STUDIES ON FORMULATION AND STANDARDIZATION OF KIWI-GUAVA MINT LEMONADE

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Abstract: Fruit and Vegetable juices are valuable source of antioxidants because it contains a significant amount of bioactive compounds. The fruits like kiwi fruit (*Actinidia deliciosa)*, guava fruit (*Psidium* guajava) and lemon (*Citrus aurantifolia)* possesses wide range of compounds like flavonoids, phenolic acid, ascorbic acid, and pigments. The formulation and standardization of blended kiwi and guava pulp was conducted in order to develop innovative formulation of beverage and study the changes in beverage quality during storage. The extraction of pulp from kiwi and guava fruit was done. Based on sensory evaluation by a panel of trained judges, the optimum quantity of kiwi pulp, guava pulp, lemon juice, mint extract, sugar, citric acid, preservative and water were reported to be 37.5 ml, 12.5 ml, 0.15 g, 7.4 g, 9.24 g, 0.15%, 0.075 % and 33 ml respectively for 100 ml beverage. The prepared health drink was packed in glass bottles with cork cap and stored at refrigerated temperature (4^0C) satisfactorily for the period of more than 90 days. Since the juice of these fruit is rich in vitamin C content, it is a healthy beverage having medicinal characteristics.

***Index Terms* – Kiwi fruit, Guava fruit, Formulation, Standardization, Sensory evaluation, Lemonade.**

I. INTRODUCTION

Lemonade is a sweetened beverage which is characterized by a lemon flavour. It is a drink made from lemon juice, citrus fruit and water sweetened with sugar. The citrus fruits like kiwi, orange, lime, guava, litchi, passion fruit, and other local fruits can be used for lemonade preparation (Tariq *et al.,* 2015). The advantage of lemonade beverage is that there is no need to dilute it whereas squash, syrup, cordial, crush are diluted with water before use. Ready-to-serve beverages are made out of juice, sugar and water and consumed as such. Fruit and Vegetable based beverages are relished when served chilled, particularly during summers. It has been reported that the organoleptic quality of RTS beverage prepared from juice could be increased by the addition of spice extracts of ginger, black pepper, mint, cardamom and cumin etc.(Sindumathi *et al.,* 2013).

Kiwi (*Actinidia deliciosa)* is tasty & juicy fruit, belongs to the family Actinidiaceae. It is originated in mountainous, forested regions of china (as Chinese gooseberry), where it is known as Macaque peach and Chinese gooseberry. It is a small fruit approximately 3 inches long. Kiwi fruit has become terribly popular during the past two decades due to its various medicinal properties. It has been used in some cultures as a traditional meat tenderizer, also contain lots of glucose and fructose and small amount of sucrose (Sachin, 2015). Kiwi pulp is an excellent source of vitamin C providing 61 % of the daily requirement of an individual (USDA nutritional Database, 2014). Fresh kiwi fruit is a very rich source of heart-healthy electrolyte "potassium." 100 g of kiwi contains 312 mg or 7 percent potassium. An increase in potassium intake along with a decrease in sodium intake is the most important dietary change that a person can make to reduce their risk of cardiovascular disease (Sachin, 2015). Some studies revealed that diseases like asthma, cough and diabetes have shown positive improvements with the daily consumption of kiwi fruit. Moreover, these fruits have the effect of increasing immune function. Consumption of kiwifruit enhances blood circulation also has preventive effect against certain cancers and cardiovascular disease. Different cancers, especially cancers of the digestive system (mainly stomach cancer), lung and liver have been treated with kiwi fruit prescriptions due to its cytotoxic and antioxidant activities (Guroo *et al*., 2017).

Guava (*Psidium* guajava**)** which belongs to the Myrtaceae family. It is originated in the tropical and subtropical areas. Guava is consumed fresh or made into processed products such as juice, nectar, puree, jam and jelly (Sirichote et al., 2007). It is a small tropical tree that grows up to 35 feet tall (Baby, 2011). Guava is a good source of energy, dietary fiber, vitamins, and minerals. The guava fruit contains vitamin C, A, E, B-vitamins, as well as potassium, phosphorus, magnesium, calcium, sodium, and zinc (USDA nutritional Database, 2014). Guava contains broad spectrum of phytochemicals including polysaccharides, vitamins, essential oils, tannins. It is a rich source of ascorbic acid than citrus (80 mg of vitamin C in 100 gm of fruit) and a good source of pectin – a dietary fiber (Baby, 2011). Guava contains four times higher vitamin C than that found in oranges, helps boost the immune system. The antioxidants in the fruit help defend the body against the proliferation of free radicals in the body, which are one of the main causes of serious conditions like cancer and heart diseases. Another tremendous benefit of guava is the presence of B vitamins, B3 and B6. Vitamin B3 can increase blood flow and stimulates cognitive function, whereas vitamin B_6 is a nutrient correlated with brain and nerve function (Meenakshi, 2019).

