PROCESSING AND PRESERVATION OF BITTER GOURD JUICE

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THESIS

BY

TOUHIDA AKTAR
Student No.: 1205052
Session: 2012-13
Semester: January – June, 2013

MASTER OF SCIENCE (MS)
IN
FOOD PROCESSING AND PRESERVATION

DEPARTMENT OF FOOD PROCESSING AND PRESERVATION

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY
UNIVERSITY, DINAJPUR

JUNE, 2013

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Submitted to the
Department of Food Processing and Preservation, Hajee Mohammad Danesh Science and Technology University, Dinajpur

In partial fulfillment of the requirement for the degree of

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Approved as to style and content by

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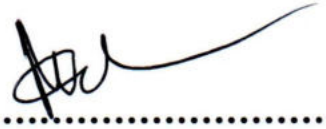
Supervisor **Co-supervisor**



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Chairman of the Board of Examination Committee

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

JUNE, 2013

DEDICATED
TO MY
BELOVED PARENTS

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JUNE, 2013 ***The Author***

ABSTRACT

The study was conducted to formulate bitter gourd juice from matured bitter gourd, to study microbial analysis and also to evaluate the sensory attributes of the prepared bitter gourd juice. Firm and Fresh bitter gourd was analyzed for its composition. The pulp was prepared from matured bitter gourd. Calculated amount of sugar, citric acid and water were mixed and heated for 60°C for 15 minutes and cooled down to 28°C temperature and 0.6 gm potassium metabisulphite (KMS) was added to it and mixed thoroughly. The finished products were kept into clean, dry and sterilized glass bottles and sealed properly. The sealed bottles were stored in cool and dry places. The samples were analyzed for proximate composition and sensory evaluation was done to detect the best sample and sample differences. The results from panel taste revealed that the color, flavor, texture and overall acceptability were the best in Sample S_2. The bests Sample S_2 contained 300 ml juice, sugar-90 gm, citric acid-3.5 gm, KMS-0.6 gm and water-605.9 ml for each 1000 ml of juice. The juice did not show any change in quality attributes during the storage period till 2 to 4 months. The keeping quality, shelf life and consumer's acceptability of the products were investigated. The juice was also analyzed for their TSS, acidity, pH and vitamin C. It was found that TSS and acidity increased slightly but vitamin C and pH were decreased gradually during the storage periods. Fading of colour and off flavour was found at the end of storage periods. This research reveals that bitter gourd can be converted to nutritious juice and this increase the preservation time as well as value of the product.

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CHAPTER I

INTRODUCTION

Bitter gourd *(Momordica charantia L.)* is an economically important medicinal plant belongs to the family Cucurbitaceae (Chakravarty, 1990) commonly known as karela or bitter melon. It is also known as "miracle fruit", is a plant original from Nepal .The immature fruits are eaten as vegetables which is highly nutritive and are a good source of fibres, carbohydrates, Vitamin C, Vitamin A, phosphorus and iron (Sultana and Bari, 2003; Paul *et al.,* 2009). But it is a fair source of protein, minerals, while poor source of sugar (Kalra *et al.*, 1988). Bitter gourd is one of the popular vegetables in Asia. Although the seeds, leaves, and vines of bitter melon all have uses, the fruit is the safest and most prevalent part of the plant used for food and medicine. Each 100g of edible portion of fruits contains 83.2g water, 2.1g protein, 1.0 fat, 1.4g minerals, 1.7g fiber, 10.6g carbohydrate, 23mg calcium, 38 mg phosphorus, 2.0 mg iron, 126 µm carotene, 0.07mg thiamine, 0.06mg riboflavin, 0.4mg niacin and 96mg vitamin C (Gopalan *et al.*, 1982). The bitter flavor is due to the alkaloid momordicine produced in fruit and leaves.

Bitter gourd has excellent medicinal virtues. The medicinal value of bitter gourd in the treatment of infectious diseases and diabetes is attracting the attention of scientists worldwide. Bitter gourd is anti-diabetic, stimulant, stomachic, laxative, blood purifier and control diabetes (Raman and Lau, 1996).

Bitter gourd is one of the most important vegetable grown in Bangladesh during summer season. Production of bitter gourd has been increased from 19 thousand tons in 1990-91 to 25 thousand tons in 2007-2008 (DAE, 2007), which indicates its significance as high-value crops. Therefore, development of dietetic and ready-to-serve soft drinks from bitter gourd will not only contribute as alternative of health hazards synthetic drinks but also will substantially to reduce post-harvest losses of this vegetable and to get fair price by the poor farmers. Keeping the above views in consideration attempts have been taken to make dietetic bitter gourd drinks for diabetes suitable for consumers who are fascinated to low calorie.

Health drinks are natural drinks that contain a balanced amount of nutrients and minimal amounts of sugar, fat and salt. High consumption of fruits and vegetables is associated

with decreased risk of coronary heart disease, prostate cancer and Alzheimer's disease (Joshipura *et al.*, 2001; Schuurman *et al.*, 1998; Dai *et al.*, 2006). Today busy families have less free time to prepare nutritious, home-cooked meals. Juices obtained by squeezing fruit without any pasteurization treatments represent an alternative way of consuming fresh fruit and vegetables. Furthermore, these products meet the needs of modern consumers, who increasingly buy ready- to- eat food to save time, without giving up the pleasure and nutritional intake linked to healthy diet (Endrizzi *et al.*, 2009).

The growing interest in new functional foods with special characteristics and health benefits has led to development of new functional beverages. The global market of functional beverage has been estimated to be at least 33 billion US$ (Hilliam, 2000). They provide means to reduce increasing burden on health care system by a continuous preventive mechanism (Shahidi, 2004). The functional beverages not only provide taste and refreshment satisfaction, but can also provide necessary nutrients to prevent nutrition-related diseases (Menrad *et al.*, 2000). Beverages are considered to be an excellent medium for the supplementation of nutraceutical components for enrichment (Kuhn, 1998) such as soluble fiber or herbal extract (Swientek, 1998).The new formulations of beverages are rapidly changing.

The market shelves are full of different beverages with not only soda pop, juices and dairy beverages but also huge number of food products taken as beverages such as iced teas, coffees, sports drinks, herbal teas, frozen carbonated beverages, mint blends, vegetable juices and smoothies (Giese, 1992). However, in current years consumers have not been choice for traditional drinks but also have more exotic beverages such as the, teas iced coffees, isotonic or sports drinks and non-carbonated beverages and ready-to-drink iced herbal teas are also gaining popularity (Swientek, 1998). Fruits and vegetable juices have become important in recent years due to overall increase in natural juice consumption as an alternative to the traditional caffeine beverages such as coffee, tea, or carbonated soft drinks. Consuming fruits and vegetables promotes health, energy and quality of life. Juices are obtained from a single fruit or from different kinds of fruits and vegetables (Tombak, 2000).

As bitter gourd is bitter in taste and not liked by masses. It needs to be processed and fortified to make it palatable and acceptable. Presently ready-to-serve beverages have

been increasingly gaining popularity among masses. Beverages are consumed by people of all the groups to quench the thirst and as health food.

Therefore, there is need to prepare ready to-serve (RTS) beverage from bitter gourd which has nutritional as well as pharmacological significance. However, information on optimization of various ingredients and quality evaluation with respect to functional and sensory properties during storage of RTS beverage of bitter gourd is scanty.

Based on the information as accumulated above a research was conducted to achive the following objectives:

1. To extract bitter gourd juices and proximate composition determination.
2. To find out suitable formulations for the bitter gourd based juice.
3. Microbiological study of the bitter gourd juices and to assess the overall acceptability of the formulations.

CHAPTER II

REVIEW OF LITERATURE

A conceptual framework for the study based on the ideas and concepts gathered from review work of existing literature of both theoretical and empirical nature will facilitate planning the study in a comprehensive manner. It also helps to know the previous research work carried out in the area and acts as a torch for new research.

The proper harvesting time of immature bitter gourd fruit for using as vegetables was 15 to 17 days after anthesis, when moisture content was 95-97%. The best bitter melons are harvested when the fruit is pale green. Fructose and glucose 20 among the sugars were detected, and the highest sugar contents were observed 5 to 9 days after anthesis (Chung-Hee Don *et al.*, 2000). No differences were ovserved between varieties for sugar content. The flesh contained 14.2-32.3% cellulose on a dry weight basis.

Nutrient compositions were determined in the fruit samples of 13 lines (varieties) of spring bitter gourd (*Momordica charantia)* and 11 cross combinations of autumn bitter gourd in Hubei, China. Results showed that cross combinations had water contents over 90%. The tested bitter gourd lines (varieties) had vitamin C contents which varied significantly, in the range 439.4-779.6 mg/kg (Xian C. *et al.,* 2000). Sixteen amino acids contents were higher than those of tomato but glutamic acid and tryptophan contents were lower. The bitter gourd lines had crude protein contents in the range 11.4-20.9 g/kg.

Bitter gourd has the highest nutritive value among cucurbits (Miniraj *et al.*, 1993; Desai and Musmade 1998). The vitamin C content of Chinese bitter gourd varies significantly (440-780 mg/kg edible portion). Considerable variation in nutrients, including carbohydrates, iron, zinc, calcium, magnesium, phosphorous, and ascorbic acid, has been observed in bitter gourd (Kale *et al.,* 1991; Yuwai *et al.*, 1991). Moreover, the crude protein content (11.420.9g/kg) of bitter gourd fruits is higher than that of tomato and cucumber (Xiang *et al.,* 2000).

The Bitter Melon fruit has considerable amount of potassium, calcium, magnesium, vitamin C, protein, and dietary fiber as compared with other commercial vegetables (Wills *et al.*, 1984). It also has niacin, thiamin, riboflavin, vitamin A, protein, organic

acids and other nutrients. Physical separation of bitter melons resulted in flesh and seed fractions. Samples of Bitter Melon flesh in general contained about 93% moisture over the four varieties; whereas, the moisture content of melon seeds varied from about 53% in Indian Green (IG) to 75% in Indian White (IW). Bitter melon flesh contained 8.4% to 9.8% protein; whereas, seed contained 27% to 31% protein.

All soluble compounds of fruits or vegetables usually play important roles in preparing juices. The distribution of these soluble compounds is not same in different parts like skin, flesh and seeds. Whole bitter gourd were divided carefully into its skin, flesh and seeds and expressed as percentage of its total weight. The results are presented flesh is the highest part of bitter gourd, followed by skin and the lowest part are seeds. On an average, 67.27% of bitter gourd is flesh. Usually, most of the soluble polysaccharides like pectin, hemicelluloses and cellulose are located in the flesh, whereas skins are the good source of plant color like chlorophylls in case of bitter gourd. For preparing dietetic soft drinks, it is necessary to know details about the sugar content and fiber content of different parts of the raw materials used bitter gourd (Aziz *et al.*, 2011).

Bitter gourd can be used as traditional medicine (TM) or complementary and alternative medicine for future drugs to counteract insulin resistance, consistent with a resurgence of interest in drug discovery from natural products (Koehn and Carter, 2005). Bitter melon extract can also be used as a broad-spectrum antibacterial agent to fight off infections caused by *E.coli, Salmonella, S.aureus, Staphylococcus, Pseudomonas, and Streptobaccilus* (Saeed and Tariq, 2005).

In a study where rats were fed a high fat diet, with or without a bitter melon juice supplement, the rats consuming the supplement gained less weight and tended to have less visceral fat than rats on the same diet without the bitter melon juice. Improved insulin resistance, lowered serum insulin and leptin but raised serum free fatty acid concentrations were also observed (Chen *et al.*, 2003).

Bitter melon is known in South-East Asia as pare. There are several dozen active substances in bitter gourd fruit. Compounds in this plant activate the enzyme AMPK, a protein that's regulates the body's metabolism and affects glucose uptake. One of the compounds increases fatty acid oxidation and glucose disposal in the body. Dr. Mon-Jia Tan of the Chinese Academy of Sciences in Shanghai isolated several compounds from

bitter gourd known as cucurbitane triterpenoids, and tested their effects on glucose (sugar) and fat metabolism in cells and in mice. When tested in muscle and fat cells the compounds stimulated the glucose receptor GLUT4 to move from the cell interior to the cell surface, thus promoting more effective glucose metabolism. Several of the tested compounds found that they promoted both glucose tolerance and fat burning, and one was particularly effective in promoting glucose tolerance in animals consuming high fat dites (Tanmon J. *et al.,* 2008)

Krawinkel & Keding (2006) cited 17 animal studies, the majority of which showed anti-diabetic properties, including blood glucose lowering effects of various forms of bitter gourd, such as juice or powdered (Karla *et al.,* 2000; Ahmed *et al.,* 2001;Chaturvedi 2005; Sathishsekar & Subramanian 2005).

The blood glucose lowering effects of juice were found to be better than those of dried fruit products (Basch *et al.,* 2003, cited in Krawinkel & Keding 2006), but fresh fruit extracts and acetone extracts of fresh fruit powder were also effective (Singh *et al.,* 1989). Australian consumers (largely Vietnamese, but also other Asians) appear to prefer dark green shiny fruit but this may be because that is what they are sold. Development prospects were rated as high for both fresh and processed bitter melon. There is also potential for fresh cut product. Pickled bitter melon is imported from India, Malaysia and the Philippines. As these are low cost producers, it is unlikely that Australia will be able to compete on this market (Vinning, 1995).

There are two basic types of soft drinks: the so-called ready-to-drink and concentrated soft drinks. Dietetic soft drink is one type of so-called ready-to-drink. It is used for diabetic patients. A ready to beverage could be prepared having pulp content 10%, refractometer solids 20%, and acidity 0.3% (Setty *et al., 1978*)

Fruit flavoured beverages could be classified into two broad categories, from the point of view of composition, those containing fruit juice with or without pulp and fruit cells, and other fruit flavoured ones and those flavoured with natural fruit oils (Jacob, 1959).

The term 'Soft drink' applies to beverages containing flavourings and fruit juices together with other constituents of technological or nutritional value designed to enhance

the appearance and stability of the product and to ensure its organoleptic properties remain inactive during a reasonable shelf period (Taylor, 1998).

Hulme (1971) stated that all beverages are inherently unstable. Microorganisms already present on the fruit or gaining access to the product during processing rapidly attack them; they are also subject to enzymatic and non-enzymatic changes. It is thus essential to destroy the microorganisms at an early stage or to prevent their development.

Bitter melon stored at 15°C in controlled atmospheres (21, 5 or 2.5% 0_2 in combination with 0, 2.5, 5 or 10% CO_2) were not different in quality from air-stored fruits at 2 weeks. Fruits stored 3 weeks in 2.5 or 5% CO_2 in combination with 2.5% 0_2 showed greater retention of green color and had less decay and splitting than air-stored fruit (Zong, 1994).

Optimal storage temperature for fruit is 7-10°C. Fruit can be stored at temperatures down to 4°C for short periods but prolonged exposure to low temperatures can cause chilling injury. Fruit stored above 10°C continue ripening. The optimum temperature for setting refrigeration may be lower, and is different for each set of storage conditions. Transport refrigeration and cool room settings need to be calibrated. A temperature setting for transportation is 5-7°C which has been quoted by suppliers. If air circulation is low, heat will build up in the cartons causing fruit temperature to be higher than air temperature (Gosbee and Lim, 2000).

Joslyn *et al.* (1961) investigated that the effects of length and temperature of storage and the relationship of oxygen, light, sugar, pH and ascorbic acid to deteriorative changes in colour of these factors. Storage temperature and oxygen content were the most specific for colour injury of both juices and isolated pigments. Exposure to light caused little deterioration in colour. Adjustment of acidity within the range of pH 2 to 4.5 or sugar addition had little effect on colour retention in fruit juices during storage.

Mitra (1997) studied on post-harvest physiology and storage of tropical and subtropical fruits. He showed in his food that tropical and subtropical fruits are becoming important food items in countries where they are produced and also in an increasing number of importing countries in non-tropical areas. His book deals with the post harvest storage, physiology and conservation of all of the economically important tropical and

subtropical fruits. It should be of particular interest to all horticulture researchers, exporters and importers within the industries concerned with tropical and subtropical fruits.

In order to extend the shelf-life of vegetables, several authors have recommended the storage of these products at 4-10°C (Wills *et al.,* 1999). Modified or controlled atmosphere storage is also a useful technique for extending shelf-life of vegetables, especially for those that deteriorate quickly (Sanchez-meta *et al.,* 2003)

Rangana & Bajaj (1966) reported that SO_2 is widely used throughout the world principally for treating food of plant origin. It is used in the preservation of fruit juices, pulps, beverages and concentrates; concentration used may vary from 350 to 2000 ppm. Soluble sulphite salts (e.g. KMS) are usually used in treating fruit products. The activity is higher at pH below 4.0 Rangana and Bijoy (1966) reported that preservatives are food additives used to prevent infection or inhibition of spoilage caused by bacteria, yeast, molds or other organisms.

Zurowietz (1996) reported that the relationship between subconscious sensory perceptions and product development in the beverage industry is discussed with particular reference to development of soft drinks and fruit juices. Aspects considered include evolutionary aspects of sensory perception; the physiology of olfaction; use of common sensory profiling for product development; advertising and physiological aspects of appeal to the senses; recommendations for providing taste/olfactory profiles for fruit juices and nectars; common profiling of other flavored products; and the concept of global profiling.

Bitter melon (*Momordica charantia L.*) has long been used in various Asian traditional medicine systems and is commonly consumed as a vegetable (Giron *et al.,* 1991; Lans and Brown, 1998). Anti-cancer activities of extracts of bitter melon against lymphoid leukemia, lymphoma, choriocarcinoma, melanoma, breast cancer, skin tumors, prostatic cancer, squamous carcinoma of the tongue and larynx, human bladder carcinomas and Hodgkin's disease have been reported (Basch *et al.*, 2003; Battelli *et al.*, 1996; Ganguly *et al.*, 2000; Licastro *et al.,* 1980; Ng *et al.*, 1994; Sun *et al.*, 2001).

The fruit is oblong and resembles as small cucumber, young fruit is emerald green that turn to orange-yellow when ripe (Grover and Yadaf, 2004). Consumers eat bitter melon when the fruit is particularly immature or unripe.

Several authors (Al-Khalifa, 1996; Kamel *et al.*, 1985; Badlfu, 1991) have reported studies about some melon seeds and compared the physicochemical characteristics of their oils with those from conventional sources. Melon (*Cucumismelo*) seeds, besides possessing medicinal qualities (Bellakhdar *et al.*, 1991), are also a rich source of protein (53.90%) and oil (37.67%) (Mariod *et al.*, 2008; Rashwan *et al.*, 1993).

As the name suggests, the fruit has an extremely bitter taste, which is due to the presence of a non-toxic alkaloid, momordicine. Before cooking the fruit is usually blanched or soaked in salt water to reduce bitterness (Krawinkel & Keding 2006). As with all plant foods, composition varies according to a number of variables, including variety. Measuring phenolic content in four Asian varieties, Horax *et al.* (2005) found variations of 27.7–53.37 mg chlorogenic acid equivalents (CAE)/g fresh weight.

Kandlakunta *et al.* (2008) measured moderate levels of carotenoids in bitter melon However, total carotenoids in 38 accessions of bitter gourd measured by Dey *et al.* (2005) ranged from 0.205 to 3.2 mg/100 g fresh weight (1.6 mg/100 g average).Various preparations of *M. Charantia* from injectable extracts to fruit juice to dried fruit bits have been traditionally used worldwide, particularly for blood-sugar lowering effects (Welihinda *et al.*, 1986; Raman and Lau, 1996).

The Bitter gourds have many health benefits and medicinal properties. These are such as kills bacteria, reduce inflammation, kill viruses, fights free radicals, kills cancer cells, kills leukemia cells, prevents tumors, cleanses blood, reduces blood sugar and balance hormones. (Leslie Taylor, 2005).

The most researched health attribute of bitter melon has been its purported antidiabetic activity. The compounds charantin, vicine, and polypeptide-p are thought to be the major hypoglycaemic agents according to a review by Krawinkel *et al.* (2006). In both animals and humans these compounds have been shown to increase glucose uptake and glycogen synthesis in the liver, muscle, and adipose tissue, and improve glucose tolerance (Sloan-Kettering 2008).

Antioxidant activity of bitter melon has been measured in a number of different studies. Using a less common method involving the oxidising of linoleic acid methyl in the presence of phenolic extracts as antioxidants, Horax *et al.* (2005) found that bitter melon extracts had moderate to good inhibition effects on oxidation in comparison to 92 edible and inedible plant materials evaluated by Kahkonen *et al.* (1999).

The antioxidant properties of carotenoids that protect plants during photosynthesis may also protect humans from carcinogens and mitigate free radical effects associated with heart disease. Natural antioxidants, primarily plant phenolics and polyphenolic compounds (e.g., in fruits and seeds of bitter gourd), are alternatives to synthetic antioxidants for alleviating oxidative deterioration in fruit. For instance, bitter gourd fruit contains as many as 14 carotenoids depending on stage of maturity (5, 6, and 14 in the immature, mature-green, and ripe stage, respectively), where cryptoxanthins becomes the principal chloroplast and chromoplast pigment found in ripe fruit (Rodriguez *et al.*, 1976).

Other carotenoids, such as 3-carotene, zeaxanthmn and lycopene (at ripe stage), and lutein and a-carotene (immature fruit) are also prevalent in the fruits, where they could serve as a model for studying carotenogenesis during ripening (Rodriguez *et al.*, 1976). For instance, carotenogenesis in bitter gourd is not affected by temperatures above 30°C (Tran and Ráymundo 1999); in contrast, in Likewise, the total carotenoid concentration of bitter gourd seeds can be a 100-fold higher in the ripe than the immature stage, which is exclusively attributable to lycopene (96% of the carotenoids in ripe seeds; Rodriguez *et al.*, 1975).

Both traditional ayurvedic uses and most recent scientific interest regarding bitter melon has focused upon anti-diabetic activity. Research suggests that bitter melon's antidiabetic properties are the result of multiple mechanisms, including the capacity to regulate impaired carbohydrate digestion, glucose metabolism and utilisation, and the ability to stimulate insulin release. Further it has been found to possesses insulin-like activities and correct compromised antioxidant defence in diabetes (Yeh *et al.*, 2003; Tiwari, 2007).

Ethno-medical reports of bitter gourd *(Momordica churantia)* indicate that it is used in folkloric medicine for treatment of various ulcers, diabetes, and infections (Gurbuz *et al.*, 2000; Scartezzini and Speroni 2000; Beloin *et al.*, 2005).While the root decoctions have

abortifacient properties, leaf and stem decoctions are used in treatment of dysentery, rheumatism, and gout (Subratty *et al.*, 2005). In addition, juice of *Momordica charantia* drawn directly from fruit traditionally has been used for medicinal purposes worldwide. Likewise, the extracted juice from leaf, fruit and even wholeplant are also used for treatment of wounds, infections, parasites(e.g., worms), measles, hepatitis, and fevers (Behera *et al.*, 2008c).

CHAPTER III
MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

The experiment was conducted in the Laboratory of food processing and preservation, Hajee Mohammad Danesh Science & Technology University, Dinajpur during the period of January 2013 to July 2013. In this chapter manufacturing process of bitter gourd juice and chemical properties of raw bitter gourd and prepared bitter gourd juice were discussed.

3.1 Materials

The fresh bitter gourd (*Momordica Charantia L*) used in this study was collected from local market. Equipments and chemicals required were used from the laboratory stock.

3.1.1 Chemical required

The chemicals required were used from the laboratory stock. The lists of chemicals used in research work are listed below;

a) KMS
b) Acetic acid
c) H_2SO_4
d) Methyl Blue red etc.

3.1.2 Equipments required

a) Balances
b) Muffle furnace
c) Electrical oven
d) pH meter
e) Juice extractor
f) Homogenizer and
g) Sealer

3.2 Methods

Firm bitter melons having solid, light green color and without blemishes and any signs of yellow or orange color on the skin were selected. Selected fresh bitter gourds were washed thoroughly. All the bitter melons were cut open lengthwise and all the seeds were collected. Then outer skins of the pieces separated carefully and the fleshes were chopped into small pieces. All the separated portions were collected, weighed and stored at refrigeration temperature. Previously stored frozen samples was bought to room temperature and analyzed for their chemical compositions (Deore *et al.*, 2008).

3.2.1 Dietetic drinks formulation

Different formulation of three bitter gourd dietetic drinks were prepared by heating 10%, 30%, 20% and 15% bitter gourd pulp in acid-sweetener solution, coded as sample S_1, Sample S_2, Sample S_3 and Sample S_4 is control. The ingredients required for different formulations are shown in Table 3.1.

Table 3.1 Composition of 1000gm of beverage from Bitter gourd

Ingredients in gram	Formulations (gm)			
	Sample S_1	Sample S_2	Sample S_3	Sample S_4
Bitter gourd	100	300	200	150
Sugar	110	90	70	100
Citric acid	3.5	3.5	3.5	-
KMS	0.6	0.6	0.6	-
Water(ml)	785.9	605.9	725.9	750

Sample S_1=10% bitter gourd pulp; Sample S_2= 30% bitter gourd pulp; Sample S_3=20% bitter gourd pulp; Sample S_4= 15% bitter gourd pulp (without KMS).

3.2.2 Preparation of dietetic bitter gourd juice

The firm and fresh bitter gourd were washed thoroughly. Then the fruits were cut into slices and the pulp was extracted by using blender machine and taken into a beaker according to the formulation given in Table 3.1. All other ingredients were mixed separately and added to the beaker containing bitter gourd pulp. The pulp was heated to

60°C for 15 min. After heating the samples were bought to the room temperature and filtered through muslin cloth. After filtration, potassium per mangenate at above mentioned rate was added to the finished product. Finally the products were bottled, hot sealed and stored.

3.2.2.1 Processing flow sheet for the preparation of dietetic bitter gourd juice

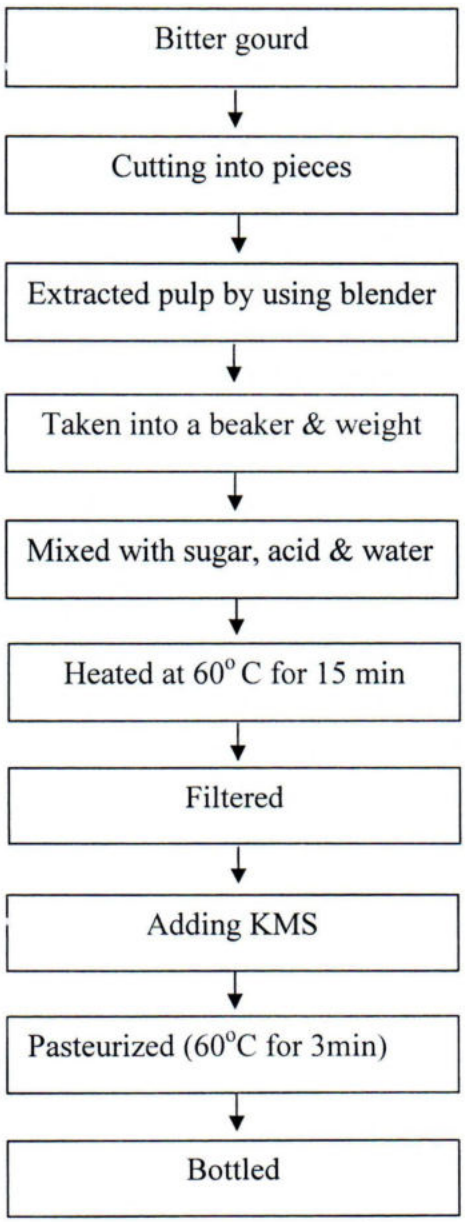

Fig. 3.1. Processing flow sheet for the preparation of dietetic bitter gourd juice

Fig. 3.2 Firm and fresh bitter gourd

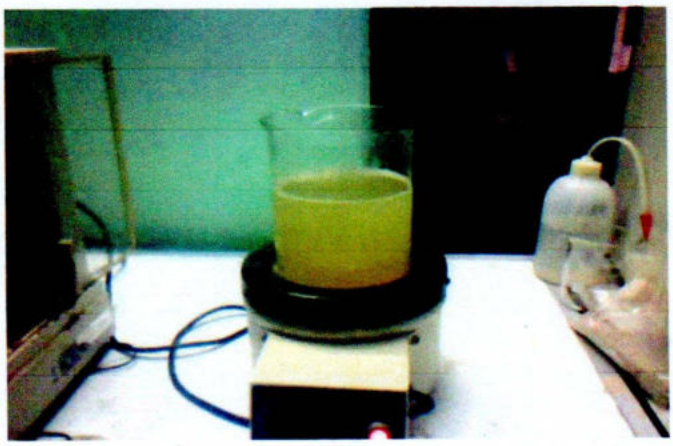

Fig. 3.3 Preparation of bitter gourd juices

Fig. 3.4 Bitter gourd juices stored in glass bottles

3.3 Chemical analysis

Moisture, ash content, TSS, reducing sugar, non-reducing sugar and total sugar, Protein, crude fiber, pH, acidity, vitamin-C were analyzed as per methods of AOAC (2000), AOAC (2004), Rangana (1992).

3.3.1 Moisture content

AOAC method (2000) was used to determine the moisture content of raw bitter gourd. 5g of sample was taken in a clean, dry and pre-weighted crucible. Then it was transferred to oven and dried at 105°C for 16 hours. After that it was cooled at desiccator and weighed. Again it was transferred to oven and dried until a constant weight was obtained. Finally it was cooled and weighed.

Moisture Content was calculated by following formula:

$$\% \text{ Moisture} = \frac{W_1 - W_2}{W} \times 100$$

Here,

W_1 = weight of sample with crucible

W_2 = weight of dried sample with crucible

W = weight of sample

3.3.2 Ash content

Total ash content of bitter gourd was determined adopting AOAC method (2004)

Procedure

Two gram of raw bitter gourd sample was weighed and taken in dry, clean porcelain dishes. Hot air oven method was applied to remove the moisture. Then the samples were burnt on an electrical heater. This was done to avoid the loss of sample in the muffle furnace under high temperature. Then the samples were transferred into the muffle furnace and burnt at 550°C temperature for 4-6 hours and ignited until light gray ash resulted (or to constant weight).The samples were then cooled in desiccators and weighed.

The ash content was calculated by the following formula:

$$\% \text{ Ash} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100$$

3.3.3 Determination of K, Ca, Mg, Zn, Fe and P

Organic matter is digested and K, Ca, Mg, Zn, Fe and P are released by digestion with nitric and perchloric acid. Ca, Mg, Zn and Fe are determined by atomic absorption spectrophotometry, K is determined by flame photometry, and P is determined by spectrophotometry and each element was determined separately for each sample. (Horwitz W. 2000; Greenfield H. and Southgate D.A.T. 1992; Kirk R.S and Sawyer R. 1991)

a) At the stage of digestion 0.5g of bitter gourd sample was weighted into a 200ml conical flask. Add 20ml 68% nitric acid and 10ml 70% prechloric acid (2:1) to the conical flask. Then the conical flask was placed in the digestion chamber on an adjustable heater and covered with the exhaust manifold. The temperature was set at 125°C and allowed to boil until the brown smoke become white and a clear solution obtained also observed that conical flask do not become dry. If the flask becomes dry add another 10ml solution (2:1) of 68% nitric acid and 10ml 70% prechloric acid.

b) After cooling, the digestion mixture was transferred to a 100ml volumetric flask. Made the flask to volume with distilled water and mixed. Filtered on a dry filter into a dry bottle, which could be closed with a screw cap. The filtrate was kept in the closed bottle. K, Ca, Mg, Zn, Fe and P were determined in the filtrate.

3.3.4 Determination of Ca, Mg, K and P

Using a pipette, 20ml filtrate was transferred into a100ml volumetric flask. Made the flask to volume with distilled water and mixed.

i) **Measurement of Ca:** 20ml diluted filtrate was transferred into a 50ml volumetric flask using a pipette. 5ml $LaCl_3$ solution was added and made the volume with distilled water and mixed. The content of Ca was measured by atomic absorption spectrometer (AAS). If the reading is higher than the reading of the highest standard solution, we have to make a larger dilution, e.g. 10ml filtrate into a50ml volumetric

flask. In this case 1:100 diluted HNO_3 must be added to the volumetric flask to make the total volume of 1:100 diluted $HNO_3$and filtrate equal to 20ml.

ii) **Measurement of Mg:** 5ml diluted filtrate was transferred into a 50ml volumetric flask using a pipette. 5ml $LaCl_3$ solution was added and made the volume with distilled water and mixed. The content of Ca was measured by atomic absorption spectrometer (AAS). If the reading is higher than the reading of the highest standard solution, we have to make a larger dilution, e.g. 2ml filtrate into a50ml volumetric flask. In this case 1:100 diluted HNO_3 must be added to the volumetric flask to make the total volume of 1:100 diluted $HNO_3$and filtrate equal to 5ml.

iii) **Measurement of K**: 10ml diluted filtrate was transferred into a 50ml volumetric flask using a pipette. 5ml $LaCl_3$ (Lanthium Chloride) solution was added and made the volume with distilled water and mixed. The content of Ca was measured by flame photometer. If the reading is higher than the reading of the highest standard solution, we have to make a larger dilution, e.g. 5ml filtrate into a50ml volumetric flask. In this case 1:100 diluted HNO_3 must be added to the volumetric flask to make the total volume of 1:100 diluted HNO_3 and filtrate equal to 10ml.

iv) **Measurement of P:** 5ml diluted filtrate was transferred into a 50ml volumetric flask using a pipette. 30ml distilled water and 10ml ammonium molybdate-ascorbic acid solution were added, made to volume with distilled water and mixed. After 15 minutes the absorbance was measured on a spectrophotometer at 890nm. If the absorbance is higher than that of the, repeat the procedure using a smaller amount of filtrate. In this case 1:100 diluted HNO_3 must be added to the volumetric flask to make the total volume of 1:100 diluted HNO_3 and filtrate equal to 5ml.

If the content of P is very high, it is necessary to dilute the filtrate further before the transfer to the 50ml flask. The dilution is made with water using pipette and volumetric flask. After transfer of 5ml diluted filtrate to the 50ml volumetric flask, 5ml 1:100 diluted HNO_3 and water to 30mlare added. Then 10mlammonium molybdate-ascorbic acid is added, the50ml volumetric flask is made to volume with water and the absorbance is measured on a spectrophotometer at 890nm after 15 minutes.

Calculations for Ca, Mg, K and P

$$\text{mg per Kg plant material} = \frac{a \times 2500}{b \times c}$$

Where,

a = mg/l Ca, Mg, K or P measured on atomic absorption spectrophotometer, flame photometer or spectrophotometer.

b = ml diluted filtrate transferred into the 50ml volumetric flask for determination of Ca, Mg, K and P.

c = g plant material weighted into the digestion flask.

3.3.5 Determination of Zn and Fe

The contents of these elements were measured by atomic absorption spectrometer (AAS) directly in the undiluted filtrate.

Calculations for Zn and Fe

$$\text{mg per Kg plant material} = \frac{d \times 100}{c}$$

Where,

d = mg/l Zn or Fe measured on atomic absorption spectrometer or spectrophotometer.

c = g plant material weighted into the digestion flask

3.3.6 TSS

Two drops of prepared juice was taken in a refractometer (Model no. HI 96801, ROMANIA) plate and the total soluble solids of the juice were read directly from the refractometer.

3.3.7 Vitamin C (Ascorbic acid)

Ascorbic acid was determined following the method of Rangana (1992).

The reagents used for the estimation of vitamin C were as follows:

1. **3% Metaphosphoric acid:** Prepare by dissolving the sticks or pellets of HPO3 in glass-distilled water.

2. **Ascorbic acid standard:** Weigh accurately 100mg of L ascorbic acid and made up to 100 ml with HPO3. Dilute 10 ml to 100 ml with 3% HPO3 mg = 0.3mg of ascorbic acid.
3. **Dye solution:** Dissolve 50 mg of the sodium salt of 2, 6 dichlorophenol indophenols in approximately 150 ml of hot glass distilled water containing 42 mg of sodium bicarbonate. Cool and dilute with glass distilled water to 200 ml. Store in a refrigerator and standardize everyday.

The dye 2, 6 Dichlorophenol-indophenol is blue in alkaline solution and reduced to light red colour by an ascorbic acid at pH range of 1-3.5

Standardization of Dye:

Take 5ml of standard ascorbic acid solution and add 5ml of HPO3. Fill a micro burette with the dye. Titrate with the dye solution to a pink colour, which should persist for 15 seconds. Determine the dye factor i.e. mg of ascorbic acid per ml of the dye, using the following formula:

$$\text{Dye factor} = \frac{0.5}{\text{Titre}}$$

Preparation of the sample:

10g sample was blended and homogenized in a blender with 3% metaphosphoric acid solution. The homogenized liquid was transferred to a 100-ml volumetric flask and made to volume 100 ml with metaphosphoric acid solution. Content of the flask was then thoroughly mixed and filtered. Then 5ml of the aliquot was taken in a flask and titrated with 2, 6 dichlorophenol-indophenol dye.

$$\text{Mg of vitamin C per 100g sample} = \frac{T \times D \times V_1}{V_2 \times W}$$

Where,

T= Titre

D= Dye factor

V_1=Volume made up

V_2= Aliquot of extract taken of estimation

W= Weight of sample taken for estimation

3.3.8 Protein

AOAC method (2000) was used with some modification to determine the protein content of the rice powder. Usually three stages are used to determine protein content. These stages are given below:

At the stage of digestion Bitter gourd (1g), Selenium powder (1g), $CuSO_4$ (0.1g), K_2SO_4 (10g) were taken into a volumetric flask. Then 25ml of H_2SO_4 (conc.) was added. After that the volumetric flask was heated at 100°C for 3 hr and cooled for 20 minute at room temperature.

After digestion 300 ml of distilled water and 125 ml of 40% NaOH were added to the volumetric flask. 25 ml of 4% boric acid solution and 2-3 drops mixed indicator were taken in a conical flask. The volumetric flask was connected with one end of the condenser and the conical flask was connected with other end. The volumetric flask was heated continuously until the conical flask was filled to 150 ml.

The conical flask was disconnected and was taken for titration. Titrated against 0.2 N of H_2SO_4 solution. The end point was indicated by orange color.

$$\% \text{ of } N_2 = \text{Burette reading} \times \text{Normality of } H_2S0_4 \times \text{ml equivalent of } N_2$$

Here;

Normality of $H_2S0_4 = 0.2$

ml equivalent of $N_2 = 1.4$

$$\% \text{ Protein} = \% \text{ of } N_2 \times \text{Protein factor}$$

Here;

Protein factor = 5.5

3.3.9 Crude fibre

The crude fiber was estimated by taking 1g moisture and fat free bitter gourd sample. The sample was treated successively with boiling solutions of 1.25% H_2SO_4 and then with 1.25% NaOH solution both for 30 min, respectively. The residue is separated by filtration and washed with ether and ethanol to reduce acidity and alkalinity.

