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Preparation of Antioxidant Rich Healthy Beverages by Using Pineapple Juice and Guava Leaves Extract Flavoured With Herbs (Mint)

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ABSTRACT

*The present study entitled "**Preparation of antioxidant rich healthy beverages by using pineapple juice and guava leaves extract flavoured with herbs (mint)**" was undertaken to analyse the antioxidant content of prepared beverages. Two beverages were prepared using pineapple juice, guava leaf extract, herbs extract and dates and to calculate the antioxidant composition of the prepared beverages. The recipes was prepared namely; "**pineapple based beverage incorporated with guava leaf extract, mint extract, dates**", using the standard ingredients and method of preparation. The five treatments were T_0(pineapple juice 92% and dates8%), T_1(pineapple juice-88%, guava leaf extract-2%, mint 2% /basil 2 %, dates-8%), T_2(pineapple juice-84%, guava leaf extract-4%, mint 4%/ basil 4%), dates 8%), T_3(pineapple juice-80%, guava leaf extract-6%, mint 6%/ basil 6%), dates 8%), T_4(pineapple juice-76%, guava leaf extract-8%, mint 8%/ basil 8%), dates 8%), T_5(pineapple juice-72%, guava leaf extract-10%, mint 10 %/basil 10 %), dates 8 %). The prepared beverages were organoleptically evaluated for the colour, consistency, taste and flavour and overall acceptability using Nine Point Hedonic Scale and the best treatment was selected. Pineapple based beverages incorporated with guava leaf extract, mint extract, dates was the best among the one beverages.*

The nutritional compositions of the beverages were evaluated through chemical analysis. The total carbohydrate content ranged from 22-24.25g/100 ml, energy content ranged from 98.45–104.7Kcal/100ml, vitamin C content ranged from 29.89 – 31 mg/100ml, total polyphenol content ranged from 131.47 – 225.48mg/100ml, the highest being in beverage flavoured with mint , total flavonoids content ranged between 147 – 293.58 mg/100ml, the highest being in beverage flavoured . It was concluded from the study that the beverages formulated using guava leaves extract, herbal extract, dates improves the total Polyphenol and total flavonoid content of the prepared beverages in addition to the fact that guava leaf , mint has several other therapeutic benefits.

Key words: Pineapple Juice, Guava Leaves Extract, Herbs Extract, Dates, Acceptability, Nine Point Hedonic Scale.

1. INTRODUCTION

One of the best ways to keep our immune system strong is to include fruits and vegetables which are rich in nutrients called antioxidants that are good for our immune system. Our bodies are battlegrounds against infection and diseases. Normal body functions such as breathing or physical activity and other lifestyle habits such as smoking produce substances called free radicals that attack healthy cells. When these healthy

cells are weakened, they are more susceptible to cardiovascular disease and certain types of cancers. Antioxidants, such as vitamins C and E and carotenoids, which include β-carotene, lycopene and lutein, help protect healthy cells from damage caused by free radicals which are tremendously found in fruits and vegetables. In perspective to Indian diet recommended 400g/day of fruits and vegetables are needed to meet the requirements of antioxidants such as β Carotene. Vitamin-C, polyphenol and flavonoids **(Brahmam 2010).**

Beverages that contain sugars and other carbohydrates, proteins and/or fats also provide calories that, like the calories found in foods, contribute to an individual's total daily energy in take. As a result, it's important that beverage calories, like food calories, are managed as part of an individual's overall energy balance strategy to maintain a healthy weight. Fruit juices have been scientifically proven to give certain health benefits, provided that they are taken in moderation. Nutritionists also suggest that fruit juices should be taken in their pure state and people should only drink 100% fruit juice.

The antioxidant mechanism of guava leaf extracts may be contributed to their radicals scavenging ability. Phenolic compounds appears to be responsible for the antioxidant activity of guava extracts (**Chen** and **Yen, 2006**)

Mint leave which is one of the oldest and most popular herb and is grown around the world. There are many different varieties of mint, each having its own subtle flavour and aroma. Mint has high polyphenol content with many health benefits (She *et al.,* 2010). In hypertensive individual, sweet basil helps in reducing blood pressure. In diabetics, basil reduced both fasting and post-prandial blood glucose. And as is usual with the herbs, basil displays some protective attributes against fatty acid oxidation.

Basil display some protective attributes against fatty acid oxidation through its omega 3 fatty acid content and also possess vitamin c content. Basil serve not only as a source antioxidant but also as inflammatory, antifungal, antibacterial and adaptogenic.

Dates have always been an important food source in Middle Eastern communities, and they play a significant role in the Muslim tradition of Ramadan. Ramadan lasts for approximately one month every year and includes daily fasting for adults from dawn to sunset. Each evening, a fast-breaking meal called an if tar is enjoyed, and dates in many forms are available **(Moskin, 2015).**

2. MATERIAL AND METHODS

This present investigation "**Preparation of antioxidant rich healthy beverage by using pine apple juice and guava leave extract flavoured with herbs (mint and basil) "** was conducted in the Nutrition Research Laboratory of Food Nutrition and Public Health Department. Ethelind College of Home Science, Sam Higginbottom University of Agriculture Technology & Sciences, Allahabad, U.P.

The basic recipe served as control (T0). Five value added treatments i. e incorporated with guava leave extract at 2%, 4%, 6%, 8% and 10% were referred to as T1, T2, T3, T4 and T5. mint in the ratio of 2%,4%,6%,8% and 10% were referred to as five treatments of the first beverage respectively and dates was added in all control and treatments. Control and treatments for each preparation were replication 5 times respectively.

Nutrient calculation: Nutritive value of the prepared herbal beverage is to be calculated using the value of raw ingredients used for preparation of antioxidant rich healthy beverage as given by **Gopalan *et.al.* (2014).**

2.1 Chemical analysis of the developed antioxidant rich herbal beverage:

2.1.1 Determination of total carbohydrate:-

Procedure:-

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1. 100 mg of the sample was weighed into a boiling tube.
2. It was hydrolysed by keeping it in a boiling water bath for three hours with 5 ml of 2.5 N HCl and cool to room temperature.
3. It was further neutralized with solid sodium carbonate until the effervescence ceases.
4. The volume was made to 100 ml and centrifuged.
5. The supernatant was collected and 0.5 was taken and 1ml aliquots for analysis.
6. The standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard. '0' serves as blank.
7. The volume was made to 1 ml in all the tubes including the sample tubes by adding distilled water.
8. 4 ml of anthrone reagent was added.
9. It was further heated for eight minutes in a boiling water bath.
10. It was cooled rapidly and reading was taken from green to dark green color at 630 nm.
11. Standard graph was drawn by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis.
12. From the graph, the amount of carbohydrate present in the sample tube was calculated.

2.2 Determination of ascorbic acid (vitamin c) in fruits-

2.2.1 Procedure:

1. Juice sample was taken and filtered through cheese cloth.
2. 10 ml of the juice was measured and pipette in to a 100ml volumetric flack and diluted to the mark with 1 % oxalic acid solution.
3. It was mixed thoroughly. The dilute sample solution was filtered through dry filter paper.
4. The first few ml of the filtrate was discarded.
5. 10 ml or 20 ml aliquot of the filtrate was pipette into a small Erlenmeyer flask and titrated immediately with the standardized dye indicator solution to a faint pink colour end point that persist for 15 seconds.
   Standardization of dye:

2.2.2Determination of Calcium

Procedure

- Took 10 ml of ash extract in a clean beaker, add 100 ml distilled water. Added two drops of Methyl orange Indicator and stirred with glass rod.
- Added drop-wise Ammonium Hydroxide solution till solution turns yellow.
- Now added drop-wise dilute hydrochloric acid solution till solution turned pink colour.
- Heated the content to boiled and added 10 ml of Ammonium Oxalate solution, boiled again.
- Added drop-wise, very diluted (10 times diluted) Ammonium Hydroxide with constant stirring till colour of the content changed to yellow.
- Covered the beaker with watch glass and kept over a very low flame (do not allow it to boil) for about 30 minutes, undisturbed.
- Filtered the supernatant through what man No. 1 filter paper. Washed the precipitate left several times with hot distilled water till fresh filtrate is free from oxalate ions. (To test oxalate ions collect about 5 ml of fresh filtrate in a clean test tube, add 2-3 drops of dilute H_2SO_4 Warm, add 1 drop of $KMnO_4$ solution. Pink colour, indicate free from oxalate ion).
- Took the filter paper contained precipitate out from funnel and opened in same beaker. Allowed it to adhere on side of beaker. Added distilled water to run down the precipitate in solution.
- Collected about 100-150 ml water in the beaker. Add 10-20 ml dilute H_2SO_4 solution. Heated the content to about 60-80 ˚ C (boiling is not allowed.)
- Titrated while hot with standard potassium permanganate solution very carefully till pink colour persists beyond 1 minute.
- Pushed down the adhering filter paper on side of beaker with glass rod. Stirred gently and completed the titration to faint pink colour end point. Recorded the burette reading.

2.2.3 Determination of Iron:-

Procedure:-

1) Added about 30ml of dilute HCl in a porcelain dish containing ash. Dissolve the residue with glass rod. Heat over boiling water for 5mins. Dilute it with 30ml of distilled water. Filter and collect the filtrate in a beaker. Dilute the filtrate to 100ml.
2) Marked three test tubes as A, B and S for ash, blank and standard solution respectively. Measure about 10ml of distilled water, working iron and ash solution in the respective test tubes.
3) Added 1ml of saturated potassium persulphate and 2ml of potassium thiocyanate. Shake thoroughly by inversions and read in the colorimeter at 560 nm (green filter) within next 20minutes.

3. DETERMINATION OF ANTIOXIDANT

3.1 DETRMINATION OF TOTAL POLYPHENOL CONTENT

Procedure:

Standard solution – 0.110 g of Gallic acid monohydrate (M=188.14) was weight in to 100 ml volumetric flask. It was dissolve in water make and mixed (stock standard). The volume as Gallic acid stock standard solution given in (table1) was transferred using pipette to 100 ml one mark volumetric flasks. It was dilute to the mark with water and mixed.

Sample preparation-

5ml of methanol was taken in test tube (duplicates) and heated in water bath set at 70˚C. And allowed at least 30 min for heating.

0.2 ml of sample (duplicates) was taken and dissolved in above test tubes. Heating was continued in water bath for 10 min.

Tubes were removed from the water bath and allowed it to cool to temperature.

The supernatant of the two test tubes was carefully merged in another test tube.Using a pipette, add 5 ml of dilute folin – ciocalteu phenol reagent into each test tube and mixed. Within 3 to 8 min after the addition of the dilute folin –ciocalteu phenol reagent, 4 ml sodium carbonate solution was pipette into each test tube and was mixed carefully (blue color appear).

Allowed to stand at room temperature for 60 min, nod the optical density was measured by spectrophotometer set at 765nm.

3.2 Determination of Total Flavanoid Content

Procedure: 1 ml of 2 % aluminium tricloride was mixed with the same volume of sample juice. Absorbance reading at 430 nm were taken after 10 minutes against a blank sample consisting of 1 ml of sample solution and 1 ml of distilled water without aluminium tricloride. The total flavonoid content was determined using a standard curve of quercentin at 0 -5 mg/ml. The average of three reading was used then expressed as milligram of quercertin equivalents / 100 ml of juice sample.

Statistical analysis:-

7.1 After tabulating the data obtained from the sensory evaluation, it was statistically analysed by using two way Analysis of variance techniques. Significant difference between the treatments was determined by using CD (critical difference) test. **(Gupta *et. al.*, 2005)**

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4. RESULT AND DISCUSSION

The carbohydrate and energy value decreases in treatment T_1, T_2, T_3, T_4.and T_5 gradually as compared to treatment T_0 (control), as the value of incorporation of guava leaf extract increases carbohydrate and energy value decreases. The iron and calcium content of T_5 is the highest that is, 45.78mg and 23.88mg respectively followed by T_4 T_3, T_2 and T_1. This has been noted to be the lowest for T_0 being 28mg and 2.8mg respectively. The result related the value of total polyphenol content , total flavonoid content with incorporation of guava leave extract at levels in the respective treatment T_1 (2ml), T_2 (4ml) ,T_3(6ml), T_4(8 ml) and T_5(10 ml) was greater than control T_0, in which guava leaf extract was not incorporated. At 10 ml / 100ml total polyphenol, total flavonoid contents were highest 293.58mg, 177.06 mg per 100ml in T_5 followed by T_4, T_3, T_2, and T_1respectively and the least was observed in T_0 (control) where no guava leaves extract was incorporation in the control sample. With the decreased in percentage of guava leaves extract incorporation, the antioxidant value further decrease.

Table No. 2.1 Nutrient content in control and treated sample of pine apple fruit beverage with Guava leaves extract, Mint and Dates.

Treatment / Nutrients	T0	T1	T2	T3	T4	T5
Carbohydrates(g)	24.25	23.64	23.28	23.03	22.54	22
Energy (kcal)	104.07	103.05	102.15	101.25	100.3	98.45
Ascorbic acid (mg)	31	30.71	30.45	30.16	30.4	29.89
Calcium (mg)	28	31.55	35.11	38.66	42.16	45.78
Iron (mg)	2.8	7.01	11.23	15.44	19.65	23.88

Table No.2.2 Antioxidants content in control and treated sample of pine apple fruit beverage with Guava leaves extract, Mint and Dates.

Antioxidant	T0	T1	T2	T3	T4	T5
Total Polyphenol content (mg)	147	237.335	251.4	265.46	279.52	293.58
Total Flavonoid content (mg)	131.47	156.69	161.78	166.87	171.97	177.06

4.1 Organoleptic Characteristic Of Prepared Beverages

Table No.2.3 The average sensory scores of different parameters in control and treated sample of prepared pine apple fruit beverages with guava leaves extract, mint and dates.

Treatment	Colour	Consistency	Flavour and Taste	Overall Acceptability
T0	7.7	7.6	7.5	7.6
T1	7.2	7.5	7.24	7.2
T2	7	7	7.28	7.18
T3	8.5	8.7	8.5	8.4
T4	6.6	6.3	6.5	6.4
T5	6.04	5.6	5.6	5.7
F	Significant	Significant	Significant	Significant
C. D	3.42	0.59	0.75	0.62

The result illustrated in the average sensory scores of different parameters in treated sample with guava leaf extract. This indicates that among all the treatments, T_3 (8.5) has highest score of colour which increased the colour acceptability of the beverage. This was followed by T_1 (7.2) and T_2 (7) respectively. The calculated value F (11.24) is smaller than its tabulated value 2.71, degree of freedom at 5% level of probability. Therefore there is significant difference between treatments regarding the colour of the prepared beverages.

The consistency of the beverage in treatment T_3 (8.7) had the highest score for consistency followed by T_0 (7.6), T_1 (7.5), T_2 (7), T_4 (6.3) and T_5 (5.6) respectively making it clear that the addition of 10% guava leaf extract decreases the consistency acceptability of the beverage.The calculated value F (29.1) is higher than the tabulated value F (2.71), degree of freedom at 5% level of probability. Therefore there is significant difference between the treatments regarding the consistency of the prepared beverages.

The taste and flavour of the prepared beverage in treatment T_3 (8.5) has the maximum score according to sensory evaluation followed by T_0 (7.5), T_2 (7.28), T_1 (7.24), T_4 (6.5) and T_5 (5.6) respectively indicating that the addition of 10% guava leaf extract decreases the taste and flavour acceptability of the beverage.

The calculated value of F (13.58) is higher than the tabulated value F (2.71), degree of freedom at 5 % level of probability. Therefore there is significance difference between the treatments regarding the taste and flavour of the prepared beverage.

The mean sensory scores in relation to overall acceptability tabulated in table 4.1 indicated that treatment T_3 (8.4) has the highest score followed by T_0 (7.6), T_1 (7.2), T_2 (7.18) and T_4 (6.4), T_5 (5.7) respectively. Making it clear that the addition of 10% guava leaf extract decreases the overall acceptability of the beverage.

The calculated value F (18.16) was higher than the tabulated value F (2.71), degree of freedom at 5% level of probability. This shows that there is significant difference between the control and treatments regarding the overall acceptability of the beverages.

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