Peppermint or mint (*Mentha piperita* L.) belongs to the Lamiaceae family. It is originated in Europe and cultivated all over the world. It is a natural hybrid between spearmint (*Mentha spicata* L.) and water mint (*Mentha aquatic* L.). It is cultivated all over the world for its use in flavor, fragrance, medicinal, and pharmaceutical applications. It is a medicinal plant that has received more attention from both food and pharmaceutical industries because of its health benefits for human society (Loolaie *et al.* 2017). It is mainly used in folk remedies and traditional medicine for treatment of digestive disorders because of its antitumor and antimicrobial properties, its renal actions, antiallergenic effects, and digestive complaints, anorexia, nausea and diarrhea. Also herbs are used for flavoring foods, culinary preparation, perfumery, cosmetics, beauty and body care. The simple aroma of peppermint enhances memory and increases alertness in human subjects.

Lemon (*Citrus aurantifolia)* is an important medicinal plant of the family Rutaceae. It is acidic in nature and serves as rich source of dietary fiber, vitamin C, phenolic components and flavonoids. It was mainly used as an ornamental plant and for medicine. Lemons are a rich source of vitamin C, providing 64% of the daily value in a 100 g serving. Lemon juice contains slightly more citric acid than lime juice. It is one of the very low glycemic fruits. Which has both culinary and cleaning uses. Lemon juice is used to make lemonade, soft drinks, and cocktails. (Wikipedia, the encyclopedia). It is helpful for people suffering from heart problems because it contains potassium. Lemon juice controls high blood pressure, dizziness, and nausea as it provides a calming sensation to both, the mind and body. Daily lemon intake and walking were effective in reducing high blood pressure. Also it is rich in vitamin C which help in preventing various coronary heart disease (Wikipedia, the encyclopedia).

II. RESEARCH MATERIAL AND METHODOLOGY

2.1. Materials

The present research work was undertaken in the Department of Food Safety and Quality Nutrition in MIT College of Food Technology, during the year 2018-2019, entitled 'Studies on formulation and standardization of kiwi-guava mint lemonade beverage. The material used and methods adopted during the tenure of study are presented in this chapter.

2.1.1. Fruits

Kiwi fruit, guava fruit, lemon, mint leaves were purchased from local market of Hadpsar, Pune. The fruits were thoroughly washed prior to processing to remove dust and dirt adhered to fruits, to prevent contamination.

2.1.2. Chemicals and Packaging material

Sugar used in the experimentation was obtained from Local Market: Hadpsar, Pune. Other chemicals and equipment required for experimentation are acquired from MIT-ADT laboratory.
Glass bottles used as packaging material for blended juice, were obtained from Pune.

2.2. Methodology

2.2.1. Moisture Content

The moisture content was determined by the procedure given in Ranganna (1986) as given below:

Procedure:

The moisture content was determined using the hot air oven method. The sample was weighed (W1) approximately 10 g and kept in a petri plate and allow to dry at 110^0C in the hot air oven with periodically weighing until constant. The dried sample was kept in desiccators for cooling. The weight (W2) of cooled sample was obtained. The moisture content was calculated as follows:

$$\text{Moisture content (\% mc)} = \frac{W1 - W2}{W1} \times 100$$

Where,
W1 – Initial weight
W2 – Final Weight

2.2.2. Protein Content

The nitrogen was determined by Kjeldahl method and protein was then calculated by using the below given formula.