Then it is dried, weighed and ashed at 500°C for 3-4 hrs. The fiber percentage was calculated according to (AACC, 1983).The loss in weight resulting from ashing corresponds to crude fibre of the sample:

$$\text{Crude Fiber \%} = \frac{\text{Wt. loss on ignition (g)}}{\text{Wt. of sample (g)}} \times 100$$

3.3.10 Reducing sugar

50g of sample was homogenized with water and transferred to 500ml beaker. It was neutralized with 0.1N NaOH and boiled gently for 1hr.Added boiling water to maintain original level. Then cooled and transferred to a 500-ml volumetric flask. Then volume was made to 500-ml volumetric flask. Then volume was made to 500 ml and filtered. Pipetted a 100 ml aliquot into a 500 ml of water and volume was made to 500 ml. It was stand for 10 min, then precipitated the excess of lead with potassium solution. The mixture was filtered and the filtrate was used to titrate against a measured amount of standard Fehling's solution (Rangana 1992). Percent reducing sugar was calculated from the following formula:

$$\% \text{ Reducing Sugar} = \frac{I \times D \times 100}{T \times W \times 1000}$$

Where,

I = mg of invert sugar required to reduced known volume of Fehling's solution.

D = Dilution factor

T = Titre

W = Weight of sample

3.3.11 Non-reducing sugar

Fifty ml of purified solution was taken in a conical flask. Fifty ml distilled water and 5 gm of citric acid were added to it. Then the conical flask was heated for 10 minutes for insertation of sucrose and finally cooled. The sample was then neutralizes by 1N NaOH solution using phenolphthalein indicator. The volume was made up to 100ml with distilled water. The mixed Fehling's solution was titrated using similar procedure followed as in the case of reducing sugar (Rangana 1992). The percent invert sugar is

then calculated by the similar procedure as in the case of reducing sugar from we got the percent non-reducing sugar by using the following way:

% Non-reducing sugar = % Invert sugar - % Reducing sugar

3.3.12 Total sugar

Total sugar can be calculated by using the following way:

% Total sugar = % Reducing sugar + % Non-reducing sugar

3.3.13 Acidity

Acidity was determined following the method of Rangana (1977). Twenty five gm sample was taken in a blender machine and homogenized with distilled water. The blended materials were then filtered and transferred to a 250 ml volumetric flask and the volume was made up to the mark with distilled water. Five ml of solution was taken in a conical flask and titrated with 0.1N NaOH solution just below the end point, using phenolphthalein indicator. The titration was done for several times for accuracy. Percent tritable acidity was calculated using to the following formula:

$$\% \text{ of Tritable acidity} = \frac{T \times N \times V_1 \times E}{V_2 \times W \times 1000} \times 100$$

Where,

T= Titre

N= Normality of NaOH

V_1= Volume made up

E = Equivalent weight of acid

V_2 = Volume of sample taken for estimation

W = Weight of sample

3.3.14 Determination of pH

The pH means the negative logarithm of hydrogen ion concentration in a solution. The pH of the selected samples was determined by the conventional procedure by a pH meter (Janin and Nema, 2007)

Materials

A pH meter (Hanna instruments- ORPP), salinity-sodium tester (ISO-9001 certified company; Woonsocket, RI 02895), the supplied pH 4.0 buffer solution, distilled water and 50 ml beakers.

Using standard buffer solution of pH 4.0 for calibration the pH buffer solution was used to calibrate the pH meter.

Procedure

The electrode assembled to the pH meter was dipped into the standard buffer solution of pH 4.0 taken in a clean and dry beaker.

The fine asymmetry potential knob was adjusted to pH 4.0. The electrode assembled pH meter was dipped into the selected bitter gourd juice, the pH was then readout washed twice with distilled water. Again it was dipped into another sample to determine the pH. The pH of all samples was determined by the procedure.

3.4 Microbiological studies

3.4.1 Determination of total viable bacteria

For total viable count of microorganism present in the samples (bitter gourd), Standard pour plate method was followed according to the method described in "Recommended method for the microbiological examination of food" (Ali, 2008).

3.4.2 Preparation of media

Table 3.2 Composition of Agar media

Ingredients	Amount
Peptone	2.5 gm
Agar	9 gm
Beef extract	1.5 gm
Sodium chloride (NaCl)	1 gm
Distilled water	500 ml

All necessary ingredients were measured with the help of electric balance and taken them in a conical flask and mixed. The conical flask was heated for proper mixing. In the time of heating, the mixture was rotted with the glass rod. When the mixture was properly mixed, the mouth of the conical flask was blocked with cotton plug and covered with aluminium foil. Then the conical flask with media was placed in autoclave for sterilization (Temperature: 121^0 C, Pressure: 15 Ib/inch^2).

3.4.3 Preparation of dilution blank

In order to dilute the sample consecutively 1ml of the original sample was diluted stepwise through a series of tubes containing 9ml of distilled water. At first 9ml of the distilled water was taken in a sterile test tubes and then 1ml of the original sample was taken to the first test-tube with a sterile pipette. Water with the sample was vigorously shaken for homogenous distribution of the bacterial population in the solution. This tube was denoted as "A". From the tube "F-1" another 1ml aliquot was transferred to the second tube and this tube was denoted as "F-2". In this way "F-3", "F-4", "F-5", "F-6" was prepared until the desired dilution is achieved. Now the tube "F-1" has got the dilution 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} respectively.

The dilutions were as follows:

Tube No.	Dilution	Volume of original fluid per ml	
1	1/10	0.1	or 10^{-1}
2	1/100	0.01	or 10^{-2}
3	1/1,000	0.001	or 10^{-3}
4	1/10,000	0.0001	or 10^{-4}
5	1/100,000	0.00001	or 10^{-5}
6	1/1,000,000	0.000001	or 10^{-6}

3.4.4 Procedure of plating

Now from the test-tube "F-1", 1ml of the sample solution was taken in a sterile Petridish containing 9ml of agar medium. The agar with bacterial sample was mixed by rotating the petridish. This petridish was marked as "A". In this way "B", "C", "D", "E", "F" marked petridishes were prepared from the tubes "F-2", "F-3", "F-4","F-5" and "F-

6"respectively. Then these petridishes were placed on a level surface for few minutes for solidifying the agar medium

3.4.5 Incubation and colony count

After solidification petridishes were placed in the incubator at 36 ^{0} C for 24 hours, the over loaded petridishes were avoided and the petridishes containing countable colony were selected. Colonies were counted with the aid of a magnifying glass and finally the total number of bacteria per gram of sample was calculated by the following equation:

Colony count (per ml) = Number of colonies (per plate) × Reciprocal of the dilution.

3.5 Sensory evaluation

The data for the characters of the present study were statistically analyzed wherever applicable. The experiments were conducted one factor Randomized Complete Block Design (RCBD). The analyses of variance (ANOVA) for different characters were performed with the help of a computer program MSTAT-C and means were compared by the Duncan's New Multiple Range Test (DMRT) (Gomez and Gomez, 1984). The standard deviations of each sample at different periods were also analyzed by MS Office Excel (2007).

3.6 Storage studies

The bitter gourd juices were processed and bottled for two months of storage. The changes of TSS, acidity and vitamin-C were observed at an interval of 30 days under refrigerant temperature (3-8^{o}C) during the storage period.

CHAPTER IV
RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Chemical analysis of the raw materials

The raw materials of fresh bitter gourd were analyzed for moisture, ash, fat, protein, total carbohydrate and mineral contents (K, Ca, Mg, Zn, Fe and P). The results are shown in Table 4.1 the raw bitter gourd contains 91.93g moisture, 1.059g protein, 0.6338g ash, 3.33g fiber, 0.618g reducing sugar, 0.00g non-reducing sugar, 0.618g total sugar, 20.0mg calcium, 16.0mg magnesium, 180.0mg potassium, 0.1mg zinc, 1.8mg iron and 70mg phosphorus per 100g. The results were more or less similar to those reported by (Aziz *et al.*, 2011) and (Wills *et al.*, 1984) due to varietals difference, agro-ecological condition and methods of analysis.

Table 4.1 Major compositions of raw bitter gourd flesh

Parameters	Bitter gourd
Moisture	91.934g/100g
Ash	0.6338g/100g
Protein	1.059g/100g
Vitamin-C	197.72mg/100g
Fiber	3.33g/100g
Reducing Sugar	0.618g/100g
Non-reducing Sugar	0.00g/100g
Total Sugar	0.618g/100g
Calcium	20mg/100g
Magnesium	16.0mg/100g
Potassium	180mg/100g
Zinc	0.1mg/100g
Iron	1.8mg/100g
Phosphorous	70mg/100g

4.2 Chemical composition of bitter gourd juice

According to formulation the bitter gourd juice was prepared by mixing different ingredients. After preparation of the products the chemical compositions were determined. The compositions of the products have been shown in the Table 4.2

Table 4.2 Composition of the formulated juices

Sample	TSS (%)	Acidity (%)	pH	Vitamin-C mg/100gm
S_1	14.03	0.26	3.14	198.43
S_2	13.93	0.32	3.25	199.62
S_3	13.77	0.33	3.32	197.47
S_4	13.97	0.34	3.48	196.56

4.3 Storage effect on the analysis of bitter gourd juice

The chemical compositions of the formulations of bitter gourd juices during 4 months of storage. As the sample S_4 was spoiled within 15 days of storage it was not considered in the later studies.

