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Prepation and Analysis of Value - Added Products from Sugarcane Juice

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Abstract: The study was conducted on freshly extracted sugarcane juice and value-added products, which were prepared using amla, raw mango, carrot and ginger. The blends formulated were sugarcane juice: amla cubes, sugarcane juice: mango pieces, sugarcane juice: carrot cubes and sugarcane juice: ginger diced. Physiochemical and phytochemical studies were carried out on the fresh juice and stored value-added products. Stored Amla candy prepared from sugarcane juice had 1818 mg/100ml of ascorbic acid. In the same way mango candy, ginger juice and carrot condiment had 436.32, 509.04 and 545.4 mg/100ml of ascorbic acid. Further, the reducing power assay of formulations were estimated and the results showed highest for candied amla followed by carrot condiment, ginger juice and candied mango. Organoleptic quality of the products didn't show any notable change.

Keywords: Ascorbic acid content, Candied amla, Candied mango, Carrot condiment, Ginger juice, Reducing power assay, Sugarcane juice, Titratable acidity and TSS.

Practical Applications: This work was done to analyze different physiochemical and phytochemical properties of the value- added products of sugarcane juice. We have prepared formulations, estimated their Total polyphenol, flavonoids, ascorbic acid content and also checked for anti-oxidative properties. Initially, our work involved preparation of formulation and for the same we took raw materials and they were subjected to treatments like blanching then, they were processed and resulted into value-added final product.

INTRODUCTION

Spices are natural plant products used in whole or ground form to impart flavor and aroma to food. They had been involved in development of eastern civilizations. It is mostly being used as flavoring substance and in few cases it is used in medicine, religious rituals, perfumes and for cosmetics.

Amla (*Emblica officinalis*) has a great importance in ayurveda. According to belief in Indian mythology, it is the first tree to be created in the universe; which belongs to the family of *Euphorbiaceae* and is also known as *Phyllanthus emblica* or Indian gooseberry. Amla has many nutritional qualities. It is rich in polyphenols, minerals and is regarded as one of the rich source of vitamin C (200 - 900 mg per 100 g of edible portion) (Kaushik Vilas Kulkarni, Shrishail M Ghurghure, 2018)

Mango (*Mangifera indica*), member of the cashew family (*Anacardiaceae*) and one of the most important and widely cultivated fruit of the tropical world. Mangiferin, being a polyphenolic antioxidant and a glucosyl xanthone has got strong antioxidant, antilipid, immunomodulation, cardiotonic, hypotensive, wound healing, antidegenerative and antidiabetic activities (**Pallab Kalita, 2014).**

Carrot "annual" or "biannual" herb belongs to family of *Umbelliferae* and is native

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of "Europe". It is a great source of Vitamin A. Carrot is major vegetable crop of India. Haryana, Andhra Pradesh, Karnataka, Punjab and Uttar Pradesh are major carrot growing states.

Carrot roots are rich sources of α and β carotenes (1890 µ g/100g) and contain sucrose 10 times that of glucose or fructose. Carrot leaves are a good source of leaf protein. It is used as fodder and for preparation of poultry feeds. Carrot has many medicinal properties. It increases quantity of urine and helps in elimination of uric acid. It has cooling effect and is beneficial for people suffering from gall stones, constipation and heat troubles [1].

Ginger (*Zingiber officinale Roscoe*) which is a bulbous plant and has been cultivated for a very long time. It is mainly used as spice and flavour agent in foods. Volatile oils, primarily consisting of zingerone, shogaols and gingerols as the major pungent compound are responsible for the characteristic fragrance and flavour of ginger. *The underground stem (rhizome) of ginger* contains fats, carbohydrates, protein, fibre, water and volatile oil. It is further very widely used in the food industry as an additive to ginger ale, candies, pastries and cakes (Keith Singletary, 2010) (Ann M. Bode and Zigang Dong, 2011).

MATERIALS AND METHODS

Initially samples were prepared using sugarcane juice, amla, raw mango, carrot and ginger.