Procedure:

0.4 g of sample was weighed and transferred to Kjeldahl flask. Around 2.5 g of digestion mixture, and 10 ml of concentrated H2SO4 was added to it. The Kjeldahl flask was kept in the digestion assembly. The assembly was heated to 420º C and the sample was digested till all the fumes of SO2 were exhumed. The flask was cooled and transferred to the digestion assembly. 50 ml of 40% NaOH was added to it and distillation was started. The ammonia gas was liberated during the distillation process and was absorbed in the 25 ml of 3 % Boric acid solution taken in a conical flask. 3-4 drops of mixed indicator were added to it and it was titrated against 0.116 N HCl till pink color end point was obtained. The titre value was noted and % nitrogen in sample was calculated using following formula:

$$\text{Nitrogen (\%)} = \frac{(\text{Sample titre} - \text{Blank titre}) \times \text{Normality of HCl} \times 14}{\text{Weight of sample} \times 1000} \times 100$$

2.2.3. Fat Content

The fat was estimated by the procedure given in Ranganna (1986) as given below:

Procedure:

A clean, dry Soxhlet flask was weighed (W1). Take 3 g of dried sample (W2) was transferred to a thimble and the top of the thimble was plugged with a cotton plug. The thimble was dropped into a Soxhlet apparatus. Approximately, 75 ml of Petroleum ether was poured through the sample into the flask. One end of the fat extraction tube was attached to the flask and other to condenser. The sample was extracted for 16 hr. After the extraction of fat from the sample into the solvent, the solvent was

recovered. The solvent in the flask was evaporated in an oven at 100º C for 1 hr., further cooled and weighed (W3). The crude fat percent was calculated as follows:

$$\text{Fat (\%)} = \frac{W3 - W1}{W2} \times 100$$

Where,
W1 - Initial weight of empty flask
W2 - Sample weight
W3 - Final weight of flask + fat

2.2.4. Carbohydrates

The carbohydrates are estimated by anthrone method.

Procedure:

Take clean and dry test tubes and mark all the tubes as per the protocol. Pipette out 0.1-0.5 ml of glucose standard solution in duplicate test tubes. In one test tube take only 1 ml of distilled water and mark it as blank. Make up the volume to 1 ml in each test tube by adding distilled water. Then add 3 ml of anthrone reagent to each test tube and mix thoroughly. Heat the test tubes for 8 min in a boiling water bath. Cool rapidly and read the green to dark green color at 630 nm. Draw a standard graph by plotting concentration of the standard on the X-axis and absorbance on the Y-axis. From the graph calculate the amount of carbohydrate present in the sample tube.

$$\text{Carbohydrate content in 100 mg of sample} = \frac{\text{mg of glucose} \times 100}{\text{Volume of test sample}}$$

2.2.5. Ash Content

The total ash of the sample (raw material and final product) was determined using the procedure explained by Ranganna (1986).

Procedure:

The silica crucible (W1) was weighed. Then, 5 g of sample was weighed (W2) in it. The contents in the crucibles were charred on a Bunsen burner and then were kept in muffle furnace at 525- 550º C for 6 hr. The crucibles were cooled overnight and weighed (W3) again. Percent total ash was calculated as follows:

$$\text{Ash content (\%)} = \frac{W3 - W1}{W2}$$

Where,

W1 – Initial weight of crucible

W2 – Sample weight

W3 – Final weight of crucible

2.2.6. Determination of Energy value

Procedure:

Energy value = (Carbohydrate + Protein) x 4 + Fat x 9. Ranganna, S. (1986).

2.2.7. Fibre Content

Fibre was estimated using the protocol given by Ranganna (1986) using Fibroton apparatus.

Procedure:

2-3 g defatted sample was weighed (W) and transferred to the crucibles for fibre estimation. The crucible was placed in the hot extraction unit. For acid extraction, 150 ml 1.25 % H_2SO_4 was poured in the crucible. The acid wash was done at 400º C for 45 min and then, wash with distilled water. The acid wash was followed by alkali wash with 1.25 % NaOH and after washing with distilled water, the crucibles were dried in hot air oven at 100º C till free from moisture. Then, the weights of crucible were taken (W1) and the crucible were placed in muffle furnace at 400º C for 5-6 h. After cooling the crucibles, weight of crucible with ash was taken (W2).

$$\text{Fibre content (\%)} = \frac{W1 - W2}{W} \times 100$$

Where,
W – Sample weight

W1 – Initial weight of crucible before ashing

W2 – Final weight of crucible after ashing

2.2.8. Ascorbic acid content

The ascorbic acid content was determined by Assay method given by Ranganna (1986) as portrayed below:

Procedure:

Preparation of sample: 10 g/ml of sample was mixed with 100 ml of 3% HPO3. It was then filtered. An aliquot (10ml) of the sample was measured and titrated against standard dye till pink color was observed as end point, which persisted for 15 s. The Ascorbic Acid content of the sample was calculated by using the following formula:

$$\text{Ascorbic acid(mg/100 g)} = \frac{\text{Titre value} \times \text{Dye factor} \times \text{Volume made up}}{\text{Aliquot of sample taken for estimation} \times \text{Weight of sample taken for estimation}} \times 100$$

2.2.9. Sensory evaluation of kiwi-guava mint lemonade

Sensory evaluation of Kiwi-Guava Mint Lemonade for color, taste, flavor, texture, appearance, and overall acceptability were carried out using 9-point hedonic scale with semi-trained panelists. Sensory attributes were rated on a scale of 1 (dislike extremely) – 9 (like extremely) Amerine et. al., (2013).

2.2.10. Statistical analysis of data

The data obtained were analyzed for statistical significance according to the procedure given by Panse and Sukhatme (1967). However, due to spoilage of some samples before the completion of storage period, simple mean values have been reported.

2.3 Flow chart for preparation of kiwi guava mint lemonade

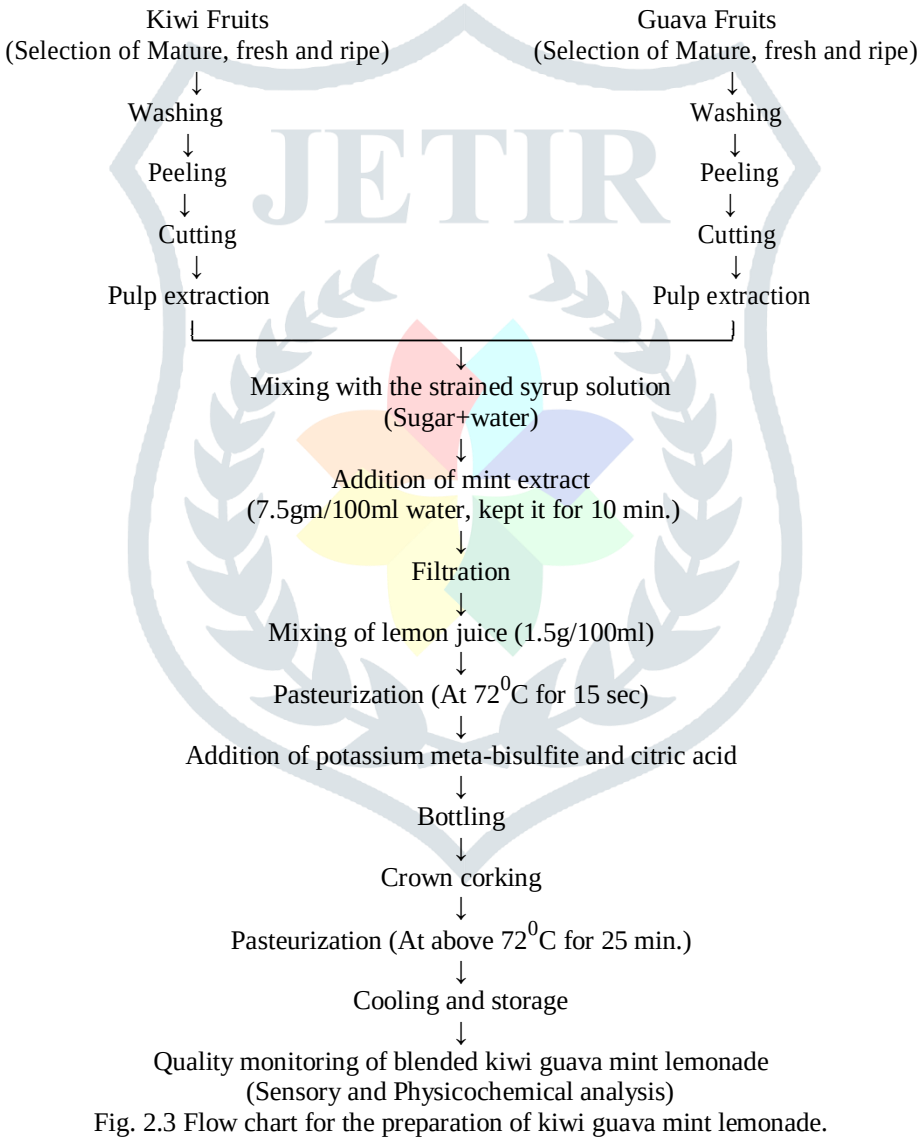

Fig. 2.3 Flow chart for the preparation of kiwi guava mint lemonade.

2.3.1. Procedure of kiwi guava mint lemonade preparation

2.3.2. Selection of raw material

Good quality of fruits were purchased from market and used in the preparation of Kiwi-Guava mint lemonade.

2.3.3. Washing

The entire ingredients were washed to remove dirt, dust and other contaminant on the surface.

2.3.4. Peeling
After washing of ingredients all the fruits were peeled out.

2.3.5. Cutting
After peeling the fruits are cut in small size required for the juice extraction by grinder.

2.3.6. Pulp extraction
Pulp extraction was done by using grinder. Fruit juice were extracted from all the fruits separately and stored. Mint juice extracted from mint leaves by mixing with sugar syrup.

2.3.7. Mixing of pulp extracts
Kiwi-Guava pulp was mixed with sugar syrup solution (upto 14 ^{0}Brix) which contains mint extract. Then add 1.5g/100ml lemon juice in it.

2.3.8. Filtration
Filter it through muslin cloth, to remove impurities.

2.3.9. Pasteurization
Lemonade is pasteurized at (At 72 ^{0}C for 15 sec) to remove the contaminants from raw pulp.

2.3.10. Addition of preservatives
After that potassium meta-bisulfite (0.6g/1liter) and citric acid (0.3gm/100ml) were added as preservative.

2.3.11. Filling and crown corking
The lemonade was filled in a glass bottles (capacity 200 ml) and crown cork it.

2.3.12. Pasteurization

Glass bottles were pasteurized above 72 ^{0}C for 25 minutes.

2.3.13. Storage
Samples were stored at refrigerator temperature (4 ^{0}C) and at ambient temperature (4°C).
The chemical and sensory evaluation of all the juice bottles within the storage period of 90 days was conducted and was recorded.

2.4. Treatment details
2.4.1. Formulations:
Different samples like sample A (control), sample B, sample C, and sample D were prepared by blending kiwi and guava pulp proportions with variations 100ml. The formulation is presented as below in table.

Table No. 2.4.1. Formulation for kiwi-guava mint lemonade

Ingredients	Sample A	Sample B	Sample C	Sample D
Kiwi pulp (ml)	50	37.5	37.5	25
Guava pulp, white guava (ml)	-	12.5	_	25
Guava pulp, pink guava (ml)	-	-	12.5	-
Sugar (g)	9.24	9.24	9.24	9.24
Mint extract (g)	7.4	7.4	7.4	7.4
Water (ml)	33	33	33	33
Lemon juice (g)	0.15	0.15	0.15	0.15
Citric acid (g)	0.15	0.15	0.15	0.15
Potassium Meta bisulphate (g)	0.075	0.075	0.075	0.075

III. RESULTS AND DISCUSSION

3.1. Formulation and Standardization of kiwi-guava mint lemonade
Several trials (Table 3.1) were conducted to select optimum quantity of kiwi and guava fruit pulp, lemon, mint extract, sugar, preservative and water for the blended beverage. Based on sensory evaluation by a panel of trained judges, the optimum quantity of kiwi fruit pulp, guava fruit pulp, sugar, mint extract, water, lemon juice and preservatives were reported to be 37.5 ml, 12.5 ml, 9.24 g, 7.4 g, 33 ml, 0.15 g, 0.15g and 0.075 g respectively for 100 ml beverage.

Table 3.1. Standardization table for kiwi-guava mint lemonade

Ingredient	Sample A	Sample B	Sample C	Sample D
Kiwi pulp (ml)	50	37.5	37.5	25
Guava pulp, white guava (ml)	-	12.5	_	25
Guava pulp, pink guava (ml)	-	-	12.5	-
Sugar (g)	9.24	9.24	9.24	9.24
Mint extract (g)	7.4	7.4	7.4	7.4
Water (ml)	33	33	33	33
Lemon juice (g)	0.15	0.15	0.15	0.15
Citric acid (g)	0.15	0.15	0.15	0.15
Potassium Meta bisulphate (g)	0.075	0.075	0.075	0.075

Based on the sensory evaluation by a panel of trained judges the sample B got highest score i.e. 8 which was having , the optimum level of kiwi fruit pulp, guava pulp, sugar, mint extract, water, lemon juice and preservatives were reported to be 37.5 ml, 12.5 ml, 9.24 g, 7.4 g, 33 ml, 0.15 g, 0.15g and 0.075 g respectively for 100 ml beverage (Table 3.2).

3.2. Sensory evaluation of kiwi-guava mint lemonade

Table 3.2. Sensory evaluation of kiwi-guava mint lemonade

Sample	Color and Appearance	Taste	Flavor	Mouth feel	Overall acceptability
Sample A	7.8	8.1	8.0	8.2	8.1
Sample B	7.7	8.0	7.7	7.8	8.0
Sample C	7.1	7.4	7.5	7.3	7.5
Sample D	7.0	7.2	7.4	7.2	7.5
Mean	7.40	7.68	7.65	7.63	7.78
SE (m)	2.0485	2.1277	2.1205	2.1722	2.1565
CD@ 5%	6.1667	6.4050	6.3833	6.5392	6.4917

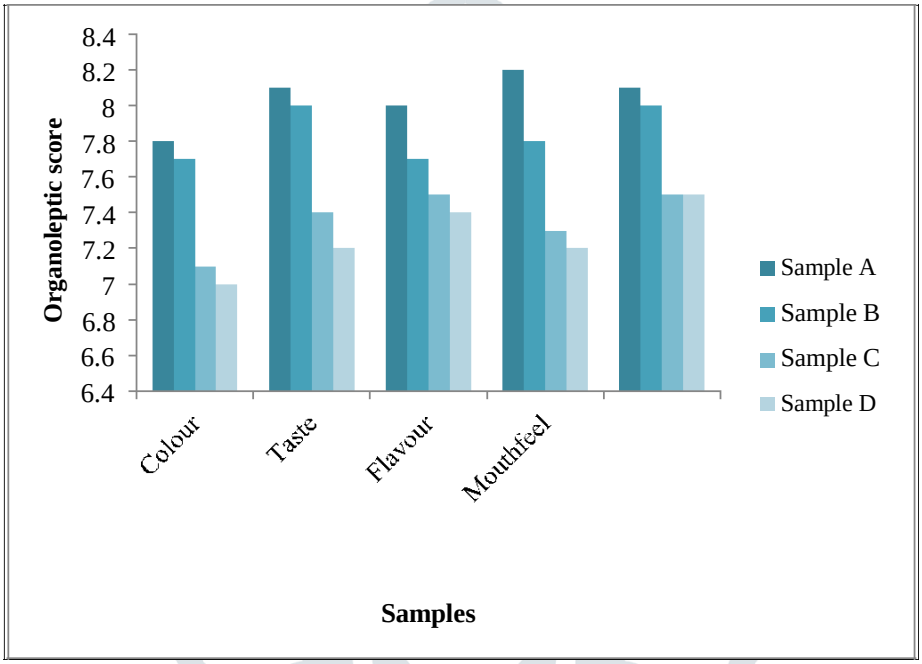

Figure 3.2. Sensory evaluation of kiwi-guava mint lemonade

3.3 Proximate analysis of beverage

The data of proximate analysis of kiwi-guava mint lemonade is presented in Table 3.3. Among the chemical composition of kiwi fruit and guava blended beverage, the values for protein content were 0.8 %, carbohydrates were 12.1%, fat were 0.3%, ash content were 0.22 %, moisture were 81.3 %, vitamin C were 26.5 mg/ml, where as energy were 67.8 Kcal.

Table 3.3 Proximate analysis of selected blended beverage

Sr. No.	Particulars	Value (%)
1	Protein (%)	0.8
2	Carbohydrates (%)	12.1
3	Fat (%)	0.3
4	Ash (%)	0.22
5	Moisture	81.3
6	Vitamin C (mg)	26.5
7	TSS (^{0}Brix)	14.0
8	Titrable acidity (%)	0.4
9	pH	4
10	Energy value (Kcal)	67.8

IV. SUMMERY AND CONCLUSION

This research work was conducted in order to make a new flavoured healthy lemonade by blending of kiwi and guava fruit pulp, along with lemon juice and mint water. To increase the shelf life chemical preservatives potassium meta-bisulfite and citric acid were used. From the results obtained after proximate analysis of prepared lemonade the amount of carbohydrate, protein, fat, and vitamin C was found to be 12.1%, 0.8%, 0.3% and 26.5 mg/100ml respectively. It is obvious from the findings of this research work that certainly it can improve the nutritional status of the population because it is rich source of vitamin C, and similar research work should be carried out with different citrus fruits individually as well as with combination.

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