45 and 49.83 g.100g^{-1} for pulp, juice and jelly respectively which showed the excellent source of energy. The range of Vitamin C was observed 32.5 mg.100g^{-1} in pulp while 16.96 and 14.8 mg.100g^{-1} was found in juice and jelly. A similar type of work was also reported by Bala *et al* 2017 which described the chemical analysis of custard apple pulp into jam. According to epidemiological studies which give influential evidence that diet prosperous in antioxidant lowers the incidence of degenerative diseases.

Mineral Content

The data on mineral composition of custard apple based products like pulp, juice and jelly were presented in table 2.

Table 2: Mineral composition of custard apple based products (mg.kg^{-1}).

S. No.	Minerals	pulp	Juice	Jelly
1.	Calcium (Ca)	642.5	581.5	582.0
2.	Potassium (Fe)	4280.0	5455.0	4119.0
3.	Sodium (Na)	627.5	467.5	190.0
4.	Iron (Fe)	28.0	22.5	26.5
5.	Copper (Cu)	27.5	0.5	6.0
6.	Magnesium (Mg)	545.0	650.0	412.0
7.	Manganese (mn)	17.5	14.5	10.5
8.	Arsenic (As)	-15.5	-12.5	-17.0

The mineral content of custard apple based products was shown in table 2. The results shown that custard apple and their products were high in calcium, potassium, sodium and magnesium but low in iron and copper. The average calcium content was 642.5, 581.5 and 582.0 mg.kg^{-1} in custard apple pulp, juice and jelly respectively. This result was found higher than 60.25 mg.100g^{-1} for sugar apple fruits reported by Bhardwaj *et al*., 2014. The amount of calcium present in these product found higher to wheat flour (18 mg.100g^{-1}), corn flour (34 mg.100g^{-1}) and rye flour (24 mg.100g^{-1}) (Marques *et al*., 2013; Villela *et al*., 2013).The processed products of custard apple also contained relatively good quantity of calcium which hold a promising source of calcium and also counted for suitability for lactose intolerance individuals, since milk and its products are considered best source of calcium. The average content of potassium was found to be 4280.0, 5455.0 and 4119.0 mg.kg^{-1} in pulp, juice and jelly respectively. This value was higher than the reported value of Nigerian sugar apple fruits 45± 0.49 mg.100g^{-1} (Hassan *et al*., 2008). According to USDA 2002 custard apple and their products could contribute with 10.5% of nutritional recommendation. The results were compared to rice flour (97.4 mg.100g^{-1}), corn flour (148.7 mg.100g^{-1}) and wheat flour (150 mg.100g^{-1}) for potassium content as reported by Hager *et al*., 2012 was very low.

The sodium content of custard apple and its products was found in a range of 627.5, 467.5 and 190.0 mg.kg^{-1} for pulp juice and jam respectively. This results compared to earlier report on custard apple fruit of Nigeria (7310 mg.100g^{-1}) was higher than present observation (Amoo *et al*., 2008) and in another report only 10 mg/100 g of sodium was reported in sugar apple fruits of Nigeria (Hassan *et al*., 2008). These fruits and their products can contribute in a large quantity the amount of sodium needed in the body since RDA value for sodium for an adult is 500 mg. On the other side, high sodium content makes them not to be ideal food material for prevention and management of hypertension.

The magnesium content in custard apple based products was found to be 545, 650 and 412 mg.kg^{-1} of pulp, juice and jelly respectively. This result was found in accordance with the reported value of 51.76 mg.100g^{-1} in custard apple pulp that may play an important role in bone formation and other essential metabolic activities in the body (Bhardwaj *et al*., 2014). These custard apple based products could be an alternative choice in place of green banana flour (30.84 mg.100g^{-1}), *Annona crassiflora flour* (14.23 mg.100g^{-1}) and bean flour (47.20 mg.100g^{-1}) (Frota *et al*., 2009; Hager *et al*., 2012 and Villela *et al*., 2013)

The iron content of custard apple processed products was recorded 28.0, 22.5 and 26.5 mg.kg^{-1} for pulp, juice and jelly respectively. This value was less than the level of 4.81 mg.100g^{-1} of custard apple reported by Bhardwaj *et al*., 2014 and higher than the level of 1.70 ± 0.05 mg.100g^{-1}. The contribution of iron by this fruits in the diet is poor as the range obtained in this study is less than the range for the RDA of 8.0-20 mg/day (National Research Council, 1989). However, this fruit and its products contain less iron but could be superior to food such as milk (0.02 mg.100g^{-1}), wheat flour (0.8 mg.100g^{-1}), oat meal (2.09 mg.100g^{-1}) and corn flour (1.1 mg.100g^{-1}) (Labanowska *et al*., 2014; Pedron *et al*., 2016).

The copper content was recorded 27.5, 0.5 and 6.0 mg.kg^{-1} in pulp, juice and jelly respectively. Custard apple juice showed the lowest value of copper while maximum level was found into pulp. A similar type of observations was also recorded 0.9 mg.100g^{-1} of custard apple pulp which was lower than this result for pulp (Bhardwaj *et al*., 2014). The RDA level of 1.2 - 3.2 mg.100g^{-1} for copper could be supplemented with custard apple pulp. The custard apple and its products presented 17.5, 14.5 and 10.5 mg.kg^{-1} of manganese in pulp, juice and jelly respectively. These products could be superior to banana flour (0.14 mg.100g-1) and wheat flour (0.43 mg.100g-1) which shows it potential in food formulations (Fasolin, 2007; Tejera *et al*., 2013). The other heavy metals like lead and arsenic was recorded in negative value which indicates absence or negligible amount of such elements. These results indicated that custard apple based products falls within safe limits for consumption (FAO/WHO). The results of compositional analysis indicated that custard apple could be a potential source of carbohydrate, vitamin c, potassium, calcium, magnesium which can act as functional food for improving health status.

Sensory evaluation of custard apple jelly

The results for sensory evaluation of custard apple jelly produced from custard apple pulp were presented in Table 3. The flavour of custard apple jelly was the most appreciated attribute, which had an average score of 8.05 The attribute consistency of custard apple jelly was also pleased by the judges (average score 7.25), suggesting this jelly was left with an appropriate texture of the gel. However, since the fruit does not have an attractive colour, the colour attributes were less appreciated by the evaluators (score of 6.04). Similar results were also observed in a study conducted by Lago *et al*. (2006) when performed sensory evaluation of jam produced with jambolan pulp. No rejection was observed for custard apple jelly, which means that 100% of the panellists certainly would purchase this product.

Table 3: Sensory evaluation of custard apple jelly

Attribute	Grade (Average)
Flavour	8.05
Aroma	7.45
Colour	6.04
Texture	7.54
Appearance	7.25
Total average	7.26 ± 0.21

Conclusions

The exotic Indian *A. squamosa* shows good nutritional composition with ample amount of moisture, TSS, reducing sugar, total sugar and titrable acidity. These important constituents of the Annona may be explored for processing to enhance the nutritional security and to redude post harvest losses. The fruit showed presence of important minerals like Calcium, potassium, sodium, magnesium in a moderate quantity which holds the potential to meet the daily requirement from such type of processed food. The other minerals like iron, copper, manganese were present in low quantity but these may be nutritionally superior to wheat flour, rice flour, bean flour and green banana flour. High amount of sugar, vitamin C, calcium and potassium in custard apple based products could be a functional source of nutraceutical and flavouring agents. Custard apple jelly was the most preferred by the panelists with an acceptance index of 85.22% for overall acceptability and 100% of the panelists reported that they possibly would purchase the product if available in the market.

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