1) Preparation of candied amla using sugarcane juice:

Add 750ml of sugarcane juice

↓

Add 600gms of amla

↓

Add 750ml of sugarcane juice again and heat.

↓

After few hours of processing measure brix on hand held refractometer.

2) Preparation of candied raw mango(kairi) using sugarcane juice:

Add 750ml of sugarcane juice

↓

Add 800 gms of raw or unripe mango

↓

Add 750ml of sugarcane juice again and heat.

↓

After few hours of processing measure brix on hand held refractometer.

↓

3) Preparation of carrot condiment using sugarcane juice:

Add 750ml of sugaracane juice

↓

Add 800 gms of carrot

↓

Add 750ml of sugarcane juice again and heat.

↓

Add 6-8 pieces of kokum, tamarind, black pepper and a pinch of sodium metabisulphite.

↓

After few hours of processing measure brix on hand held refractometer.

↓

4) Preparation of ginger flavored juice using sugarcane juice:

Add 974 gms of ginger.

↓

Add 1 ½ litres of sugarcane juice and start to heat them.

↓

Later, that day add another 2 litres of juice and heat.

Next day, again add 2 litres of juice and heat again.

In the same way add juice for another 2 days in order to decrease strongness of ginger and moisture.

PROCEDURES FOR POLYPHENOLS AND PHYSICOCHEMICAL PROPERTIES

Estimation of Total Polyphenol Content

Standard preparation: Gallic acid (10mg) was dissolved in 10 ml distilled water, this 10 ml solution was taken and made up to 100 ml with distilled water.

Procedure: Total phenolic content of the sample was determined by using the Folin- Ciocalteau Assay**.** An aliquot (0.1 ml) of extracts or standard solution of gallic acid (10,15,20,25,30,35,40µg/ml) was added to a 200ml test tube. Reagent blank was prepared using distilled water. 0.5ml of Folin- Ciocalteu phenol reagent and 1.5ml of 20% saturated Na_2CO_3 was added to these extracts. The volume was made up to 10ml with distilled water**.** After incubation for 90 min at room temperature, the absorbance against the prepared reagent blank was determined at 765nm with a UV-VIS spectrophotometer (Schimadzu UV- 1800). The standard curve was obtained and total phenolic content of the extracts was expressed as mg Gallic acid equivalents (GAE)/100g of the extracts. All samples were analyzed in triplicates (Charalampos Petal., 2007).

Further samples of candied amla, candied raw mango, carrot condiment and ginger juice were analyzed in triplicates.

Estimation of Total Flavanoids Content

Standard preparation: (+) catechin hydrate (100 mg) was dissolved in 4ml methanol and this mixture was made up to 100 ml with distilled water.

Procedure: Total flavanoid content was measured by the aluminium chloride calorimetric assay (zhishenet *et al*., 1999). An aliquot of 0.1 ml and 0.2 ml of extracts (10mg in 10ml) or standard solution of catechin (10,20,30,40,50,60,80, 100mg/100ml) was added to the 10ml test tubes containing 4ml of distilled water. To these test tubes 0.3ml of 5% $NaNo_2$ (sodium nitrite) and 0.3ml of 10% $Alcl_3$ was added. After 5 minutes 2ml of 1M NaOH was added and the volume was made upto 10ml with distilled water. The absorbance was prepared against reagent blank at 510nm. The standard curve was obtained and the total flavonoid content in the extracts was expressed as mg catechin equivalents (CE)/100g of the extract. Samples were analyzed in triplicates.

Estimation of Radical Scavenging Activity (DPPH ASSAY)

Reagent and standard preparation: DPPH solution was prepared by dissolving 10mg of DPPH in 250 ml methanol in a standard flask. Standard BHA was prepared by dissolving 10 mg of BHA in 10 ml methanol.

Procedure: Radical scavenging activity of the extracts (10 mg in 10 ml) was determined by the DPPH assay. An aliquot (0.25ml (50ppm), 0.5 ml (100 ppm), 1 ml (200ppm) of extracts were made up to 1 ml with methanol. To this mixture 4ml of DPPH was added and shaken. A control was also prepared containing 1 ml methanol and 4 ml DPPH solution and pure methanol was taken as the blank. After incubation in dark for 20 minutes at room temperature, the absorbance was measured at 517nm. The samples were analyzed in duplicates. Radical scavenging activity was measured in percentage using the following formula (Amarowicz et al., 2003).

Inhibition (%) = (control OD – sample OD/ control OD) *100.

Estimation of TSS

Total soluble solids (TSS) were measured using a refractometer (Erma, Japan), which was calibrated with ultrapure water just before use by ensuring 0° B reading.

Estimation of Titratable Acidity

Titratable acidity was determined by the method as described by Ranganna (1977).

Reagents

a) Sodium hydroxide (N/10)
b) Phenolphthalein indicator (1%)

Procedure

To 5ml of fresh or stored sugarcane juice/blend, 20ml of the distilled water was added and titrated against 0.1N NaOH using Phenolphthalein as an indicator. Appearance of light pink color denoted the end point.

The titratable acidity was calculated as per percent citric acid using the formula:

$$Acidity(\%) = \frac{Vol.(NaOH) * N(NaOH) * eq.wt.\ of\ citric\ acid * 100}{Vol.of\ sample * 10}$$

Estimation of Ascorbic Acid

Reagents

1. Oxalic Acid (4%)
2. Dye Solution: Weigh 42mg sodium bicarbonate into a small volume of distilled water. Dissolve 52mg 2,6-dichlorophenol indophenol in it and make up to 200ml with distilled water.
3. Stock Standard Solution: Dissolve 100mg ascorbic acid in 100ml of 4% oxalic acid solution in a standard flask (1mg/ml).
4. Working Standard: Dilute 10ml of stock solution to 100ml with 4% oxalic acid. The concentration of working standard is 100ug/ml.

Procedure

1. Pipette out 5ml of the working standard solution into a 100ml of conical flask.
2. Add 10ml of 4% oxalic acid and titrate against the dye (V1 ml). End point is the appearance of pink colour which persists for a few minutes. The amount of dye consumed is equivalent to the amount of ascorbic acid.
3. Extract the sample (0.5-5g depending on the sample) in 4% oxalic acid and make up to a known volume (100ml) and centrifuge.
4. Pipette out 5ml of this supernatant, add 10ml of 4% oxalic acid and titrate against the dye (V2 ml) [2]

Calculations

Amount of ascorbic acid mg/100ml sample

0.5mg / V1ml xV2 ml/5 ml x100 / Wt. of the sample x100.

Estimation of Reducing Power Assay

Various concentrations of the extracts in corresponding solvents were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). This mixture was kept at 50°C in water -bath for 20 minutes. After cooling, 2.5 ml of 10% trichloro acetic acid was added and centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml).The absorbance was measured at 700 nm.Ascorbic acid at various concentrations was used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power. Reducing power was measured by varying the concentration of the extract (Nayan R. Bhalodia, Pankaj B. Nariya, R. N. Acharya, and V. J. Shukla, 2013).

RESULT AND DISCUSSIONS

Sugarcane juice beverage formulations and juice based other products were prepared with combination of sugarcane juice and other ingredients.

Total Polyphenolic Content

The total phenolic content in the examined extracts using the Folin-Ciocalteu's reagent is expressed in terms of Gallic acid equivalent (the standard curve equation: y=0.00x, R^2= 0.9969). Phenolic compounds have redox properties, which allow them to act as antioxidants. As their free radical scavenging ability is facilitated by their hydroxyl groups, the total phenolic concentration could be used as a basis for rapid screening of antioxidant activity. Percentage of phenolic content of samples is shown in table 1 and standard curve is shown in figure 1.

(Tyagi T. *and* Agarwal M., 2017)

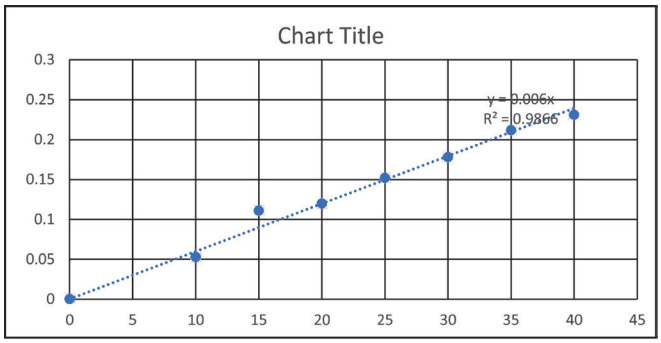

Figure 1: Standard curve

Table 1: % of total Polyphenolic content in samples

Sample	*content gm %*	*Standard Deviation*
Candied raw mango	5.29 ± 0.75	0.0236
Candied amla	5.80 ± 0.89	0.0321
Carrot condiment	0.39 ± 0.06	0.017
Ginger juice	0.28 ± 0.02	0.0074

Total Flavanoids Content

The concentrations of flavonoids in the extracts were determined using spectrophotometric method with aluminium chloride. Flavonoids, including flavones, flavanols and condensed tannins, are plant secondary metabolites, the antioxidant activity of which depends on the presence of free OH groups, especially 3-OH. Plant flavonoids have antioxidant activity in vitro and also act as antioxidants in vivo. % flavanoids content in samples is shown in table 2. Figure 2 depicts standard curve of total flavanoids content (Shoib A. Baba, Shahid A. Malik, 2015).

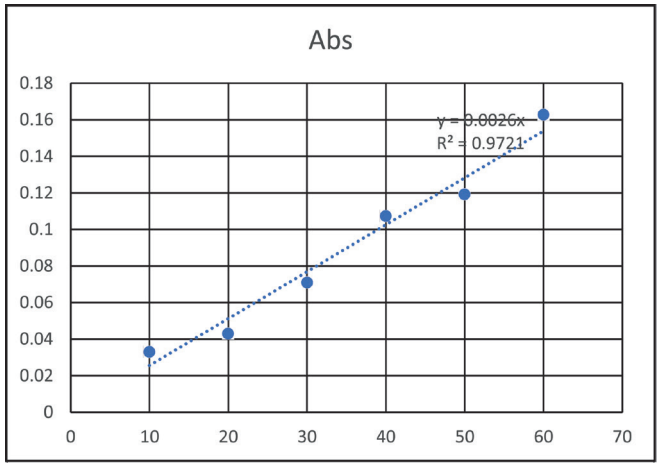

Figure 2: Standard curve (Total flavanoids content)

Table 2: Flavanoids content in samples (%)

Sample	*% content (in gms)*	*Standard Deviation*
Candied amla	0.4720% ± 0.0381	0.002
Candied mango	0.1674% ± 0.0323	0.0008
Ginger juice	0.6221% ± 0.047	0.0033
Carrot condiment	0.3422% ± 0.0094	0.0038

Radical Scavenging Activity

The antioxidant activity of different samples was determined using a methanol solution of DPPH reagent. DPPH is very stable free radical. A freshly prepared DPPH solution exhibits a deep purple colour with absorption maximum at 517 nm. This purple colour generally fades when antioxidant molecules quench DPPH free radicals (i.e., by providing hydrogen atoms or by electron donation, conceivably via a free radical attack on DPPH molecule) and convert them into a colourless/bleached product (i.e., 2,2- diphenyl 1-1-hydrazine, or a substituted analogous hydrazine), resulting in a decrease in absorbance at 517nm (Amarowicz et *al*., 2003). Parallel to examinations of antioxidant activity of formulated sugarcane juice blends, the values for a standard compound was obtained and compared to the values of antioxidant activity. The standard substance was BHA. % of RSA in samples is shown in table 3

(Laçine Aksoy[a], Erdi Kolay[a], Yasin Ağılönü[a], Zeyneb Aslan[a], Mustafa Kargıoğlu[b], 2013).

Table 3: % of RSA in samples

Sample	*0.25 ml*	*0.5 ml*	*1.0 ml*
Candied amla	96.4893	97.0425	97.6382
Candied mango	44.9332	61.8141	78.5714
Ginger juice	21.105	32.85	45.765
Carrot condiment	9.46	18.265	21.31

Estimation of TSS

The TSS of was determined by handheld refractometer and the results were expressed in degree brix.

The sugarcane juice which was used for formulations had TSS value of 20°brix.

Titratable Acidity

Titratable acidity of sugarcane juice was calculate using formula

$$Acidity(\%) = \frac{Vol.(NaOH) * N(NaOH) * eq.wt.\ of\ citric\ acid * 100}{Vol.of\ sample * 10}$$

Estimation of Ascorbic Acid

The titrimetric method is a redox titration method which depends on the reduction of the blue dye 2,6 dichlorophenol indophenol to a colourless leuco compound by ascorbic acid in solution or in extracts made out of foodstuff. Ascorbic acid, is a strong reducing agent because of which it reduces the dye 2,6 dichlorophenolindophenol and itself gets converted to dehydro ascorbic acid. After equivalence point is reached the next drop of dye gives a pink colour to the solution indicating the end point. The dye in this titration is coloured in the oxidized form and colourless in the reduced form also the dye is pink in acidic solution and blue in alkaline solution. In acidic solution the pink dye is reduced to a colourless substance in presence of ascorbic acid. The solution remains colourless when we start to add dye and remains colourless until all the ascorbic acid has reacted. As soon as the next drop of dye solution is added the solution it becomes light pink due to excess dye indicating that the end point of the titration has reached. The ascorbic acid content of various raw materials and samples is presented in table 4 and table 5 respectively [3].

Table 4: Ascorbic acid content of raw materials

Sample	Experimental value (mg/100 ml)	References (mg/100 ml)
Amla	545.4	600 [Ref no: 4]
Mango	181.8	13.2 - 92.8 [Ref no: 5]
Totapari mango	36.36	54.78 ± 2.19 [Ref no: 6]
Ginger	18.18	5 [Ref no: 7]
Carrot	18.18	5.8 [Ref no: 8]
Green chilli	109.08	242.5 [Ref no: 9]
Red chilli	145.5	45.33 ± 317 [Ref no: 10]
Sugarcane juice	18.18	3.39 [Ref no: 11]
Lemon juice	36.36	31.05 ± 4.23 [Ref no: 12]
Lime	72.72	27.78 [Ref no: 13]

Table 5: Ascorbic acid content of samples

Sample	Experiment value (mg/100 ml)
Candied amla	1818
Candied mango	436.32
Ginger juice	509.04
Carrot condiment	545.4

Estimation of Reducing Power Assay

Reducing power assay (RPA) of samples: The reducing power assay is often used to evaluate the ability of an antioxidant to donate an electron. This assay is used to show the ability of extracts to reduce Fe^{3+} to Fe^{2+}. The presence of antioxidants in the extracts resulted into reduction of the ferric cyanide complex (Fe^{3+}) to the ferrous cyanide form (Fe^{2+}). In reducing power assay, antioxidants cause the reduction of the Fe^{3+} into Fe^{2+}, thereby changing the solution into various shades from green to blue, depending on the reducing power of the compounds. Reducing power assay of samples is shown in table 6,7,8 and 9 respectively (Md. Irshad, Md. Zafaryab, Man Singh, and M. Moshahid A. Rizvi , 2012).

Table 6: RPA of Candied Amla

Sample	*Water*	*Deionized water*	*Phosphate buffer*	*Potassium ferricyanide*	*Incubation In water bath for 20 mins*	*TCL*	*Centrifuge at 3000 rpm for 10 mins*	*Upper layer*	*Distilled water*	*$Fecl_3$ solution*	*Abs at 700 nm*
Blank	1 ml	1 ml	2.5 ml	2.5 ml		2.5 ml		2.5 ml	2.5 ml	0.5	0
0.1	0.9	1 ml	2.5 ml	2.5 ml		2.5 ml		2.5 ml	2.5 ml	0.5	1.9645
0.2	0.8	1 ml	2.5 ml	2.5 ml		2.5 ml		2.5 ml	2.5 ml	0.5	3.3888
0.3	0.7	1 ml	2.5 ml	2.5 ml		2.5 ml		2.5 ml	2.5 ml	0.5	4.0000

Table 7: RPA of Candied Mango

Sample	*Water*	*Deionized water*	*Phosphate buffer*	*Potassium ferricyanide*	*Incu-bation In water bath for 20 mins*	*TCL*	*Centr-ifuge at 3000 rpm for 10 mins*	*Upper layer*	*Distilled water*	*$Fecl_3$ soluti-on*	*Abs at 700 nm*
Blank	1 ml	1 ml	2.5 ml	2.5 ml		2.5 ml		2.5 ml	2.5 ml	0.5	0
0.1	0.9	1 ml	2.5 ml	2.5 ml		2.5 ml		2.5 ml	2.5 ml	0.5	0.0066
0.2	0.8	1 ml	2.5 ml	2.5 ml		2.5 ml		2.5 ml	2.5 ml	0.5	0.001595
0.3	0.7	1 ml	2.5 ml	2.5 ml		2.5 ml		2.5 ml	2.5 ml	0.5	0.0283

Table 8: RPA of Ginger juice

Sample	*Water*	*Deionized water*	*Phosphate buffer*	*Potassium ferricyanide*	*Incu-bation In water bath for 20 mins*	*TCL*	*Centr-ifuge at 3000 rpm for 10 mins*	*Upper layer*	*Distilled water*	*$Fecl_3$ soluti-on*	*Abs at 700 nm*
Blank	1 ml	1 ml	2.5 ml	2.5 ml		2.5 ml		2.5 ml	2.5 ml	0.5	0
0.1	0.9	1 ml	2.5 ml	2.5 ml		2.5 ml		2.5 ml	2.5 ml	0.5	0.0087
0.2	0.8	1 ml	2.5 ml	2.5 ml		2.5 ml		2.5 ml	2.5 ml	0.5	0.0208
0.3	0.7	1 ml	2.5 ml	2.5 ml		2.5 ml		2.5 ml	2.5 ml	0.5	0.0320

Table 9: RPA of Carrot condiment

Sample	*Water*	*Deionized water*	*Phosphate buffer*	*Potassium ferricyanide*	*Incu-ba-tion In water bath for 20 mins*	*TCL*	*Cen-tr-ifuge at 3000 rpm for 10 mins*	*Upper layer*	*Distilled water*	*$Fecl_3$ solu-ti-on*	*Abs at 700 nm*
Blank	1 ml	1 ml	2.5 ml	2.5 ml		2.5 ml		2.5 ml	2.5 ml	0.5	0
0.1	0.9	1 ml	2.5 ml	2.5 ml		2.5 ml		2.5 ml	2.5 ml	0.5	0.0046
0.2	0.8	1 ml	2.5 ml	2.5 ml		2.5 ml		2.5 ml	2.5 ml	0.5	0.0188
0.3	0.7	1 ml	2.5 ml	2.5 ml		2.5 ml		2.5 ml	2.5 ml	0.5	0.0570

CONCLUSION

The formulations were prepared from sugarcane juice and other ingredients (amla, raw mango, ginger and carrot). These formulations were analyzed for various parameters. The results of the analysis are given below in brief.

The sugarcane juice used for preparing various formulations had 20% TSS, 0.5376% acidity and 18.18mg/100ml ascorbic acid content.

Among the selected formulations candied amla had highest polyphenol content followed by candied mango, carrot condiment and ginger juice. Flavonoids content was highest in ginger juice followed by carrot condiment, candied amla and candied mango. Radical scavenging activity (RSA) was highest in candied amla followed by candied mango, ginger juice and carrot condiment.

Ascorbic acid content was highest in candied amla followed by carrot condiment, ginger juice and candied mango. Reducing power assay was highest in candied amla followed by carrot condiment, ginger juice and candied mango.

The present investigation "analysis and preparation of value-added products from sugarcane juice" was conducted at Central Food Technological Research Institute, Mysuru.

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