

Development of Whey Based Guava Beverage Incorporated With Papaya Leaf Extract



THESIS

Submitted in partial fulfillment of the requirements for the award of the degree
of

Master of Technology

in

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Supervisor

Dr. Amrita Poonia

Submitted By

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(Dr. Amrita Poonia)
Supervisor

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By

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FROM

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ABBREVIATIONS

ANOVA	:	Analysis of variance
AOAC	:	Association of Official Analytical Chemists
PLE	:	Papaya Leaf Extract
WGB	:	Whey Guava Beverage
DPPH	:	1,1-Diphenyl-2-picryl-hydrazyl
GAE	:	Gallic acid equivalents
CFU	:	Colony Forming Units
HCL	:	Hydrochloric acid
ml	:	mililitre
Min	:	Minute
°C	:	Degree celcius
ppm	:	parts per million
TPC	:	Total phenolic content.
%	:	Percent
TSS	:	Total Soluble Solids
TS	:	Total solids
TA	:	Titratable acidity

Chapter 1

INTRODUCTION

The public awareness for nutritive and functional food greatly increased during the Covid-19 pandemic, so did the demand for functional and nutritive food. People currently pay more attention to the nutritional makeup of food and beverages than to their taste. The food and beverage business is attracting more attention due to the rising customer demand around the world. One of the largest food processing industries is beverage production. Drinks give energy and water for digestion, body temperature control, preventing dehydration, soothing thirst and reducing psychological stress (**Shaikh *et al.*, 2001**). Milk is a high nutrient, low-calorie, protein rich liquid that has been a staple diet since man first began making cheese around 5000B.C. Whey, the watery portion that separates from the coagulating milk, was considered a waste product and was disposed or used for feeding animals. In ancient Greece and during the Middle Ages, new utilizations of whey were sought, eg., in medicine and skin balms. In recent years, consumer interest in high protein diets has grown.

Following coagulation, whey separates from milk as a semi-translucent greenish yellow liquid. Lactose is the most prominent component, followed by whey proteins, water soluble vitamins, and minerals. In whey, you'll find proteins including α -lactalbumin, β -lactoglobulin, serum albumin, immunoglobulins plus lactose, milk salts, and more (Puranika and Rao,1996). Whey is a nutrition dense by-product created by the cheese, chana, and paneer industries. Whey accounts for 45-50 percent of total milk solids, 70% of lactose(milk sugar), 20% of milk proteins, 70-90 percent of milk minerals, and almost all water-soluble vitamins.

With the increased recognition of nutritional and functional value whey proteins have found their whey into numerous food products such as desserts, bakery, sauces, clinical nutrition, functional foods, meat products, and protein bars and beverages (**Onwulata and Huth, 2008**).

Whey is a fantastic beverage basis since it contains all the essential nutrients like lactose, proteins, minerals and vitamins. Whey proteins have a light, neutral flavor

that goes well with a wide range of fruits and vegetables in beverages. whey protein contains calcium, phosphorus and essential amino acids. Hence, highly nutritious product **(Saravana et.al., 2005)**. Whey production in the globe is currently estimated to be at 85 million tones, India contributes about 8 million tones to the total. Due to its high organic matter content, raw whey accounts for roughly 40% of total global production **(Reddy et al., 1987)** causing severe environmental contamination hazards (6-7 percent).

Whey predisposal treatment, on the other hand is a very costly process that has an impact on the economics of dairy plant operations **(Kumar and Tiwari, 2005)**. With the use of whey as water substitute, product diversity is now possible without a considerable change in composition **(Patel et al., 2007)**. On a commercial scale, turning whey into a beverage is cost effective and no residues are left behind **(Shendurse et al., 2009)**.

Whey proteins have distinct nutritional and functional qualities that can be used to provide customers with high protein foods. Dairy proteins can be used to make beverages with variety of nutritional and functional benefits due to their neutral pH. Whey proteins are present in small levels, however when compared to all other protein sources available, they have a high protein efficiency ratio(3.6), net protein utilization(95) and biological value (104) in terms of nutritional value, they are only second to egg proteins.

Whey proteins contain lactoalbumin, lactoglobulin, bovine serum albumin, caseinomacropeptide, immunoglobulins, lactoferrin and lysozyme which have all been linked to health benefits such as improved immunity, anticancer properties, anti-adhesion properties against pathogens, antiviral, antimicrobial (iron-binding properties) and anti-hypertensive effects.

On the other hand, Guava (*Psidium guajava* L.), a member of the botanical family Myrtaceae is one of the major fruit crops grown in tropical and subtropical regions of the world. Owing to its nutritional superiority, cheaper prices and characteristic flavor, it has gained wide popularity amongst rich as well as poor strata of the society. It has good potential for marketing both in raw and processed forms because of its taste, texture, color and potentially for processing into products and is often marketed

as “super-fruits” which has a considerable nutritional importance in terms of vitamins A and C with seeds that are rich in omega-3, omega-6 polyunsaturated fatty acids especially dietary fibre, riboflavin, as well as in proteins, and mineral salts. Also guava is one of the richest sources of vitamins, and is known to contain five folds more vitamin C than oranges (**Conway, 2001**).

The high content of vitamin C (ascorbic acid) in guava makes it a powerhouse in combating free radicals and oxidation that are key enemies that cause many degenerative diseases. It has been reported that consumption guava plays a key role in preventing the diseases such as scurvy, cancer, stroke, heart disease and urinary tract infection. The antioxidant virtue in guavas is believed to help reducing the risk to cancers of the stomach, esophagus, larynx, oral cavity and pancreas. The vitamin C in guava makes absorption of vitamin C much more effective in reducing the oxidation of the (bad) LDL cholesterol and increasing the (good) HDL cholesterol. The fibers in guavas promote digestion and ease bowel movements. The high content of Vitamin A in guava plays an important role in maintaining the quality and health of eyesight, skin, teeth, bones and the mucus membranes (**Chandel *et al.*, 2018**). Allahabad, a district in the state of Uttar Pradesh, is well known for the production of excellent quality guava fruits, especially the leading variety of guava in India viz. Allahabad Safeda. This elite variety not only ranks first in the cultivation of raw fruits production, but also for use in processing. Guava fruit consists of 20% peel, 50% flesh portion and 30% seed core. It also contains 74-84% moisture, 13-26% dry matter, 0.8-1.5 protein, 0.4-0.7% fat and 0.5-1.0% ash and the fruit is considered as an excellent source of vitamin C(299mg/100g) and pectin(1.15%) as reported by Wilson,(1980). The fruits have an appreciable amount of minerals such as phosphorus(23-37mg/100g), calcium(14-30mg/100g), iron(0.6-1.4mg/100g) as well as vitamins like Niacin, Thiamine, Riboflavin and Vitamin A (**Paul and Goo,1983 and Bose and Mitra, 1999**).

The fresh guava has a short shelf life (one week), for its high moisture content (**Singh *et al.*, 1990**) . The post harvest losses occur to reach about 22% (**Bons and Dhawan, 2006**). Guava fruit is normally consumed as fresh as a dessert fruit or in processed form as puree, juice, concentrate, jam, jelly, toffee, fruit flakes, squash, syrup, nectar, powder, wine, vinegar, ready to use snacks, drinks and dehydrated canned products

(Lalit et al., 2014). Considering the unique blend of taste and nutrition, guava is one of the most suited candidate fruits for value addition.

Carica papaya L. (papaya) from family *Caricaceae*, a native to Mexico and northern South America, is now neutralized in many parts of the world including tropical and subtropical regions. According to FAO (2020), global production of papaya is estimated to rise by 2.1% each year upto 16.6 million tones in 2029 (FAO,2020). India is the largest producer of papaya and its share of world production is expected to rise from 59% to 69% in 2029.

It is a semi- herbaceous tree like plant that grows upto 8 feet with stem, hollow, and cylindrical. Papaya is known by various names in many parts of the world and different languages. Papaya is the source of vitamins A,C and E, minerals (potassium, magnesium), carotenoids, phenolic compounds, fiber, and folate. Papaya is mixed with other herbs to treat diseases **(Anuar et al., 2008)**. In Nigeria, it is used to treatment of diabetes by traditional doctors **(Gbolade, 2009)**, in malaria and fungal infections **(Okpe et al., 2016)**, as an anti-malarial herbal mixture with other herbs in Cameroon **(Tarkang et al., 2013)**, decoctions of leaf reported as anticancer remedies by aboriginal Australians **(Nguyen et al., 2016)**.

Papaya also reported being a rich source of the digestive enzyme papain, which has applications in cosmetics, brewing industries, in tenderizing meat and pharmaceuticals. The raw fruit of papaya has laxative property and along with the fruit, the leaves are also useful in pyrexia, diabetes, syphilis, and in the healing of wounds **(Sudhakar and Theivanai Vidhya, 2014)**. Leaf and fruit extract of papaya exhibit antimicrobial and antioxidant activity due to the presence of phenols, vitamins, and enzymes **(Addai et al., 2013; Maisarah et al., 2013; Zuhair et al., 2013)**. Fruit and leaf extract papaya was reported to cure dengue fever and help to increase blood pallets count (Ahmad et al., 2011; Dharmarathna et al., 2013). The reported remedial properties of various parts of *C. papaya* include anti- hypertensive, antibacterial, diuretic, antifertility, antifungal, and antitumor effect **(Vij and Pasha, 2015; Singh et al., 2020)**. Papaya plants have also shown a beneficial effect on wound healing, immunomodulation; hypolipidemic and hypoglycemic conditions **(Nayak et al., 2007; Juárez-Rojop et al., 2012; Pandey et al., 2016; Santana et al., 2019)**. Further, some recent reports have revealed the use of *C. papaya* in different discipline, for instance, corrosion inhibitor **(Tan et al., 2020)**, multi dye degradation **(Jain et al., 2020; Bhuiyan et al., 2020)**, biodiesel/diesel blends **(Devarajan et al., 2020 Minakshi et al., 2020)** and nanoparticle fabrication **(Jain et al., 2020; Bhuiyan et al., 2020; Singh et al., 2020)** and so on.

Coconut sugar is prepared by concentration of inflorescence sap, popularly called as neera or Kalparasa, collected by tapping the unopened coconut spadix. The sap in its original form contains 14–16% sucrose which upon heating at 90–95°C turns into long threads followed by agitation or beating to form a crystalline or amorphous sugar. The coconut sugar is brown in color and contains 2–3% moisture. Unlike the commercially available sugars which are laden with high calories, palm-based sugars are rich in amino acids, vitamins, minerals, polyphenols and antioxidants. Moreover, the glycemic index (GI) of the sugar is relatively low making it a suitable healthy sweetener for all the age groups. Production of natural sweeteners with low glycemic index (GI) can be a solution to diabetes problem. GI is defined as a system (ranks 0–100) which is used to measure blood glucose raised after the consumption of carbohydrate-containing foods. A food with low GI raises blood glucose less than that of the food with high GI; thus, a healthy product is always associated with low GI value (ADA, 2018). Coconut (*Cocos nucifera* L.) sugar was reported to have a GI value of 35 (Kusumawaty, Maharani, & Edwina, 2012; Trinidad, Mallillin, Sagum, & Encabo, 2010) while the sugar from palm sugar (*Borassus flabellifer*) and sugarcane (*Saccharum officinarum* L.) has GI values of 42 and 58–82, respectively (Saputro *et al.*, 2017). The lower GI value of coconut sugar suggests that it can be a better source of healthier sugar.

Coconut sugar is made by evaporation of coconut sap and is a nutrient-rich crystalline sugar/sweetener that looks, tastes, dissolves, and melts almost exactly the same as regular sugar, but it is completely natural and unrefined and has a far superior taste (Abdullah *et al.*, 2014). The texture and flavor of coconut sugar are also similar to those of brown sugar (Appetit, 2018; Beck, 2014). Thus, it can easily replace regular table sugar.

The present investigation aims at development of a whey based guava beverage incorporated with papaya leaf extract.

Objective :

The overall objective of the present research was to develop a whey based guava beverage incorporated with papaya leaf extract (PLE).

The specific objectives are:

- To carry out comparative analysis of two different methods of papaya leaf extraction.
- Preparation of whey based guava beverage incorporated with papaya leaf extract (PLE).
- To carry out physiochemical analysis of whey based guava beverage incorporated with papaya leaf extract.
- To study the shelf –life analysis and cost estimation of the beverage.

Chapter 2

REVIEW OF LITERATURE

Different types of work have been done on the utilization of whey for the preparation of nutritious drinks, beverages etc. Various workers had reviewed the work done related to beverages, therefore, in this chapter the relevant literature to the present investigations has been reviewed. In India, milk is consumed in many forms such as fluid milk, butter, ghee, cheese, chena and paneer also the by-products like buttermilk and whey are also obtained.

2.1 Milk & Whey

Milk is a complex colloidal solution composed of 87.5% water, 3.9% fat, 3.4% protein, 4.8% lactose and 0.8% minerals. Milk proteins are of two types, casein and whey proteins. Casein are the dominant proteins at 80%. During the process of cheese manufacture, casein coagulates to form the cheese curd, trapping the milk fats within the casein complex. The serum containing lactose, whey proteins, minerals and water and is collectively referred to as “ whole whey”. Whole whey is approximately 93% water and 0.6% protein.

Whey proteins are mainly comprised of α -lactalbumin and β -lactoglobulin but also include bovine serum albumin, lactoferrin, lactoperoxidase enzymes and glomaclopeptides (**Driskell, 2007**). Immunoglobulins are also protein component of whey, but they exist in very small quantities (**Atamer, 2013**). Whey proteins are very high in essential and branched chain amino acids, which are necessary for serum replenishment during exercise and the anabolic period post- exercise and are also easily soluble at the pH of the digestive tract. As compared to the other proteins that need to be “ pre-digested” to be soluble in the gut, whey protein ingestion quickly spikes blood amino acid levels in a much shorter period of time after ingestion (**Bounous et al., 1991**).

2.1.1 Benefits of Whey

Muscles are primarily made of proteins. Proteins are responsible for creating most of the structural adaptations that make muscles bigger, stronger, faster and more fatigue

resistant in response to exercise. By supplementing the diet with whey protein, one can accelerate and enhance these beneficial changes in muscle structure and also boost heart and immune health **(Kinsella and Whitehead, 1989)**.

2.1.2 Nutritional Benefits of Whey Proteins

Whey protein supplements have shown to enhance muscle building and fat burning effects of exercise. They also improve performance and workouts, speed recovery and may even reduce the chances of injury **(Papenburg and et al., 1990)**.

The benefits of whey protein have been demonstrated in a number of studies, including a study published in the *Advanced Food Nutrition Research*, which looked at the effects of whey protein supplementation on muscle growth in men undertaking a 6-wk resistance training programme. In this study, subjects combining resistance training with whey protein supplementation increased their lean muscle mass and strength significantly more than subjects who completed the same exercise program without supplementation **(Kinsella and Whitehead, 1989)**.

Every hard workout causes microscopic damage to muscle fibers. One of the symptoms of this damage is a type of muscle pain known as DOMES (Delayed onset muscle soreness). The faster the body can repair this damage and reduce the associated soreness, the better will be its ability to perform in the next workout. **(Kinsella and Whitehead, 1989)**. This particular benefit of whey protein was demonstrated in a study from Vanderbilt University that involved Marine recruits as subjects. During bootcamp these subjects were randomly assigned to receive either a recovery supplement containing whey protein or a placebo after physical training each day. By the end of bootcamp, those Marines who had received the recovery supplement with whey protein had experienced 37% fewer joint and muscle injuries than the Marines in the placebo group **(Kinsella and Whitehead, 1989)**.

Whey proteins are advantageous not only to athletes but to the general population as well. They are beneficial for physically active individuals to repair and rejuvenate muscle fibers, help maintain body weight and keep the heart and immune system healthy. Adequate consumption of whey protein assists in maintaining body weight as whey proteins play a role in balancing blood sugar levels. An individual with normal blood sugar levels has a greater rate of fat loss, more energy, and lesser tendency to

overeat. Research has also shown that whey protein is good for the heart, helps fight cancer, and also improves the immune system of people suffering from.

In addition, inclusion of whey proteins in the diet has also shown to reduce cholesterol (**Kinsella and Whitehead, 1989**) and helps maintain strong bones and muscles during ageing. In fact, a study showed that older men who ate whey protein regularly had an increase in protein synthesis that helps limit muscle loss over time. The same study showed that elderly people who consumed lower amount of protein had a loss of bone density particularly in the hip and spine. Research indicates that whey protein can help reduce the amount of bone density loss.

In a study conducted in 2000 by **Hakkak** and others at the University of Arkansas, whey and soy rich diets were shown to protect against mammary tumours in rats. According to the study, soy protein in the diet reduced tumour incidents by 20% and whey proteins were shown to be twice as effective as soy (**Hakkak et al.,2000**). In short, whey protein consumption is beneficial irrespective of age and fitness goals.

2.1.3 Nutritional Benefits major Whey Minerals

Whey contains several minerals with magnesium, calcium, and phosphorus being the most prominent.

2.1.3.1 Magnesium

Magnesium is the fourth most common cation found in the human body and is an essential co factor in over 300 enzymatic reactions It is mainly found in bone (53%). Intracellular muscles contain 27%, and 19% of the body's magnesium is found in soft tissues leaving less than 1% in serum and red blood cells .Amongst other functional properties such as hormonal receptor binding, transmembrane ion flux, neurotransmitter release, & control of cardiac excitability, and vasomotor tone, magnesium plays a major role in exercise physiology. This includes muscle contraction, regulation of adenylate cyclase (Adenylate Triphosphate synthesizing enzyme), and synthesis of carbohydrates as well as nucleic acids (including proteins that are required for muscle restoration) Some of the enzymatic reactions catalyzed by magnesium involve energy synthesis, making magnesium an essential mineral for athletes. Low magnesium levels during endurance type activities could prove

detrimental and athletes competing with low magnesium levels could suffer from severe muscle spasms causing cramps (**Rosenbloom, 2000**). The daily estimated average requirement for magnesium is 200 mg in females and 250 mgs in males

2.1.3.2 Calcium

The fact that 99% of the body's calcium is stored in the bones and teeth (**Rosenbloom, 2000**) illustrates its importance in bone structure and formation. Calcium is responsible for bone metabolism, blood coagulation, nerve impulse transmission, cellular adhesiveness, and neuromuscular excitability aside from being involved as a cofactor in several enzymatic reactions and hormone release. The serum level of calcium is maintained at 2.2 to 2.5 millimoles/L; however, the detrimental effects of calcium deficiency have not yet been pinpointed due to its slow turnover and the detrimental effects are too gradual to be effectively demonstrated in humans (**Nordin, 2000**). Studies on animals have shown a direct correlation on deprivation of calcium and onset of osteoporosis (**Nordin, 1960**). This can be attributed to reduced calcium absorption and increased calcium re-absorption due to an effect of estrogen on bone, especially in post-menopausal women (**Nordin et al., 1999**). Also, athletes have a higher calcium requirement, due to loss in sweat during exercise (**Rosenbloom, 2000**). Recent studies have also shown that increase in muscle mass from physical activity (weight training as well as various day-to-day activity) is followed by an increase in skeletal growth. This increase in skeletal mass requires additional calcium intake (**Anderson, 2000**).

2.1.3.3 Potassium

Potassium is one of the four minerals essential for cell functionality. It is an ionic salt and is similar to sodium in structure. Potassium naturally occurs in the diet in meats, fish, dairy, fresh fruits, and vegetables. Potassium intake is essential for maintaining normal heart health. Its main functionality in the human body is in maintaining osmotic balance between cells and interstitial fluids and in facilitating neural transmissions in the brain (**Hellgren et al., 2006**). Potassium is an integral cofactor in many major biochemical functions of the body, thus maintaining osmotic and electrolytic balance and preventing involuntary muscle contraction (that could lead to

cramping), and is thus necessary to be constantly replenished in athletes, as salts such as potassium are lost in heavy amounts in sweat and urine **(Olsen, 2003)**.

2.1.4 Need for development of whey-based beverage

Using cheese whey as a beverage in human nutrition, especially for therapeutic purposes, can be traced back to the ancient Greeks; Hippocrates, in 460 B.C. prescribed whey for an assortment of human ailments. The market dynamics is driven by five key factor groupings: increased concentration in the global beverages market; diverging functional beverage trends worldwide; flavor innovations; product differentiation; and cross-category innovations. Whey beverages are manufactured and formulated keeping in consideration the nutritional values, biological and functional properties **(Chavan et al., 2015)**.

The major problems which are generally encountered in whey-based fruit juice beverages are:

1. Crystallization of lactose during storage at refrigerated temperature.
2. Coagulation of whey proteins during thermal treatments.
3. Higher viscosity of concentrates affects the effectiveness of thermal treatments.
4. Depleted shelf-life at room temperatures.
5. High content of minerals in the whey are responsible for undesired salty-sour flavour of whey.

Despite of the limitations of using whey in manufacturing beverages, whey is used on larger amounts due to the following reasons **(Chavan et al., 2015)**:

1. Whey is having a broad range of solubility i.e. from pH 3-8.
2. Whey is having a bland flavor and on higher temperature, casein they can act as carrier for the aroma compounds.
3. Buffering capacity of whey can be explored for survival of probiotic bacteria in the gastro-intestinal tract.
4. Addition of whey improves the 'mouthfeel' of the drink by increasing the viscosity of the beverage.
5. Whey can be also used to solve the problems associated with cloudiness of tropical fruit juices and produce a cloud stable juice.

2.1.5 Whey based beverages

Whey proteins hold high proteins efficiency ratio which makes them among one of the high quality food proteins, they are stable in acidic medium (4 pH) and compared to other proteins whey proteins are less chalky in a mouthful, which makes them highly appropriate for the emergence of value-added functional quenchers (**Beristain et al., 2006**). Whey also comprises a significant amount of nutritional values as it contains water-soluble vitamins like folic acid, cobalamin and riboflavin (**Irena et al., 2008; Naik et al., 2009; Sakhale et al., 2012**). A whey-based strawberry beverage was successfully developed from two different varieties of strawberries namely "Camarosa" and "Sweet Charlie" with different compositions of whey i.e... 25%, 50% and 100% (**Panghal et al; 2009**).

A successful attempt was done for the development of whey based guava beverage and with different temperature for pasteurization was used and the effect was studied (**Joshi et al., 2020**) Similarly whey protein is also utilized with different other fruit and herbal extracts like carbonated whey beverage using orange juice was developed (**Pareek et al., 2013**), utilization of whey was done in development of orange- whey beverage, pear whey beverage, peach-whey beverage, apple- whey beverage (**Djurić et al., 2004**), banana-whey beverage (**Koffi et al., 2005**), and pineapple- bottle gourd-whey mixed herbal beverage (**Balijeet et al., 2013**) were also developed. Whey based fruit beverages are more suitable for health as compared to other drinks (**Sarvana, 2005**). Whey and its biological components have proven its effects in treatments of cervical chronic diseases like cancer, cardiovascular, HIV etc. As it is nutritionally too rich, it can also be used in beverages of infant Geriatric and Athletic foods (**Devera, 2005**).

Watermelons also contain small amounts of phenolic compounds. Combining the health benefits of whey and watermelons, successful development of a nutritious low-calorie whey based watermelon beverage with high fiber content was done (**Saxena and Chakraborty, 2013**). Beverage manufacturers are taking a serious look at how to add valuable nutrition to their product and 'whey' is an ideal option. Hence, development of such a value-added product can provide a new outlet for the use of whey in a more nutritious product (**Seethalakshmi and Shankar, 2009**). Nowadays, there is a growing demand for functional fruit beverages as an alternative to

traditional beverages and soft drinks which provide specific taste profile and disease preventing properties beyond general nutrition, popularly known as functional beverages (**Kausar et al., 2012; Sharma and Tandon, 2015**).

Several researchers have suggested use of herbs/spices extract to enhance the palatability of fruit-based beverages (**Barwal and Sharma, 2001; Bhat et al., 2013; Sharma and Tandon, 2015**). Many efforts have been made in the development of beverages with the addition of fruit concentrates in order to produce a drink with acceptable sensory properties especially regarding flavour (**Koffi et al., 2005**).

(**Yadav et al., 2010**) conducted a study on development and storage of whey-based banana herbal beverage: (Ingale et al., 2009) developed a whey-based custard apple beverage. Similarly. (**Patel et al., 2006**) developed a whey-based mango herbal (ginger) beverage and (**Shekilango et al., 1997**) prepared whey-based beverage from acid whey and over ripe banana. Whey possesses preventive and curative elements and is particularly used to treat a wide variety of ailments such as arthritis, anemia and liver complaints (**Cruz et al., 2009**). Thus, technological approaches have been attempted by a large number of researchers (**Kravchenko, 1988**). The manufacture of whey based beverage requires the mixing of appropriate fruit juices and minimally processed whey with selection of suitable stabilizers and acidulants to develop acceptable whey based fruit beverages (**Singh et al., 2005**).

2.2 Guava

Guava is popularly known as “poor man’s apple” available in plenty at a low price during the season. It emits a sweet aroma which is pleasant sweet refreshing and a fleshy texture. Guava fruits are highly perishable in nature and they cannot be stored at room temperature for long time. Therefore to utilize the produce at the time of glut and save it from spoilage, the development of low cost *Corresponding author: Divya Singh processing for guava fruit is needed. Five commercially grown winter cultivators for guava viz. „Allahabadi safeda“, „Lucknow-49“, „Red fleshed“, „Chikkidar“ and „Apple colour“. The pulp of „Allahabadi safeda“ had the highest TSS content followed by „Lucknow-49“, „Red fleshed“, „Apple colour“ and „Chikkidar“ (**Divya et al., 2014**).

Guava is often marketed as "super-fruits" which has a considerable nutritional importance in terms of vitamins A and C with seeds that are rich in omega-3, omega-6 polyunsaturated fatty acids and especially dietary fiber, riboflavin, as well as in proteins, and mineral salts. The high content of vitamin C (ascorbic acid) in guava makes it a powerhouse in combating free radicals and oxidation that are key enemies that cause many degenerative diseases. The antioxidant virtue in guavas is believed to help reducing the risk of cancers of the stomach, esophagus, larynx, oral cavity and pancreas. The vitamin C in guava makes absorption of vitamin E much more effective in reducing the oxidation of the (bad) LDL cholesterol and increasing the (good) HDL cholesterol. The fibers in guavas promote digestion and ease bowel movements. The high content of vitamin A in guava plays an important role in maintaining the quality and health of eyesight, skin, teeth, bones and the mucus membranes **(Dattatreya et al., 2012)**.

Guava fruit consists of 20% peel, 50% flesh portion and 30% seed core. It also contains 74-84% moisture, 13-26% dry matter, 0.8-1.5% protein, 0.4-0.7% fat and 0.5-1.0% ash and the fruit is considered as an excellent source of vitamin C (299 mg/100 g) and pectin (1.15%) as reported by Wilson, (1980). The fruit has an appreciable amount of minerals such as phosphorus (23 - 37 mg/100 g), calcium (14 - 30 mg/100 g), iron (0.6 - 1.4 mg/100g) as well as vitamins like Niacin, Thiamine, Riboflavin and Vitamin A. The fresh guava has a short shelf life (one week), for its high moisture content **(Singh et al., 1990)**. The post-harvest losses occur to reach about 22% **(Bons and Dhawan, 2006)**. Guava fruit is normally consumed as fresh as a dessert fruit or in processed form as puree, juice, concentrate, jam, jelly, toffee, fruit flakes, squash, syrup, nectar, powder, wine, vinegar, ready to use snacks, drinks and dehydrated canned products **(Lalit et al., 2014)**.

Addition of guava adds nutritive value, flavour and medicinal properties and show great potential for processing into valuable products. It is highly perishable in nature leading to spoilage during storage.

Guava fruits beverage are cholesterol, saturated and sodium free, plus low in fat are taken. It contains key nutrients like: vitamin, carotenoids (vitamin A), folate, potassium, fiber, calcium and iron. Addition of guava which adds nutritive value, flavor and medicinal properties and show great potential for processing into

valuable products (**Zietemann et al., 2007**). It is useful in survey, cough and digestion. Because of availability of fruit in abundance during the season of production, it causes glut in the market. In addition to this fruit are highly perishable in a nature and there is a lot of spoilage in rainy season due to insect and rain. So the fruit is available at a very remunerative price during the season of processing.

It emits a sweet aroma which is pleasant sweet refreshing and a fleshy texture. Guavas are often considered as super fruits being rich in vitamins A and C in the pericarp, omega-3 and -6 polyunsaturated fatty acids in the seeds and especially have high levels of dietary fibre. A single guava fruit weighing 160-170 g contains over four times more of vitamin C compared to a single orange (220-230mg/100g) and also has adequate levels of dietary minerals, potassium, magnesium and an otherwise broad, low-calorie profile of essential nutrients (**Mahendran, 2010**). Single guava fruit without seed (90g) provides 46 kilocalories energy, contains 78% water, 5 gram dietary fiber, 1g fat, 11g carbohydrate, 1 g protein, 18mg calcium, 256 mg potassium, 9mg magnesium, 23 mg phosphorus, 71 RE vitamin A, 165 mg vitamin C and 1mg of niacin and vitamin E each (**Murdock, 2002**). Addition of guava adds nutritive value, flavor and meditational properties and show great potential for processing into valuable products. It is highly perishable in nature leading to spoilage during storage.

2.3 Papaya Leaf

Papaya (*Carica papaya* L.) member of family Caricaceae, usually called versatile horticultural plant or wonder fruit of tropical and subtropical regions is a large quick growing soft stemmed plant with large leaves (**F.Husin et al., 2019**). The different parts of papaya plant (the roots, stem, latex, leaves, fruits and seeds) are used for various medicinal purposes as claimed traditionally for the treatment of different ailments such as malaria, dengue, diabetes, etc (**C. Wiart, 2002**). The herbal leaves of *Carica papaya* are known to increase the antioxidant activity in the blood. Papaya leaf extract are rich in tannins, Flavonoids, glycosides, folic acid, vitamin B, Vitamin A and C, alkaloids, saponins, etc. and also possess anti-inflammatory, anticancer activity and protection against the oxidative damage (**Indra et al., 2008**). The juice of papaya herbal leaf acts as a synergistic therapeutic dietary supplement in patients with oxidative stress related diseases (**Parzarini et al., 2014**). Papaya leaf extract helps to increase platelet counts and shorten the hospitalization period during dengue fever

(Yunita *et al.*, 2012). The mechanism of action of *C. papaya* leaf extract has shown very good stabilizing properties to prevent lysis of platelets (Ranasinghe *et al.*, 2012). There are plenty of studies which reported that juice of papaya leaves increases the platelet production in the treated patient (Charan *et al.*, 2016). Thus, papaya leaf juice is considered to exert positive effect for the cure of many diseases (C.P. Kala, 2012). In traditional medicines, the leaves are added into tea to cure malaria while dry leaves are used as cigar (Yogiraj *et al.*, 2014). The papaya leaf drink encourages digestion and aid in treatment of ailment such as high blood pressure, chronic indigestion, obesity, overweight and weakening of heart (C.Mantok, 2005). In India, papaya leaves are being used specifically for treatment of fever, beriberi and asthma (Krishna Prabha *et al.*, 2014). Though, the papaya leaves are store house of many pharmaceutical properties yet these have not been utilized on a commercial scale for preparation of functional food products. Development of products like functional immune boosting beverages from papaya leaf can be an alternative for efficient utilization of leaves. However, bitter taste of the leaf extract makes the product unacceptable by the masses, Preparation of leaf extract and blending of extract with fruit pulps for preparation of different beverages is an alternative for utilization of leaf extract with good functional properties.

Carica papaya, commonly known as papaya, is a treelike herbaceous plant belonging to the family *Caricaceae*. It is cultivated globally, mainly for its fruit, but other parts of the plant are used for various purposes, and some have anti-bacterial properties (Navita *et al.*, 2019). Papaya leaves have been reported to reduce symptoms of asthma, worm infestations and dysentery (Parle M and Guruditta G., 2011) and to have anti-cancer properties (Noriko O *et al.*, 2010). *Carica papaya* leaf extracts are prescribed as a tonic for the heart and also for the treatment of fever, pyrexia, diabetes, gonorrhea, syphilis, and inflammation and for dressing infected wounds (Mehdipour *et al.*, 2006). Previous phytochemical analysis has shown the presence of flavonoids, alkaloids, carbohydrates, saponins, glycosides, phytosterols, phenolics, terpenoids and tannins in the leaves of *Carica papaya*. A few studies have reported an increase in platelet count after administration of PLE to dengue patients (Yunita *et al.*, 2012), but the effect of PLE on dengue virus infection *in vitro* has not been studied so far.

Several other studies have linked papaya leaf extract with health benefits, such as the healing of wounds (**Mahmood et al., 2005**), reducing cardiovascular disease risk (**Runnie et al., 2004**), anti-inflammatory activity (**Owoyele et al., 2008**), antitumour activity, alleviating allergic disorders serving an immune-adjuvant for vaccine therapy (**Otsuki et al., 2010**). As such, it is necessary to prepare papaya leaf powder using optimal extraction conditions, and further characterize its bioactivity for potential utilization by herbal practitioners and for commercial use. . Its complementary medicine uses have included healing of burns, relieving the symptoms of asthma, treating intestinal worms, treating gastric digestion problems, controlling fever and treating amoebic dysentery (**Canini et al., 2007; Starley et al., 1999; Zunjar et al., 2011**). The Papaya (*Carica Papaya*) plant has ample medicinal properties such as uterotonic, nephroprotective, anti-inflammatory, anti-tumour, etc. Many cohort studies have shown that the leaves of Papaya contain ample active components that can improve the overall antioxidant potential in the blood along with reduced lipid peroxidation levels; for example, it contains components like papain, chymopapain, tocopherol, flavonoids, cystatin, ascorbic acid, glucosinolates and cyanogenic glucosides (**Roshan et al., 2014**). In addition to this, the Papaya plant also produces natural compounds like annonaceous acetogenins in its Leaf bark along with twig tissues that have highly anti-tumour & pesticide properties. The presence of a greater number of natural self-defence factors in the tree gives the plant the resistance power against infestation of insect and diseases. Papaya leaf extract, or the tea made of it, has an immense reputation as a “tumour destroying agent”

In a recent study, a therapeutic whey beverage was developed using the Extract from Papaya leaves. Various levels of papaya leaf extract were tried at 15, 20, and 25 per cent, sucrose at 7, 10 and 15 per cent and Stevia at 1, 1.5 and 2 per cent. Based on the sensory scores of sensory evaluations by expert panellists, amongst all, 20 per cent papaya leaf extract, 15 per cent sucrose, and Stevia up to 1.5 per cent were ideal for preparing this therapeutic beverage (**Singh et al., 2021**)

According to a research article provided, the prevalence of viral infections, cancer, and diabetes is increasing at an alarming rate around the world, and these diseases are now considered to be the most serious risks to human well-being in the modern period. There is a widespread practice in Asian countries of using papaya leaves (*C.*

papaya L.) as herbal medicine, either alone or in combination with prescribed medications, to treat a variety of ailments. The importance of conducting the necessary descriptive studies in order to determine the safety of papaya leaf consumption is also emphasized in the context of their application in the healthcare sector. Electronic databases such as Google Scholar, Scopus, and PubMed were used to gather information on papaya leaves, their therapeutic potential, and clinical evidence-based studies. The literature was gathered from publications on papaya leaves, their therapeutic potential, and clinical evidence-based studies. The antidengue, anticancer, antidiabetic, neuroprotective, and anti-inflammatory effects of papaya leaves discussed in this article are supported by evidence from preclinical, in vivo, in vitro, and clinical trial studies, as well as from other sources. Leaves have been investigated for their mechanism of action as well as their potential to be used in the development of novel herbal products for the health business. According to the reports gathered, only a small number of research demonstrated that leaf extract at high concentrations was hazardous to certain organs. The collective literature reviewed in the review provides insights into the use of papaya leaves as a cure for epidemic diseases, highlighting the phytochemical composition and pharmacological attributes of papaya leaves, as well as the results of various preclinical and clinical studies that have been conducted so far on the subject. The review clearly demonstrated the successful medical evidence for the use of papaya leaf extracts in the healthcare system as a supplemental herbal medication in a variety of clinical settings (**Sharma et al., 2022**).

A successful attempt was done by (**Manoj et al., 2020**) for extraction of papaya leaf extract by using cold extraction and hot extraction method with and without water. Further, to overcome the harsh taste of papaya leaf juice, blending with mango pulp was done to optimize the best blend in order to prepare nectar with 20% fruit juice/pulp, 15 TSS and 0.3% titratable acidity. Standardized functional nectar was further analysed for chemical and sensory analysis and stored under ambient and low temperature conditions. The hot juice extract method with 20% water was optimized on the basis of best quality attributes. For preparation of nectar, mango pulp and papaya juice in 70:30 ratio was standardized. Chemically, the prepared beverage had 133.06mg/mL of total sugars, 42µg/100mL of ascorbic acid, 1.07mg GAE/mL of total phenols with 23.39 % antioxidant activity which did not show prominent

changes during 90 days of storage. Leaves of *C. papaya* have been reported quite effective in curing various diseases.

2.5 Coconut Sugar

Coconut sugar is prepared by concentration of inflorescence sap, popularly called as neera or Kalparasa, collected by tapping the unopened coconut spadix. The sap in its original form contains 14–16% sucrose which upon heating at 90–95°C turns into long threads followed by agitation or beating to form a crystalline or amorphous sugar. The coconut sugar is brown in color and contains 2–3% moisture. Unlike the commercially available sugars which are laden with high calories, palm-based sugars are rich in amino acids, vitamins, minerals, polyphenols and antioxidants. Moreover, the glycemic index (GI) of the sugar is relatively low making it a suitable healthy sweetener for all the age groups. A kilogram of sugar can be produced from around 7L of sap. A coconut tree can produce on an average 400–500 L of sap equivalent to 60–70 kg sugar year⁻¹. A tree which produces 100 nuts per year provides a glucose equivalent of ~ 38.5 kg in its economic yield, while tapping and producing sugar offers a nearly 3.5 folds high glucose equivalent of 133 kg. In addition to coconut sugar a number of second line products can be developed using the sugar as a base ingredient. As it is a healthy product it fetches premium price both in domestic and international markets. Thus, tapping trees and processing sap into sugar not only empower farmers but also generate enormous employment for the tappers, technicians and sales personnel and also assures a quality product to consumers.

In palms like coconut, the sap is tapped from unopened spadix. As it is a phloem sap, it is rich in sugars, proteins, amino acids, minerals, vitamins, and phenolics with immense antioxidant potential etc., therefore, healthier than most of the other juices of plant origin. Coconut produces around 10 to 14 spadix in a year depending upon its health and the prevailing climatic conditions of the region. On an average a spadix can produce 2 L sap day⁻¹ and a coconut tree can yield 400 L year⁻¹. If the sap remains unfermented, it can be sold as a healthy beverage or it could be effortlessly concentrated into syrup, jaggery, and brown sugar or on the other hand, the fermented sap is used in the preparation of toddy or wine. In the traditional method, where the sap is collected in an open earthen pot, due to long collection time (10–12 h) the sugar present in the sap degrades, initially into alcohol and gradually into acid,

emanating a harsh odour. It not only makes the sap unfit for consumption as a healthy beverage but also the fermented sap doesn't get crystallized while preparing the jaggery or sugar thus necessitating the use of chemicals. Therefore the collection of quality sap, which is a prime raw material for the production of coconut sugar, is of utmost importance in this process. Now license is being issued for tapping the sap, its processing and marketing either to individual entrepreneurs or farmer in states like Goa or to farmer producer organizations (CPC- Coconut Producer Company or CPF—Coconut Producer Federation) in states like Karnataka, Kerala, Tamil Nadu, and Telengana. Some of these entrepreneurs/CPCs have started producing coconut sugar by adopting the latest, and advanced scientific techniques as a commercial venture. Because of its high nutritive quality, low glycemic index (GI) palm sugar is branded as a premium product and fetches relatively high prices in domestic and international market. It ensures reasonably good monetary returns to the growers and has played a greater role in the employment generation as tappers, technicians and marketing personnel are involved in the production chain besides offering a nutritious, quality product to the consumers. At present Philippines, Indonesia and Sri Lanka are the major producers of palm sugar and their export destinations are USA and European countries where there is a huge demand (**Hebbar *et al.*, 2022**).

(Karseno *et al.*, 2018) conducted a study on effect of pH and temperature on browning intensity of coconut sugar and its antioxidant activity. In the study, the effect of pH and temperature on browning intensity and antioxidant activity of coconut sugar were examined. The pH of coconut neera was adjusted at 6 and 8 and the temperature of its heating was 100°C, 105 °C, 110°C, and 115°C, respectively. The browning intensity of sugar was determined by spectrophotometrically at 420 nm. Total phenolic content of sugar was estimated by FolinCiocalteu method and antioxidant activity was expressed as DPPH scavenging activity. It was concluded that browning intensity and antioxidant activity of sugars was increased with increasing pH of coconut neera and temperature. It was found that the effect of pH at 8 and temperature at 115°C show highest total phenolics (0.48%) and browning intensity (0.35) of sugar. The treatment also exhibited good antioxidant activity (DPPH scavenging activity) as high as 40%. The result also indicated that there is a significant correlation between browning intensity and antioxidant activity of coconut sugar.

Chapter 3

MATERIALS & METHODS

Various resources and techniques used during the study period are described in this chapter in chronological order.

3.1 Plan of work

The research was done in the Department of Dairy Science and Food Technology, Banaras Hindu University Varanasi, Uttar Pradesh, India. The thesis “**Development of Whey Based Guava Beverage Incorporated With Papaya Leaf Extract**” was done to make whey based guava beverage with added coconut sugar and Papaya Leaf extract (PLE).

Table 3.1: Raw materials for study

Raw Material	Source
Milk	Milk was procured from local dairy of Varanasi, U.P having 3% fat and 8% SNF
Guava	Guava was procured from Sunderpur market, Varanasi
Papaya Leaf Extract (PLE)	Papaya leaf was procured from Agricultural Farm of B.H.U
Coconut Sugar	Coconut sugar was purchased online from Flipkart of B&B Organics
Chemicals	All the chemicals used in this study were of Analytical Grade

The chemicals were procured from Sigma Chemicals Co. (St. Louise, M.O., USA); HiMedia Laboratories Pvt. Ltd., Mumbai, India; Fisher Scientific, Mumbai, India; Merck Specialities Pvt. Ltd., Mumbai, India.

3.2 Basic Laboratory Requirements

All glasswares, metal ware and containers used for the investigation were of good quality and intact. The basic requirements included glasswares like test tubes, petriplates, glass rods, beakers, conical flasks, flat bottom flasks, pipette, burette etc.

Other requirements included tubes (Tarson's Centrifuge Tubes), Eppendorf tubes, Test tube stands, Micropipettes etc.

Table 3.2: Equipment used for the study

INSTRUMENTS	MANUFACTURER
Analytical Balance	Metler Toledo, JB1603-C/Fact, Switzerland
pH meter	Thermo Scientific, Sn B21899, Singapore
Spectrophotometer	Shimadzu UV-1800, Japan
Laminar Air Flow	Labtech LCB 120V, Daihan Pvt. Ltd, India
Autoclave	Tony SX-500, UK
Incubator	Eyela Lti-700
Water Bath	Vision Scientific Co., Ltd
Micropipette	Tarsons
Refractometer	Belingham and Stanley, UK

3.3 Packaging Materials

The bottles of 300ml capacity were used as packing material for Whey Guava beverage incorporated with Papaya Leaf extract and coconut sugar. The bottles were purchased online via Flipkart.

3.4 Methodology

3.4.1 Milk

Standard pasteurized milk bought from the local dairy of Varanasi having 3% fat and 8% SNF was taken for the preparation of whey.

3.4.2 Preparation of Whey

Standardized milk was used for the preparation of good quality whey. Milk was heated to 80°C and when temperature decreased to 40 – 45°C, 2% citric acid solution (4-5ml per 3 litre of milk) gradually added followed by continuous stirring which resulted in complete coagulation of milk protein (casein). The liquid (whey) was filtered using muslin cloth and stored for further use. The flowchart of the process

adopted for the preparation of the whey is presented in Fig.3.1 and the composition of whey is mentioned in Table 3.1.

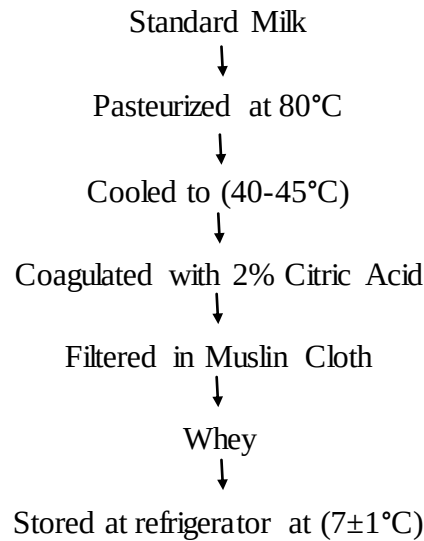


Fig 3.1 Flowchart of preparation of Whey (Source : Awsi and Dorcus 2012))

Table 3.3 Composition of Whey (Source: Judy A. Driskell. 2007. Sports Nutrition: Fats and Proteins. New York)

Constituent	Sweet Whey	Acid Whey
Water	93-94	94-95
Dry Matter	6-6.5	5-6
Lactose	4-4.5	3.8-4.3
Lactic Acid	Traces	≤ 0.8
Total Protein	0.8-1	0.8-1
Whey Protein	0.6-0.65	0.6-0.65
Citric Acid	0.1	0.1
Minerals	0.5-0.7	0.5-0.7

*Amount in (%w/w)

3.4.3 Guava

Allahabadi Safeda variety of guava was obtained from local Sunderpur market of Varanasi. It was washed thoroughly with water to remove foreign materials.

3.4.4 Preparation of Guava Pulp

With minor modifications, the Guava pulp was prepared according to the method described by (Singh *et al.*, 2014)

For the preparation of Guava pulp, fresh variety of Allahabadi Safeda was chosen. The fruits were washed under running water, to eliminate dirt and dust. Guavas were cut into small cubes and placed in screw type juicer to extract the juice/pulp. The flowchart of the process adopted for the preparation Guava juice/pulp presented in fig. 3.2 and the composition of Guava pulp/juice.

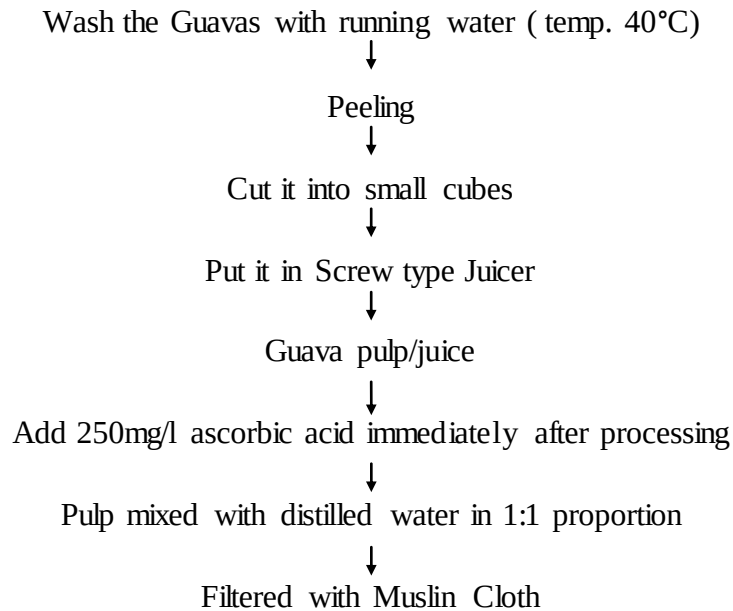


Fig 3.2 Flowchart of preparation of Whey (Source : Singh et al., 2014))

Table 3.4 Composition of Guava (Naseer et al.,2018)

Nutritional information (per 100g)	Approximate values
Carbohydrates	14.32g
Sugars	8.92g
Dietary fiber	5.4g
Fat	0.95g
Protein	2.55g
Vitamin A	31µg
Thiamine	0.067mg
Riboflavin	0.04mg
Niacin	0.084mg
Pantothenic acid	0.451mg
Vitamin B6	0.11mg
Folate (B9)	0.49µg

Vitamin C	228.3mg
Vitamin K	2.2µg
Calcium	18mg
Potassium	417mg

3.4.5 Coconut Sugar

Coconut sugar was used as substitute to sugar and also for its low glycemic index. The coconut sugar was used to prepare the whey guava beverage incorporated with Papaya Leaf Extract and Coconut Sugar and was purchased online from Flipkart of B &B Organics. The composition of coconut sugar is given below.

Table 3.5 Composition of Coconut Sugar

Nutritional Information (per 100mg)	Approximate Values
Energy (Kcal)	381.84
Fat (g)	0.1
Protein (g)	1
Sodium (mg)	607.6
Potassium (mg)	959.1
Carbohydrate (g)	95.46

3.4.6 Papaya leaf

Fresh green papaya leaves of *Pusa Dwarf* were collected from local papaya orchard in Bhagwanpur, Varanasi, India. Leaves were washed in running tap water followed by shade drying to remove surface moisture. The leaves were cut into smaller sizes for easy blending for both Hot and Cold Extraction methods. The flowchart of the process adopted for the preparation of papaya leaf extract is presented in Fig 3.3 and in Table 3.5.

HOT EXTRACTION

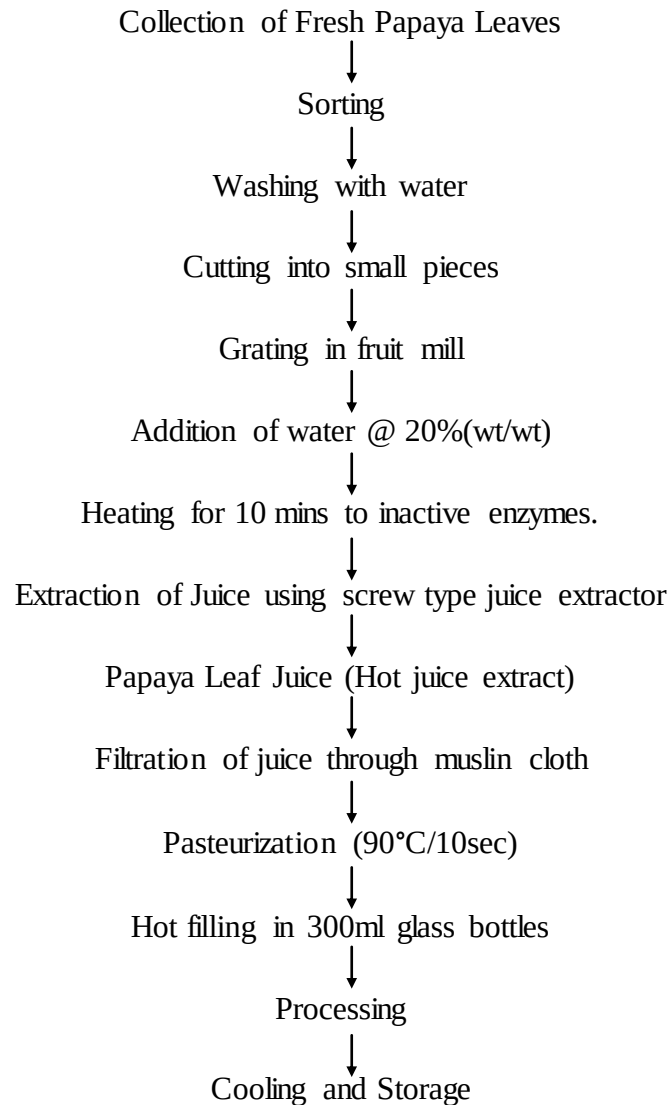
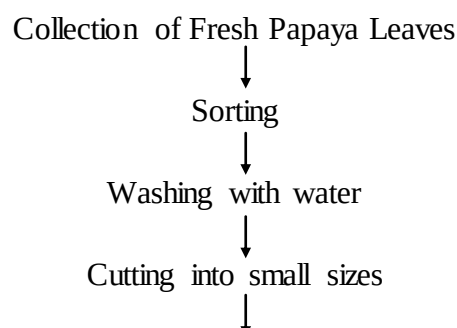


Fig 3.3 Flowchart of preparation of Papaya Leaf Extract (Hot Extraction) (Source : *Monoj et al., 2020*)

COLD EXTRACTION



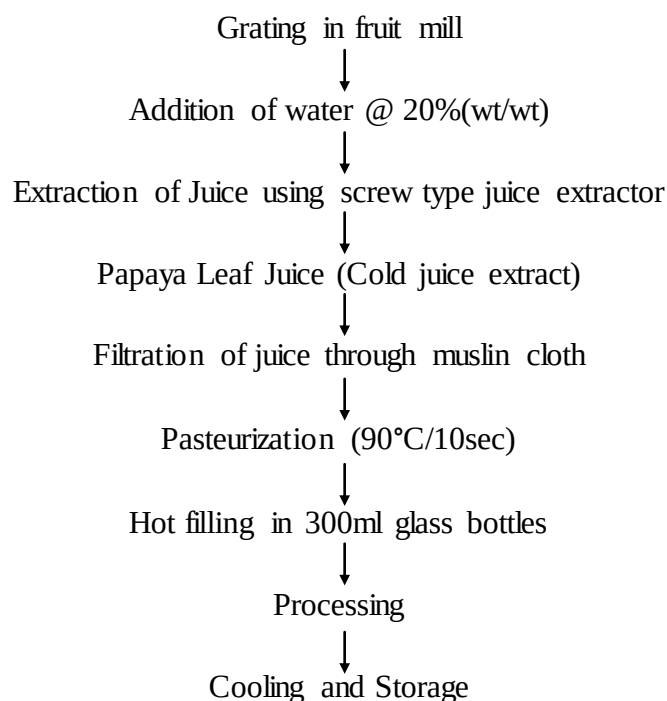


Fig 3.4 Flowchart of preparation of Papaya Leaf Extract (PLE) (Source : Monoj et al., 2020))

3.5 Product Development

The samples of whey and guava pulp were then optimized at different levels. Ascorbic acid also inhibits the pectinolytic enzymes and maintains the fresh guava aroma, possibly by preventing oxidation of volatile aldehydes. The various blends of whey guava using the constant amount of ascorbic acid 0.3% were in the ratio of 2:1. The highest score for color, taste, aroma and appearance was given to beverage containing guava juice and whey in 9 different proportions. Among the 9, T5 was chosen the best and was used for further processing.

Table 3.6 Formulation Combinations of Whey Guava Beverage

Formulations	Whey (ml)	Guava juice (ml)
T1	100	-
T2	82.5	5
T3	77.5	10
T4	72.5	15
T5	67.5	20
T6	62.5	25
T7	57.5	30
T8	52.5	35
T9	47.5	40

3.6 Development of Whey Guava Beverage

Various combinations were made which were labeled as T1, T2, T3, T4, T5, T6 and on the basis of sensory evaluation scoring (9- point hedonic scale rating), development was recorded. It was concluded that T4 labelled product had got higher acceptance than others. 1% Sodium Alginate as stabilizer was used after the addition of coconut sugar. The beverage was heated slightly for the proper mixing of the contents added into it. After that the beverage prepared was homogenized using a homogenizer at 3000 RPM. Based on the sensory evaluation, the three optimized formulations were further used for the optimization of Papaya Leaf extract to be incorporated.

Table 3.7 Formulation Combinations of Whey Guava Beverage with Coconut sugar

Formulations	Whey (ml)	Guava juice (ml)	Coconut Sugar(%)
T1	67.5	20	5
T2	67.5	20	10
T3	67.5	20	15
T4	67.5	20	20
T5	67.5	20	25
T6	67.5	20	30

3.6.1 Development of Whey Guava Beverage Incorporated with Papaya Leaf Extract & Sensory Analysis

The three formulations were chosen T3, T4 & T5 having 15%, 20% and 25% of coconut sugar was added with 1%, 2% 3% 4% PLE extract were served in front of a

panel of semi trained judges and were asked to score the treatments in respect to its color and appearance, flavor, taste and overall acceptability, on the basis of 9-point hedonic scale and samples were labeled in order to prevent biasness. The scores assigned by the judges were then statistically analyzed. Best out of 4 were then considered for comparison with control having no PLE extract added in further scoring in which judges were asked to assign sensory scoring to compare the experimental product with control. Codes were assigned to samples so as to avoid identification and bias.

Table 3.8 Formulation Combinations of Whey Guava Beverage with PLE Extract

Formulations	Whey (ml)	Guava juice (ml)	Coconut Sugar(%)	Papaya Leaf Extract (%)
T1	67.5	20	15	1%
T2	67.5	20	15	2%
T3	67.5	20	15	3%
T4	67.5	20	15	4%
T5	67.5	20	20	1%
T6	67.5	20	20	2%
T7	67.5	20	20	3%
T8	67.5	20	20	4%
T9	67.5	20	25	1%
T10	67.5	20	25	2%
T11	67.5	20	25	3%
T12	67.5	20	25	4%

It was concluded that the formulation T8 having 20% coconut sugar 4% PLE extract was best and was chosen for further physiochemical and microbial analysis.

3.7 Proximate Analysis

3.7.1 Moisture

Association of Official Analytical Chemists method (AOAC, 1995) was considered for the calculation of the moisture content of whey guava beverage incorporated with Papaya leaf extract (PLE). 5 g of each sample were taken in Petri dish and were kept in an oven (Hot Air Oven, 992, Perfit, India) at 105°C for 3 hours. Reading of each petri dish was repeated until a constant value was obtained. Using a formula, the moisture content was calculated as the percentage of the dry weight of the sample.

Calculation:

$$M \% = (W1-W2) / W \times 100$$

Where,

M is Moisture,

W1 is Weight of Petri-dish along with sample before drying,

W2 is Weight of Petri-dish along with samples after drying.

3.7.2 Total Solid

Total solid was calculated as a method recommended by **Ranganna, (2001)**. The weight of residue obtained from moisture content analysis was expressed as percentage total solids using the formula mentioned below

Calculations:

$$\text{Total solid (\%)} = \frac{\text{Weight of Dish} + \text{Dry weight of sample}}{\text{weight of sample}} \times 100$$

3.7.3 Titratable Acidity

Acidity of various samples were determined using the method as recommended by **Ranganna, (2001)** . 10 ml of sample was taken and volume was made up to 100 ml using distilled water. 10 ml of aliquot was taken and titrated against 0.1 N NaOH using a few drops of phenolphthalein solution as indicator. The end point was noted by the appearance of pink colour. Titrated value was noted and result was calculated as % total acid using the following formula,

Calculations:

$$\text{Acidity (\%)} = \frac{\text{Titre value} \times \text{Normality} \times \text{Eq. wt. of Lactic acid} \times \text{volume made up}}{\text{Weight of sample taken} \times \text{Sample taken for estimation} \times 1000}$$

$$\text{Weight of sample taken} \times \text{Sample taken for estimation} \times 1000$$

3.7.4 Ash Content (AOAC, 1980)

5 g of completely homogenized sample (Vortex shaker, MSW-308 (Deluxe Model), Micro Scientific works Pvt. Ltd, Delhi) was taken accurately in moisture free silica crucible. The crucibles were then placed on a hot plate at 130 °C till smoke disappeared. The crucibles were then placed in a muffle furnace (Muffle Furnace, SNOL 8.2/1100-1LZ, Pagaminta Lietuvoje, Lithuania) at 550°C for 6 hours. Crucibles were cooled down in desiccators and weights. (Electronic weighing balance, Labtech LCB 1201 v, Daihan Lab Tech India Pvt.Ltd.) of the crucibles were noted down at room temperature.

Calculations:

$$\text{Ash\%} = \text{Weight of ash (g) / Weight of sample taken} \times 100$$

3.7.5 pH

pH is the measurement of the logarithm of inverse of hydrogen ions in the solution.

$$\text{pH} = -\log[\text{H}^+]$$

Where, H^+ Hydrogen ion Concentration (g/lit)

The pH values were determined with the help of electronic pH meter. The electronic pH meter was calibrated using 7 pH and 4 pH standard buffer solutions. The function selector switch was set to pH and reading of digital display was allowed to get stable before it was noted.

3.7.6 Determination Total Soluble Solids (%)

TSS is an index of soluble solids present in fruits and vegetable. The TSS content of the samples was determined using the refractometer (Bellingham and Stanley, UK) and the results were expressed as percentage by weight. Sample was thoroughly blended in a blender and it was filtered through a whatman filter paper. A few drops of the clear homogenate was taken on the prism of the refractometer and direct reading was observed on the screen as per the method of AOAC (1994).

3.8 Physiochemical Analysis

3.8.1 Protein Determination

The protein content of the samples were estimated by following the protocol of AOAC (2000).

3.8.1.1 Digestion

The system was switched ON, and the digestion unit was pre-heated up to 350°C. 0.2ml sample was weighed (W) Electronic weighing balance, Labtech LCB 1201 v. Daihan Lab Tech India Pvt.Ltd in the filter paper and wrapped inside it, in triplicates. The sample was taken in a 250ml Macro DTL tube. Then to the sample, 10ml conc. H₂SO₄ was added, followed by addition of 5g of catalyst mixture [(5:1) (Potassium Sulphate: Copper Sulphate). The sample tube was then loaded in the digestion unit with manifold and KEL FLOW(E-Exlite, Pelicans Equipments)etup. Tap water was connected with maximum pressure for KEL FLOW(E-Exlite, Pelican Equipments). The temperature was increased to 420° C. Digestion was carried out till a clear green color appeared. According to the sample, ninety minutes were taken for digestion. Then the digested mixture was cooled on the cooling rack for approximately thirty minutes.

3.8.1.2 Distillation

The system was switched ON and the solutions viz. 4% Boric acid, 40% Alkali and 0.1N HCL were prepared and kept. The Alkali, Boric acid and KMnO₄ were loaded to the system through silicon hoses provided at the back of the equipment while waiting for READY signal. In a 250ml conical flask 25ml Boric acid was taken with an indicator and placed at the receiver end. The sample was diluted with distilled water (dilution 10ml to 20ml). Then the sample tube was loaded on the sample side. Before starting the sample testing, the water was allowed to flow through the system for cooling purposes (check the INLET and the OUTLET). The sample testing was started after the READY signal appeared. 40ml of the 40% Alkali was added to the above (until dark brown color appears). Then the process started. During the process, liquid ammonia was collected into Boric acid and its color changed according to the

indicator used After the completion of the process the conical flask was removed from the receiver end and then titrated. The tube from the sample side was now removed.

3.8.1.3 Titration

IN HCL was taken in the burette and titrated first against blank and then against the sample.

Tire value was noted down.

Calculation

$$\% \text{ Nitrogen} = \frac{14.01 \times 0.1N \times (TV - BV)}{W \times 1000} \times 100$$

$$\% \text{ Protein} = \% \text{ N} \times 6.25 \text{ (for food samples)}$$

Where,

TV = Titre value

BV = Blank value

W = Sample weights

3.8.2 Fat Analysis

Fat was determined by the Gerber method using butyrometer. For this, 10 ml of conc. Sulphuric acid (Sp. Gr. 1.820-1.824 at 27 C) was transferred into a milk butyrometer using automatic tilt measure taking due care as not to wet the neck of the butyrometer. The thoroughly mixed sample of (WGB) was added slowly using 10.75ml pipette followed by 1ml of amyl alcohol (Sp. Gr. 0.803- 0.805) using an automatic tilt measure. The mouth of the butyrometer was firmly stopped and contents were shaken well by applying gentle swirling motion until most of the organic constituents were dissolved.

The contents were warmed in a water bath (Water bath, VS-1205 wp, Vision scientific comapny, Limited, Korea) (maintained at 70 C-Z C) for 5 minutes. The butyrometer, after wiping it dry, was subsequently loaded in a Gerber's centrifuge (Gerber centrifuge, Nova 127. Chadha Sales Pvt. Ltd, Delhi) for 5 minutes at 1200

rpm. Again, the butyrometer was transferred to the water bath (Water bath, VS-1205 wp, Vision scientific company, Limited, Korea (70 C) for 3 minutes. Before recording the reading, the fat column was adjusted in such a way that it makes it readable.

3.8.3 Total Phenolic Content

25 ml of 0.2 N Folin- Ciocalteu reagent was taken and 0.5 ml of diluted extract was added in it and kept for 5 minutes and then 2 ml of 75g/L of Na:CO₃ was added and this prepared solution was kept for incubation at room temperature for 2 hours. Using a 1 cm cuvette UV 1800 spectrometer (Spectrophotometer, UV-1800, Shimadzu corporation, Japan) (Shimadzu Japan), absorbance was taken at 760 nm.

For obtaining the standard calibration curve, Gallic acid (0-800 mg/L) was used. The total phenolic content was determined as mg of Gallic acid equivalent (GAE)/ml of extract after using the dilution factor in the formula. **(Stankovic, 2011).**

3.8.4 Antioxidant Activity (DPPH Inhibition)

Determination of antioxidant activity of the sample was done by the DPPH inhibition method (Nishino et al. 2000). 2 ml Sample was taken in 25 ml ethanol and was kept overnight in dark at room temperature. 0.2 ml of eluted extract was taken and to it 1 ml of DPPH solution (80 ug/ml ethanol) was added.

A control was set up with 0.2 ml distilled water as blank and left at room temperature for 30 min. The sample sets were centrifuged (Centrifuge machine, 3-30K, Sigma, Germany) at 3000 rpm for 15 min. In the cuvette 0.5 ml of centrifuged solution was taken and 1ml of ethanol was added. Absorbance was taken at 517 nm (Spectrophotometer, UV-1800, Shimadzu corporation, Japan) separately for blank and samples using ethanol as reference.

$$\% \text{ DPPH Inhibition} = \frac{A_n - A_s}{A_s} \times 100$$

Where,

A_B = OD for Blank

$A_s = \text{OD for Sample}$

3.8.5 Determination of Ascorbic Acid (mg/100g)

Ascorbic Acid of various samples were determined using the method as recommended by **Roe (1954)**

3.8.5.1 Reagents

Metaphosphoric acid (HPO₃) solution (3%)

Prepared by dissolving the 3 gm of metaphosphoric acid stick in 100 ml of distilled water.

3.8.5.1 Dye solution

52 mg 2, 6-dichlorophenol indophenol was Dissolve on 150 ml of distilled water, containing 42 mg of Sodium Bicarbonate and make the volume of 500ml.

3.8.5.2 Ascorbic acid standard

Dissolve 100 mg of L-ascorbic acid in 3% (w/v) Metaphosphoric acid and makeup the final volume with metaphosphoric acid to 100 ml with same solution. 10 ml of this stock solution was diluted to 100 ml with 3% metaphosphoric acid (0.1 mg ascorbic acid/ml).

3.8.5.3 Standardization of the dye

25 ml of the standard ascorbic acid solution and 3 percent metaphosphoric acid each was taken in volumetric flask and was titrated with dye solution filled in the microburette, until pink colour persist for 10 second. Dye factor was calculated (mg of ascorbic acid/ml of dye) as follows.

$$\text{Dye Factor} = \frac{0.5}{\text{Titer value}}$$

3.8.5.4 Sample preparation and titration

10 ml of sample was taken and made up to 100 ml with 3 percent metaphosphoric acid and filtered. 10 ml of filtrate was pipet out into a conical flask and was titrated with standard dye till pink red end point appears.

Ascorbic Acid =

$$\frac{\text{Titer value} \times \text{Dye Factor} \times \text{Volume made up} \times 100}{\text{volume of filtrate taken} \times \text{volume taken of sample for titration}}$$

3.8.6 Sugar (%)

Sugars were determined by Lane and Enyon method as described by **Raganna (1977)**

3.8.6.1 Reagents

- 1) Fehlings solution A: Copper sulphate 69,28g and volume made up to 1 litre
- 2) Fehlings solution B: Potassium sodium tartarate 346g and sodium hydroxide 100g and volume made up to 1 litre,
- 3) Methylene blue indicator: 1% Aqueous methylene blue.
- 4) