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QUALITY ASSESSMENT OF DIFFERENT BRANDS OF MANGO JUICE AVAILABLE IN INDIAN MARKET FOR CARBOHYDRATES AND ACIDS (ASCORBIC ACID) BY CONVENTIONAL TITRATION METHOD

V. Pithava¹ and A. Pandey^{*2}

Food Research Laboratory¹, Institute of Forensic Science², Gujarat Forensic Sciences University, Sector - 9, Gandhinagar - 382007, Gujarat, India.

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Correspondence to Author:

Dr. Astha Pandey

Assistant Professor,
Institute of Forensic Science,
Gujarat Forensic Sciences University,
Sector - 9, Gandhinagar - 382007,
Gujarat, India.

E-mail: astha.pandey@gfsu.edu.in

ABSTRACT: Fruits play an important role in human life because it provides valuable nutrients such as carbohydrates protein and fibres. Fruits are commercially processed to produce fruit juices, jams, jellies *etc.* Fruit juices sometime naturally contaminates with certain substances like carbohydrates, heavy metals *etc.* However, sometime manufacturer intentionally adds some substance like carbohydrates and acids to increase the life span of the products and vitamin C to increase product sale. Presence of carbohydrates and acid helps in the quality assessment of the fruit juices. Color tests mainly used for the identification of carbohydrates such as sugar, reducing sugar and invert sugar *etc.* Titration is a common laboratory method of quantitative/chemical analysis that can be used to determine the unknown concentration of a known reactant. In the present study, total six branded mango juice samples were collected from local market of Gandhinagar. Acid content, fruit content, reducing sugar, total reducing sugar and glucose: fructose ratio and vitamin C were analysed with titrimetric method and this were found in the range of 0.038 to 0.061 mg/ml, 9% to 17.25%, 2.6 to 50.5%, 6.764 to 125.4%, 0.997 to 1% and 0.214 to 1.546 mg/ml respectively.

INTRODUCTION: Fruits play an important role in human life because it provides a valuable nutrient such as carbohydrates protein and fibres. So that most of the people include fruit in their daily diet. Fruits are commercially processed to produce fruit juices, jams, jellies *etc.* Juices are available in their natural concentrations or in processed forms. Fruit juice is mainly produced by mechanically pressing fresh fruits or is extracted by water. Most of the commercially prepared juices are fat-free, nutrient-dense beverages rich in vitamins, minerals and naturally occurring phytonutrients¹.

The quality of fruit juices strictly maintained in developing country like India under the Prevention of Food Adulteration Act, 1954. Due to the large population size and demand of consumer products, fruit juices sometime naturally contaminated with certain substances like carbohydrates, heavy metals *etc.* However, sometime manufacturer adds some substance like carbohydrates to increase the life span of the products.

However, carbohydrates and vitamin C are naturally present in fruits. Carbohydrates are polyhydroxy aldehydes or ketones, or substances that yield such compounds on hydrolysis². Carbohydrates are the main source of energy that is ingested by human body³. This mainly includes the reducing sugar and invert sugar. The sugars added to processed foods such as soft drinks, fruit juice, cereals, biscuits, cakes, pastries mainly for preservation purpose.

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A high sugar diet increases the risk of tooth decay and weight gain, and high consumption of sugar-sweetened drinks is associated with type 2 diabetes⁴. Invert sugar is a mixture of equal parts glucose and fructose. It manufactured artificially as a sweetener for use in the baking, drink, canning, confectionary and dairy industries. Fructose is sweeter than pure sucrose, so invert sugar is in great demand because they are sweeter and because that manufacturer uses less of it to sweeten their products⁵. Determination of fruit content is another parameter, which also helps in the quality assessment. It measures in terms of sugar / acid ratio, which contributes towards giving many fruits their distinctive taste, and so is an indicator of commercial and organoleptic ripeness. At the commencement of the ripening process, the sugar/acid ratio is small, because of low sugar content and high fruit acid content, this makes the fruit taste sour.

During the ripening process the fruit acids are ruined, the sugar content increases and the sugar/acid ratio achieves a higher value. Overripe fruits have very low levels of fruit acid and therefore lack characteristic taste. An added sugar shows variation in this sugar / acid ratio. Titration is a chemical process used in determining the amount of constituent substance in a sample, *e.g.* acids, by using a standard counter-active reagent, *e.g.* an alkali (NaOH). Once the acid level in a sample has been determined, it can be used to find the ratio of sugar to acid⁶. Vitamin C, also known as ascorbic acid, is a valuable food constituent because of its antioxidant and therapeutic assets. It benefits the body in forming connective tissues, bones, teeth, blood vessels and plays a major role as an antioxidant that forms part of the body defense system against reactive oxygen species and free radicals, thereby stopping tissue destruction⁷.

Sometime manufacturer added this vitamin C in the fruit juices mainly to attract consumer towards their product and ultimately to increase their product sales. Such additives may show certain adverse effect on consumer health. It may cause Osmotic diarrhea, nausea, abdominal cramps, Oxalate kidney stones, decreases uric acid and re-absorption resulting in increased risk of gout, Affects diagnostic tests in feces⁸. Presence of carbohydrates and acid helps in the quality

assessment of the fruit juices. Color tests mainly used for the identification of carbohydrates such as sugar, reducing sugar and invert sugar *etc.* Titration is a common laboratory method of quantitative/chemical analysis that can be used to determine the unknown concentration of a known reactant⁹. In present study, titrimetric methods are used to determine the carbohydrates mainly invert sugar and acid content mainly the vitamin C.

MATERIALS AND METHODS:

Sampling: In the present work six different brands of packaged mango fruit juices were selected for analysis and further labelled as sample no. 1 to sample no. 2.

Material: The Laboratory grade solution of NaOH solution, formaldehyde solution, iodine solution, phenolphthalein indicator, starch indicator, Fehling A, Fehling B, methylene blue indicator, conc. HCl, Na₂CO₃ solution NaHCO₃, H₂SO₄, zinc acetate, acetic acid in a 100 ml, potassium ferrocyanide and thiosulphate solution were selected for the analysis.

Method: Selected fruit juice samples were analysed and their acid content, fruit content, vitamin C were determined using following method.

Determination of Acid Content by Titration

Method: The 10 ml of each fruit juice sample was first diluted with 50 ml of distilled water and further titrated with 0.1M NaOH standard solution and Phenolphthalein used as indicator. Colour changes in the titration were noted for the readings in ml. Total acidity was determined by using following formula¹⁰.

Calculation:

Citric acid MW = 192.43

Average reading = Total Readings / No. of readings

Total acidity = $0.1 \times \text{volume of NaOH} \times 10^{-3} \times 192.43 / 3$

Determination of Fruit Content: 10 ml of each fruit juice sample was first diluted with 0.25N NaOH solution at pH 8.1 on the pH meter. Further 10 ml of formaldehyde solution added. After 1 min solution was titrated potentiometrically at pH 8.1 with 0.25N sodium hydroxide.

If more than 20 ml of 0.25N sodium hydroxide required, the titration to be repeated using 15 ml of formaldehyde solution instead of 10 ml. If sulphur dioxide present in the samples, the sample treated with a few drops of 30% hydrogen peroxide before neutralization.

Calculation:

$$\% \text{ of Fruit Content} = 1.05 F / 1.4$$

Where F= Formol Number ¹¹.

Estimation of Sugars by Titration Method:

Determination of Reducing Sugars: 10 ml of sample diluted with distilled water upto the 100 ml. Burette filled with this solution. In conical flask, 5ml of Fehling A and 5 ml of Fehling solution B were taken and further 10 ml of distilled water added. Solution was heated and further titrated with this burette solution until the red precipitate observed with the help of methylene blue indicator. End point noted for each sample.

$$\text{Reducing sugar \% (As inert sugar)} = \frac{\text{Dilution} \times \text{Factor of Fehling (gm)}}{\text{Weight of sample} \times \text{Titre}} \times 100$$

Determination of Total Reducing Sugars: 5 ml of conc. HCl was added to the 50 ml of each fruit juice sample. Heated for about 10 min 5 ml of conc. NaOH solution was added to neutralize the solution. Further diluted with 0.1N NaOH solution. In another conical flask 5 ml of Fehling A solution and 5 ml of Fehling B solution were taken and head for 1-3 min further this solution titrated against sample solution till the yellow red ppt with methylene blue used as an indicator. End point for each sample was noted down. Total reducing sugar % were Calculated given as follows.

$$\text{Reducing sugar \% (As inert sugar)} = \frac{\text{Dilution} \times \text{Factor of Fehling (gm)}}{\text{Weight of sample} \times \text{Titre}} \times 100$$

Determination of Factor (for Invert Sugar) of Fehling Solution: Accurately weighed around 4.75 g of Analar grade sucrose. Transferred it to 500 ml volume flask and diluted with 50 ml distilled water. 5 ml conc. HCl added and allowed to stand for 24 h. Neutralized with NaOH solution and made up to volume. Mixed well and transferred 50 ml to a 100 ml volumetric flask and made up to volume. Transferred to a burette having an offset tip. Perform the titration of Fehling solution following the similar procedure as above:

$$\text{Fehling factor (As inert sugar)} = \frac{\text{Titre} \times \text{Weight of sucrose in gm}}{500}$$

Determination of Sucrose: Sucrose content was calculated with following formula ^{12, 13}:

$$\text{Sucrose \%} = (\text{Total reducing sugars} / \text{Invert sugar \% - reducing sugars \%}) \times 0.95.$$

Determination of Glucose: Fructose Ratio: 2 gm of fruit juice sample diluted with distilled water up to the 220 ml in a volumetric flask and transferred an aliquot of 25 ml to a 250 ml of iodine flask. Pipette out 50 ml of 0.1N iodine into ml of 0.2N Na₂CO₃ and 50 ml of 0.2N NaHCO₃ added. Allowed to stand in dark for 2 hours, acidified with 12 ml 25% H₂SO₄, and further titrated it with a standard sodium thiosulphate using starch indicator. Blank estimation was carried out simultaneously. Following formula was used to determine the glucose: fructose ratio:

Calculations:

$$\text{Glucose\%} = \text{Normality of thiosulphate} \times \text{Dilution} \times (\text{B-S}) \times 0.009005 \times 100 / 0.1\text{N} \times \text{Weight of sample}$$

$$\text{Fructose} = \text{Reducing sugars\%} - \text{Glucose\%}$$

$$\text{Fructose: Glucose ratio} = \text{Fructose\%} / \text{Glucose\%}$$

Estimation of Vitamin C in Fruit Juice by Titration Method: 5 ml of each fruit samples was titrated with standard iodine solution in which starch indicator was used to determine the end point. The procedure was repeated for 3 times for each sample.

Calculations: Vitamin C was estimated by using following formula:

1. Calculate the ml of titrant used for each flask. Take the measurements you obtained and average them.

$$\text{Average volume} = \text{total volume} / \text{number of trials}$$

$$\text{Estimation of vitamin C} = 10.00 \text{ ml iodine solution} / 0.250 \text{ g vit C} = 6.00 \text{ ml iodine solution} / \text{X ml vitamin C}$$

$$40.00 \text{ X} = 6.00$$

$$\text{X} = 0.15 \text{ g vitamin C}$$

RESULTS AND DISCUSSION: Mango juice sample collected were analysed with titrimetric method and different contents like acid, fruit content, carbohydrates were successfully determined.

Determination of Acid Content by Titration

Method: All the calculated values for acid content determination by titrimetric method were observed in **Table 1**. According to that the acid content in a juice samples were found to be in range of 0.038 to 0.061 mg/ml. Different amounts of acid were found in all the samples with small variations in their values. Same amount of acid *i.e.* 0.049 mg/ml was observed in the sample 2 and sample 3. Highest concentration *i.e.* 0.061 mg/ml of was observed in the sample 4, whereas lowest concentration of acid content *i.e.* 0.038 mg/ml was observed in the sample 5. The sour taste of many fruit juices mainly due to the presence of acids. Citric acid considered as a one of the important acid presents in the juices. Titrimetric analysis considered as one of the excellent method for the acid content determination.

TABLE 1: DETERMINATION OF ACID CONTENT BY TITRATION METHOD

Sample no.	Burette readings in ml			Acid content per 100 ml (mg/ml)
	Initial	Middle	Final	
1	0.8	0.9	0.9	0.055 mg
2	0.7	0.8	0.8	0.049 mg
3	0.7	0.7	0.8	0.049 mg
4	1	1	0.9	0.061 mg
5	0.6	0.6	0.6	0.038 mg
6	1	0.9	1	0.055 mg

Determination of Fruit Content: All the calculated values for fruit content determination by titrimetric method were observed in **Table 2**. According to that, the fruit content in a juice samples were found to be in range of 9% to 17.25%. Highest amount of the fruit content *i.e.* 17.25% was found in the sample 3, whereas lowest amount of fruit content *i.e.* 8.25% was found in sample 2.

TABLE 2: DETERMINATION OF FRUIT CONTENT

Samples	Burette reading in ml	% Fruit content
Sample 1	1.3	9.75
Sample 2	1.1	8.25
Sample 3	2.3	17.25
Sample 4	2.2	16.5
Sample 5	1.2	9
Sample 6	1.3	9.75

As fruits ripen, the formol number of the juice tends to decrease, as a rule; conversely, on storage of the juice a slight increase may be noticed. Various factors can lead to a lowering of the formol number of a fruit juice *e.g.* treatment with ion exchangers or addition of ascorbic acid. In the literature, the formol number found defined as ml N alkali for each 100 ml sample, which corresponds to values 10 times smaller than those given by the preceding method of calculation¹⁴.

Estimation of Sugars by Titration Method:

Determination of Reducing Sugar: All the calculated values for reducing sugar determination by titrimetric method were observed in **Table 3**. According to that, the reducing sugar in a juice samples were found to be in the range of 2.6 to 50.5%. Highest percentage reducing sugar *i.e.* 50.5% was found in the sample 2, whereas lowest percentage reducing sugar *i.e.* 2.6% was found in sample 5. Highest amount of reducing sugar indicate the possible presence of sugar additives in the fruit juice samples. Tasnim *et al.*, (2010)¹ conducted study on determination of reducing sugar in fruit juices. They had found that highest quantity of reducing sugar (9.99%) recorded in mango juices while the lowest in orange juices *i.e.* 2.24%.

TABLE 3: DETERMINATION OF REDUCING SUGAR

Sample	Burette readings in ml			% Reducing sugars
	Initial	Middle	Final	
Sample 1	25	25	25	28
Sample 2	14	9.1	18.5	50.5
Sample 3	21	21	21	3.3
Sample 4	11.5	8.9	14	6.1
Sample 5	27.3	25.6	25	2.6
Sample 6	16.5	20.6	16.7	3.9

Determination of Total Reducing Sugar: All the calculated values for total reducing sugar determination by titrimetric method were observed in **Table 4**.

TABLE 4: DETERMINATION OF TOTAL REDUCING SUGAR

Sample	Burette reading	% Total reducing sugars
Sample 1	2.2	6.764
Sample 2	0.9	24.22
Sample 3	0.9	28.9
Sample 4	1.1	23
Sample 5	1.3	19.95
Sample 6	2	125.4

According to that, the total reducing sugar in a juice samples were found to be in the range of 6.764 to 125.4%. Highest percentage reducing sugar *i.e.* 125.4% was found in the sample 6, whereas lowest percentage reducing sugar *i.e.* 6.764% was found in sample 1.

Determination of Glucose: Fructose Ratio: All the calculated values for glucose: fructose ratio determination by were observed in **Table 5**. According to that, the glucose: fructose ratio in a juice samples were found to be in the range of 0.997 to 1%. Small variations in the calculated readings was observed. Highest percentage glucose: fructose ratio *i.e.* 1% was found in the sample 5, whereas lowest percentage reducing sugar *i.e.* 0.997% was found in sample 2. In sample 1 and 3 same percentage of glucose: fructose ratio *i.e.* 0.998 was observed. In sample 2 and 6 same percentage of glucose: fructose ratio *i.e.* 0.999 was observed.

TABLE 5: DETERMINATION OF GLUCOSE: FRUCTOSE RATIO

Sample	Burette reading	Glucose/fructose ratio (%)
Sample 1	23.9	0.998%
Sample 2	23.6	0.997%
Sample 3	23.7	0.998%
Sample 4	23	0.999%
Sample 5	25	1%
Sample 6	24	0.999%

Estimation of Vitamin C in Fruit Juice by Titration Method: All the calculated values for vitamin C determination by titration method were observed in **Table 6**. According to that, the vitamin C in a juice samples were found to be in the range of 0.214 to 1.546 mg/ml. Small variations in the calculated readings was observed. Highest amount of vitamin C *i.e.* 1.546 mg/ml was found in the sample 3, whereas lowest amount of vitamin C *i.e.* 0.214 mg/ml was found in sample 5. In sample 1 and 2, same amount of vitamin C *i.e.* 0.25 was observed. The vitamin C found to be important for the health however if taken in excess quantity it possess adverse health effect to human life. Govindappa *et al.*, (2013)¹⁴ conducted similar study, on fruits and vegetables in which they had determined the vitamin C content by titration method and which was found lemon consisted of highest amount of vitamin C *i.e.* 40.48 ± 0.098 mg per 100 grams of sample.

TABLE 6: ESTIMATION OF VITAMIN C IN FRUIT JUICE BY TITRATION METHOD

Sample	Readings in ml			Vitamin C content per 100 ml (mg/ml)
	Initial	Middle	Final	
Sample 1	0.2	0.3	0.2	0.25
Sample 2	0.1	0.1	0.3	0.25
Sample 3	1.4	1.4	1.5	1.546
Sample 4	0.2	0.4	0.4	0.356
Sample 5	0.2	0.2	0.2	0.214
Sample 6	0.3	0.3	0.3	0.322

CONCLUSION: Food plays an important role in everyone's life. As a consumer people expect that the food like milk, fruit juices, vegetables *etc.* should of good quality to protect consumer health. If quality of the dietary products is not appropriate as per the guideline it will create undesirable health risk to human life. In present study quality assessment of mango fruit juices successfully checked with titrimetric method and reducing sugar, total reducing sugar, fruit content and presence of vitamin V content were successfully determined. Titrimetric analysis proved itself as a simple method in determination of possible sugar adulterant present in the sample.

From the above study, it was concluded that titrimetric analysis could be used for identification of acids like citric acid and vitamin C. For future study, instrumental methods such as UV-Vis spectrophotometer, High Performance Liquid Chromatography, Ion Chromatography can be used for quantitative and qualitative analysis of fruit juices.

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