4.3.1 TSS

Changes of TSS of bitter gourd juices was observed that the TSS of different formulation was shown change up to 120 days of storage. From the Table 4.3, it was observed that the initial TSS was found 14.03%, 13.93%, 13.77% and 13.97% in Samples S_1, S_2, S_3 and S_4 respectively. Maximum TSS was recorded in Sample S_1 (14.47%) followed by Sample S_3 (14.23%) against minimum in Sample S_2 (14.0) after four months of storage at refrigerated temperature. The increased TSS might be due to conversion of sugar i.e. hydrolysis of polysaccharides into monosaccharide and olegosaccharides during the storage periods. The highest value of TSS was recorded to be 14.37^0Brix at the end of 15 or 20 days of storage for the juice containing 10% bitter gourd pulp. Increase in TSS might be due to the solubilization of insoluble portion of the products due to presence of acids (ascorbic and citric acid) during storage as reported by Sethi (1992). Barwal *et al.* (2005) also observed an increase in the TSS of the developed bitter gourd RTS drink during storage. Increase in TSS was also reported by Yadav *et al.* (2010) in whey based banana herbal beverage.

4.3.2 Acidity

The acidity was calculated on the basis on titrated acidity. Acidity for all the formulations during storage was determined and the results were shown in Table 4.3.Variation in acidity ranged from 0.26 to 0.31 for formulation S_1, 0.32 to 0.35 for S_2, 0.33 to 0.38 for S_3 and o.34 to 0.39 for S_4. Alessandra *et al.* (2004) also reported for increase in acidity during storage. This increase is attributed to production of CO_2 that forms weak acid on dissolution. The decrease in pH and increase in acidity during storage might be due to degradation of artificial sweetener and carbohydrate present in the bitter gourd extract by the action of microorganisms which causes production of acids in beverage. Rangana (1977) recommended acidity (as anhydrous citric acid) ranging from 0.12 to 0.23% for various fruit flavoured carbonated beverages.

4.3.3 P^H

The values of pH of different formulations S_1, S_2, S_3 and S_4 for different storage period were presented in Table 4.3. Variation in pH ranged from 3.14 to 2.82 for formulation S_1, 3.25 to 2.95 for S_2, 3.32 to 3.02 for S_3 and 3.48 to 3.12 for S_4.This variation in pH were observed throughout the storage period. The variation in pH was due to variation of acidity occurred during the storage period at refrigeration temperature. Miguel *et al.*, (2004) found a decreasing trend of pH in beverages during storage. High acid and low pH may be due to production of acetic acid and lactic acid during storage. Such types of changes in pH vales have been demonstrated by Souci *et al.* (1987).

4.3.4 Ascorbic acid (vitamin -C)

The vitamin-C of different formulations was determined at various storage periods and shown in Table 4.3

Fruits and vegetables are important source of ascorbic acid but it is sensitive to oxidation. Ascorbic acid prolongs the shelf life of a product by reacting with residual oxygen and retarding the development of off-flavor (Pollard and Timberlake, 1971). During processing the juice had undergone heat treatment and aeration which accelerated the destruction of vitamin-C in the product. It has been shown in Table 4.3 the ascorbic acid reduced remarkably when increasing storage time (0-60 days) and the reduction was prominent with different treatments. For sample S_1 in vitamin-C content decreases from

198.43 mg/100gm to 187.13 mg/100gm, for sample S_2 199.62 mg/100gm to 190.13 mg/100gm, for sample S_3 197.47 mg/100gm to 186.13 mg/100gm, and for sample S_4 196.56 mg/100 gm to 126.34 mg/100gm throughout storage period. The loss of vitamin-C is dependent on temperature and storage time. The findings of present study are in line with the work reported by Maria *et al.* (2003) who observed loss of ascorbic acid (25 to 26%) during storage. In the present study the ascorbic acid content decreased with the increase in storage periods, because ascorbic acid can inhibit browning reactions by reducing the quinines back to the original phenol compounds. This decrease might be due to the factors such as storage temperature, oxidative enzymes, processing techniques, metal contamination, and the presence of atmospheric oxygen in the head space. Sufi (1976) found that vitamin-C content decreased from 35.1 to 2.8 mg per 100 gm in the guava juice based carbonated beverage for the storage period of 35 days at 20-25°C

Table 4.3 Analysis of bitter gourd juice at various storage periods

Sample No.	Constituents	Day 1	Day 30	Day 60	Day 90	Day 120
Sample 1	TSS (%)	14.03	14.19	14.37	14.39	14.47
	Acidity (%)	0.26	0.27	0.28	0.29	0.31
	pH	3.14	3.12	3.10	3.02	2.82
	Vitamin C (mg/100g)	198.43	195.48	191.98	189.39	187.13
Sample 2	TSS (%)	13.93	13.96	13.99	14.01	14.02
	Acidity (%)	0.32	0.33	0.35	0.35	0.35
	pH	3.25	3.22	3.18	3.16	2.95
	Vitamin C (mg/100g)	199.62	197.29	195.32	192.18	190.13
Sample 3	TSS (%)	13.77	13.86	14.16	14.17	14.23
	Acidity (%)	0.33	0.35	0.36	0.37	0.38
	pH	3.32	3.27	3.18	3.16	3.02
	Vitamin C (mg/100g)	197.47	194.56	190.34	187.24	186.13
Sample 4	TSS (%)	13.97	14.17	14.20	14.25	14.31
	Acidity (%)	0.34	0.35	0.36	0.37	0.39
	pH	3.48	3.38	3.26	3.16	3.12
	Vitamin C (mg/100g)	196.56	185.75	150.45	130.56	126.34

4.4 Microbiological study of the formulated bitter gourd juice

4.4.1 Total number of viable bacteria in formulated bitter gourd juice

This study was performed by standard pour plate method. Colonies were developed after 24 hours of incubation. Then the colonies were counted in the formulated juice. The total viable bacterial load was not uniform. The total number of viable bacteria per ml of

sample was obtained by multiplying the number of colony forming units (cfu) on the plate with dilution factor then it was converted into logarithmic form. The total numbers of viable bacteria count in different samples have been shown in Table 4.4.The variation of viable bacteria count has been shown. It was observed that the total viable bacteria in sample S_4 (15% juice without KMS) was greater and after 15 days as sample S4 was spoiled so that it was not detectable. The sample S_2 showed the minimum total viable count compared to other samples.

Table 4.4: Growth of bacteria in bitter gourd juice

Storage condition	Storage period	Sample no	Total count (log.cfu/ml)
Refrigeration Temperature ($4^{o}C$)	1 day	Sample S_1	1.53
		Sample S_2	1.02
		Sample S_3	1.65
		Sample S_4	1.75
	30 day	Sample S_1	2.78
		Sample S_2	2.55
		Sample S_3	2.56
		Sample S_4	3.13
	60 day	Sample S_1	2.91
		Sample S_2	2.62
		Sample S_3	2.69
		Sample S_4	ND
	90 day	Sample S_1	3.05
		Sample S_2	2.67
		Sample S_3	2.72
		Sample S_4	ND
	120 day	Sample S_1	3.13
		Sample S_2	2.75
		Sample S_3	2.76
		Sample S_4	ND

ND: not determined

4.5 Sensory evaluation of bitter gourd juice

The samples of bitter gourds juice were subjected to sensory evaluation. After four months of storage the colour, flavor, taste and overall acceptability of the products were evaluated by a panel of 15 judges. The mean scores for colour, flavor, taste and overall acceptability preference of the samples are presented in Table 4.5.The statistical analysis and ANOVA tables are given in Appendix (I-IV). A two way analysis of variance indicated that all the sensory attributes of different samples were significantly ($p<0.05$) different and thus the sensory attributes of the samples showed various degrees of acceptability.

Table 4.5 Mean sensory score of bitter gourd juices

Sample	Sensory attributes			
	Colour	**Flavor**	**Taste**	**Overall acceptability**
S_1	5.3^{c}	5.8^{b}	5.7^{c}	6.0^{c}
S_2	7.3^{a}	7.9^{a}	8.3^{a}	8.3^{a}
S_3	6.3^{b}	6.3^{b}	7.3^{b}	7.1^{b}
S_4	5.9^{bc}	5.6^{b}	6.2^{c}	5.4^{c}
LSD at 5% level	0.6443	0.823	0.8878	0.7797

Color

The results in Table 4.5 revealed that bitter gourd juice was significantly ($p<0.05$) different in colour acceptability (details in Appendix I, II, III and IV). In other words the colour of different formulation was not equally acceptable. In (Appendix I Table 1.3) there was significant difference among the samples and the sample S_2 scored significantly better colour. Sample S_2 was the most preferred securing 7.3 while the lowest score secured by sample S_1 was 5.3. This result indicates sample S_1 shown least colour acceptability than other samples.

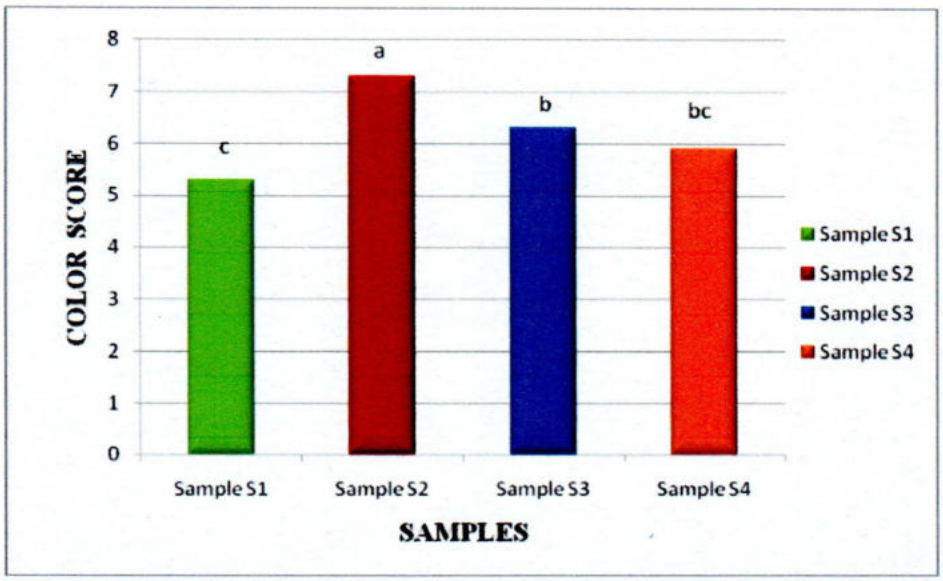

Fig 4.1 Acceptability of colour preference of prepared bitter gourd juice based on mean score

Flavour

In case of flavour preference among the samples a two way analysis of variance (Appendix II, Table 2.1 & 2.2) showed that the samples were significantly ($p<0.05$) different as far as flavour acceptability is concerned in Appendix III table 3.3. from Appendix II Table 2.3.it was obtained that the sample S_2 was the most preferred one securing 7.9 and other samples S_1, S_3, and control were equally acceptable securing scores ranging from 5.6 to 6.3 for flavour preference.

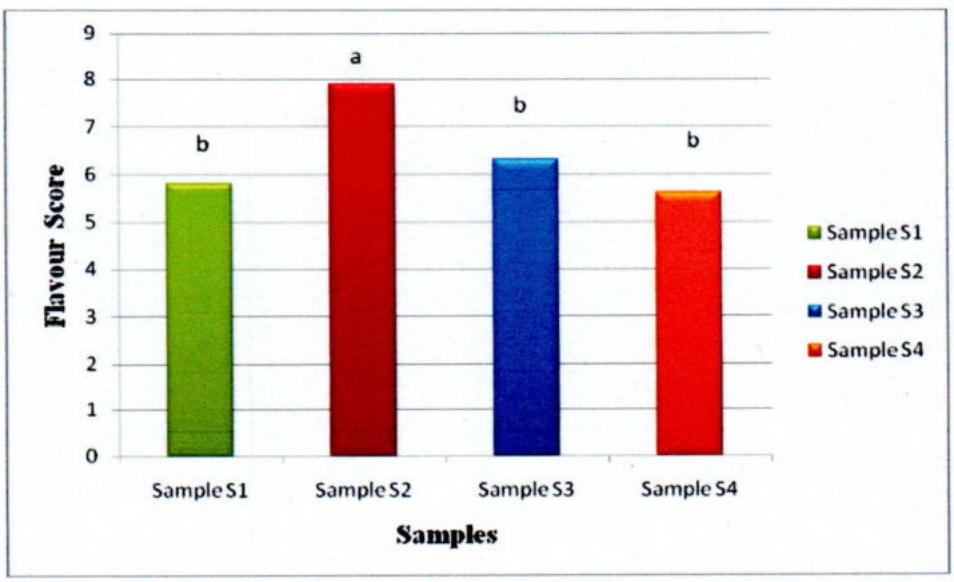

Fig 4.2 Acceptability of flavour preference of prepared bitter gourd juice based on mean score

Taste

In case of taste among the different formulations, a two way analysis of variance (Appendix III Table 3.1, 3.2 &3.3) was carried out and results in Table 3.3 revealed that there was significant ($p<0.05$) difference in taste acceptability among the products. Sample S_2 gave the highest score (8.3) for taste and was significantly different from other accept S_3 which secured in the second highest score (7.3). In terms of taste the other samples equally acceptable securing scores ranging from 5.7 to 6.3.

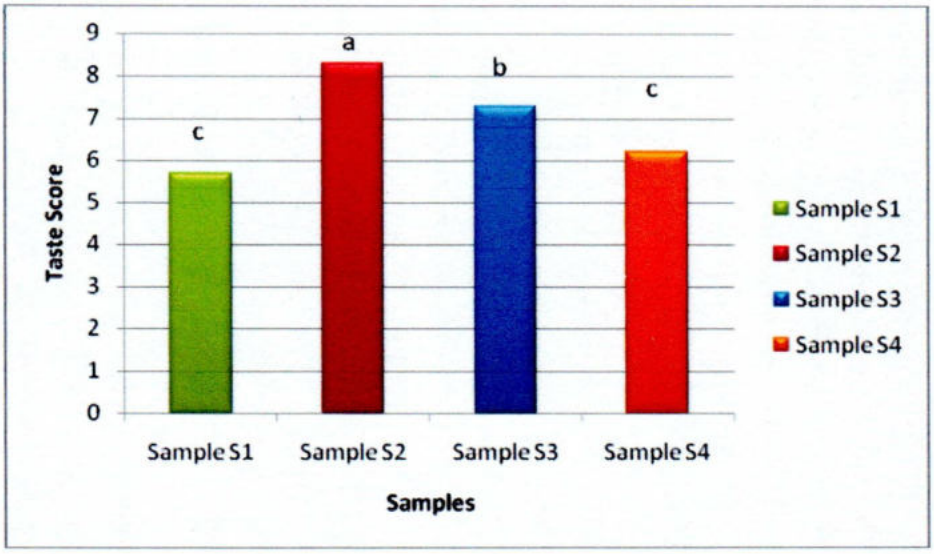

Fig 4.3 Acceptability of taste preference of prepared bitter gourd juice based on mean score

Overall acceptability

From the Table 4.5 it was revealed that sample S_2 were highly preferred securing 8.4 out of 9 for overall acceptability. The DMRT test for overall acceptability was presented in Appendix V Table 5.3. From the result, there was a significant ($p<0.05$) difference among the samples S_2 and others but the samples S_1, S_2, and control were statistically equally acceptable.

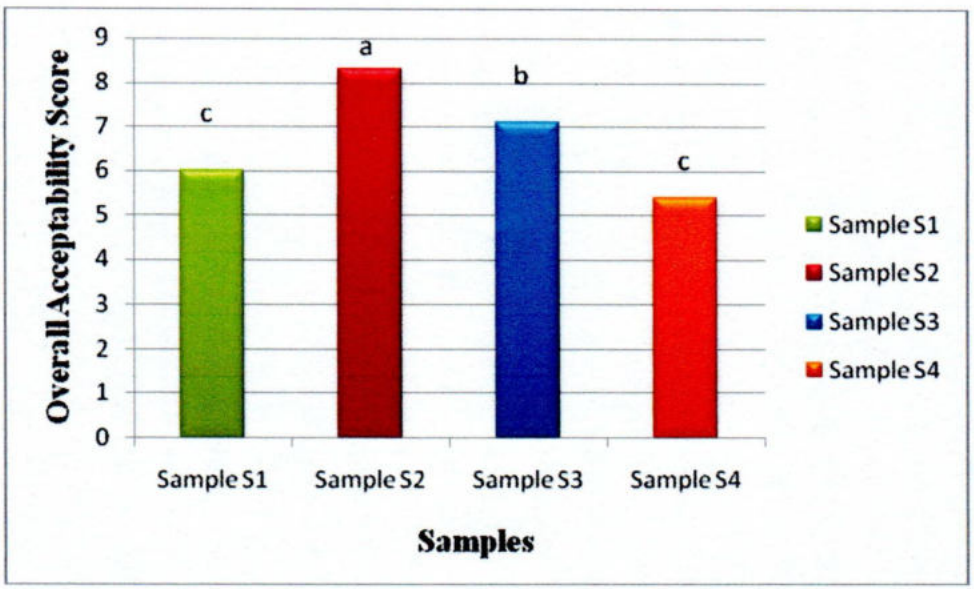

Fig 4.4 Overall acceptability of prepared bitter gourd juice based on mean score

Bitter gourd juice Sample S_2 containing 30% bitter gourd pulp, secured the highest score for colour, flavour, taste and overall acceptability among all the treatment and was closely followed by Sample S_3 having 20% bitter gourd pulp. So, Sample S_2 bitter gourd juice may be regarded as the best juice among the four samples.

4.6 Preservation observation

Preservation observation of bitter gourd juices from four samples during storage period at refrigeration temperature (RFT) were judged on basis of color, flavor, and sedimentation. The observations have been shown in Table 4.6

Table 4.6 Preservation Observation of Prepared Juice

Storage condition	Storage period	Sample	color	Flavor	sedimentation
Refrigeration Temperature (4°C)	At the day of preparation	Sample S_1	Green	Natural	Uniformly distributed
		Sample S_2	Dark Green	Natural	Uniformly distributed
		Sample S_3	Green	Natural	Uniformly distributed
		Sample S_4	Green	Natural	Uniformly distributed
	After 30 days	Sample S_1	Green	Natural	Uniformly distributed
		Sample S_2	Dark Green	Natural	Uniformly distributed
		Sample S_3	Green	Natural	Uniformly distributed
		Sample S_4	Green	Off flavor	Uniformly distributed
	After 60 days	Sample S_1	Green	Natural	Slightly sedimentation
		Sample S_2	Green	Natural	Uniformly distributed
		Sample S_3	Green	Natural	Slightly sedimentation
		Sample S_4	Fade	Off flavor	Sedimentation
	After 90 days	Sample S_1	Fade	Off flavor	Slightly sedimentation
		Sample S_2	Fade	Off flavor	Slightly sedimentation
		Sample S_3	Fade	Off flavor	Slightly sedimentation
		Sample S_4	Fade	Off flavor	Sedimentation
	After120 days	Sample S_1	Fade	Off flavor	Slightly sedimentation
		Sample S_2	Fade	Off flavor	Slightly sedimentation
		SampleS_3	Fade	Off flavor	Slightly sedimentation
		Sample S_4	Fade	Off flavor	sedimentation

From the above table it was found that there was a slight variation in color among four samples of bitter gourd juices during 120 days storage period. Color was found green/dark green on the day of preparation. The color faded and flavor deteriorated gradually with the increase of storage period both at room and refrigeration temperature. The color, flavor and taste all were found same as the day of preparation. After 30 days discoloration and off flavor was found. But sample S_1, sample S_2, sample S_3 are prepared with KMS (Potassium meta bisulphate) which is act as preservative and sample S_4 is prepared without KMS. As a result sample S_1, sample S_2 and sample S_3 spoiled after 30 days and sample S_4 spoiled after 15 days.

4.7 Studies on Sedimentation of the juice during storage period

Sedimentation was observed in the glass bottles during the storage period. The sediment settles gradually on the bottom of the glass bottles. However, formation of sediment settles was settled rapidly just after bottling. Transparent appearance in the upper portion of the bottles was observed. Formulation S1 and S4 showed the greater sedimentation than S2, S3.

This formation of sediment might be due to the solid contents of the juice. This is the body of the bitter gourd juice. If it would be shaken before use then it would be seen to be fresh homogeneous juice. Sedimentation can be removed by proper homogenization, proper water treatment practices, proper filtration practices of water and preventation of microbial activity etc. Rangana (1977) suggested some useful method for juice clarification. By using pectic enzyme, tannin and gelatin or by the combination of these two or by centrifusing and filtering the juice might be successfully clarified.

CHAPTER V

SUMMARY AND CONCLUSION

CHAPTER V

SUMMARY AND CONCLUSION

The study was conducted in the Laboratory of food processing and preservation, Hajee Mohammad Danesh Science and Technology University, Dinajpur. The microbiological study, storage study and consumer's acceptability of the formulated bitter gourd juices were studied. The products were then stored in dry and cool place at room temperature. Sensory evaluation was done at an interval of 30 days up to 120 days by a taste testing panel.

The mature and fresh bitter gourds were collected from the local market. The flesh was separated from seeds and crushed by a blender. Four formulations coded as Sample S_1 (10% bitter gourd pulp), Sample S_2 (30% bitter gourd pulp), Sample S_3 (20% bitter gourd pulp) and Sample S_4 (15% bitter gourd pulp without KMS) were prepared. The required quantity of sugar and acid were dissolved in measured amount of water, which was mixed with weighed amount of bitter gourd pulp, KMS, and citric acid. The juices were prepared and bottled to store at refrigerated temperature (3-8^{o}C).

The microbiological examination of the samples was carried out during storage period to examine the total count (cfu/ml) of viable bacteria. Maximum number of bacteria was found in sample S_4 (15% bitter gourd pulp without KMS).The sample S_2 showed the minimum total viable count. Negligible change was observed (except the vitamin-C) in the composition of the prepared juice throughout the storage period. Remarkable decrease of vitamin-C was found in the formulations during storage period and acidity increased slightly.

The acceptability of the juice was tested by a panel of 10 judges. The scores obtained after tasting the samples were analysed and the acceptability of the finished products were found out. A statistical analysis of the score response by the taste testing panelists on the sensory attributes on juices revealed that colour, flavor , taste, texture and overall acceptability of the juices were significantly($p<0.05$) different. It was found that sample S_2 (300 gm bitter gourd pulp, sugar-90 gm, citric acid-2.5 gm, KMS-0.6 gm and water-605.9 gm for eash 1000 gm of juice) was highly acceptable by the taste panelists.

The storage stability of the accepted products was studied up to four months at an interval of 30 days and the quality attributes were found satisfactory. The preservative (KMS) was also effective against microbial growth to prevent spoilage except the sample S_4 (without KMS) of the bottled juices.

The study was carried out to develop suitable formulae for the preparation of bitter gourd juice. Another important objective was to explore the new area for the utilization of bitter gourd by developing easily implementable process at rural level or in a small scale industry in urban areas. Thus by processing and preserving bitter gourd as juice may provide better nutrition to the consumers who were sensitive to intake high calorie.

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APPENDICES

APPENDICES

Appendix-I

Table 1.1 Rating score for colour of bitter gourd juice

No. of taster	Sample			
	S_1	S_2	S_3	S_4
1	6	9	6	6
2	5	7	7	6
3	5	8	6	6
4	5	6	6	5
5	5	7	7	6
6	6	8	6	5
7	5	7	6	7
8	5	6	7	6
9	6	7	7	6
10	5	8	5	6
Total	53	73	63	59
Mean	5.3	7.3	6.3	5.90

Hedonic rating score9=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=Like extremely

Table 1.2 Analysis of variance (ANOVA) for color

Sources of variance	Degrees of freedom	Sum of square	Mean square	Calculated Value F	Tabulated Value F_t
					5%
Samples	3	21.200	7.067	14.3459	2.960
Panelist	9	3.900	0.433	0.8797	2.250
Error	27	13.300	0.493		
Total	39	38.400			

Since $F > F_t$ at 5% level of significance, samples are significantly different

Table 1.3 Duncan's Multiple Range Test (DMRT) for color

LSD value= 0.6443; P < 0.05

Sample	Original order of means	Sample	Ranked order of means
S_1	5.3^c	S_2	7.3^a
S_2	7.3^a	S_3	6.3^b
S_3	6.3^b	S_4	5.9^{bc}
S_4	5.9^{bc}	S_1	5.3^c

Appendix-II

Table 2.1 Rating score for flavor of bitter gourd juice

No. of taster	Sample			
	S_1	S_2	S_3	S_4
1	6	9	6	5
2	5	8	7	6
3	7	9	6	5
4	6	8	6	5
5	6	7	7	6
6	5	8	6	7
7	7	6	6	6
8	5	9	7	5
9	5	7	7	6
10	6	8	5	5
Total	58	72	63	57
Mean	5.80	7.20	6.30	5.70

Hedonic rating score

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

Table 2.2 Analysis of variance (ANOVA) for flavor

Sources of variance	Degrees of freedom	Sum of square	Mean square	Calculated Value F	Tabulated Value F_t
					5%
Samples	3	32.600	10.867	13.7103	2.960
Panelist	9	1.600	0.178	0.2243	2.250
Error	27	21.400	0.793		
Total	39	55.600			

Since $F > F_t$ at 5% level of significance, samples are significantly different.

Table 2.3 Duncan's Multiple Range Test (DMRT) for flavor

LSD value= 0.823; $P < 0.05$

Sample	Original order of means	Sample	Ranked order of means
S_1	5.8^b	S_2	7.9^a
S_2	7.9^a	S_3	6.3^b
S_3	6.3^b	S_1	5.8^b
S_4	5.6^b	S_4	5.6^b

Appendix-III

Table 3.1 Rating score for taste of bitter gourd juice

No. of taster	Sample			
	S_1	S_2	S_3	S_4
1	6	9	6	8
2	5	8	9	6
3	7	9	8	7
4	5	8	6	5
5	6	7	9	6
6	5	9	6	7
7	7	8	8	6
8	5	9	7	5
9	5	7	8	6
10	6	9	6	6
Total	57	83	73	62
Mean	5.70	8.30	7.30	6.20

Hedonic rating score

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

Table 3.2 Analysis of variance (ANOVA) for taste

Sources of variance	Degrees of freedom	Sum of square	Mean square	Calculated Value F	Tabulated Value F_t 5%
Samples	3	40.475	13.492	14.4125	2.960
Panelist	9	8.625	0.958	1.0237	2.250
Error	27	25.275	0.936		
Total	39	74.375			

Since F > Ft at 5% level of significance, samples are significantly different

Table 3.3 Duncan's Multiple Range Test (DMRT) for taste

LSD value= 0.8878; P < 0.05

Sample	Original order of means	Sample	Ranked order of means
S_1	5.7^c	S_2	8.3^a
S_2	8.3^a	S_3	7.3^b
S_3	7.3^b	S_4	6.2^c
S_4	6.2^c	S_1	5.7^c

Appendix-IV

Table 4.1 Rating score for overall acceptability of bitter gourd juice

No. of taster	Sample			
	S_1	S_2	S_3	S_4
1	6	9	8	5
2	5	8	7	6
3	7	7	9	5
4	6	8	6	5
5	6	9	7	6
6	5	8	7	5
7	7	9	6	6
8	7	9	9	5
9	5	8	7	6
10	6	8	5	5
Total	60	83	71	54
Mean	6.00	8.30	7.10	5.40

Hedonic rating score

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

Table 4.2 Analysis of variance (ANOVA) for overall acceptability

Sources of variance	Degrees of freedom	Sum of square	Mean square	Calculated Value F	Tabulated Value F_t 5%
Samples	3	49.000	16.333	22.6154	2.960
Panelist	9	7.900	0.878	1.2154	2.250
Error	27	19.500	0.722		
Total	39	76.400			

Since $F > F_t$ at 5% level of significance, samples are significantly different

Table 4.3 Duncan's Multiple Range Test (DMRT) for overall acceptability

LSD value= 0.7797; P < 0.05

Sample	Original order of means	Sample	Ranked order of means
S_1	6.0^c	S_2	8.3^a
S_2	8.3^a	S_3	7.1^b
S_3	7.1^b	S_4	6.0^c
S_4	5.4^c	S_1	5.4^c

Appendix V

TASTE TESTING OF BITTER GOURD JUICE

Name of Tester: ..... Date: .....

Please taste these samples and check how much you like or dislike each one on four sensory attributes such as Colour, Flavour, Taste and Overall Acceptability. Use the appropriate scale to show your attitude by checking at the point that best describes your feeling about the sample. Please give a reason for this attitude. Remember you are the only one who can tell what you like. An honest expression of your personal feeling will help me.

HEDONIC	COLOUR				FLAVOUR				TASTE				OVERALL ACCEPTABILITY			
	Sample				Sample				Sample				Sample			
	S_1	S_2	S_3	S_4	S_1	S_2	S_3	S_4	S_1	S_2	S_3	S_4	S_1	S_2	S_3	S_4
Like extremely																
Like very much																
Like moderately																
Like Slightly																
Neither like nor dislike																
Dislike slightly																
Dislike moderately																
Dislike very much																
Dislike extremely																

Extra comments on each sample if any:

N.B. Overall Evaluation:

Hedonic scale used: 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.

Signature of Judge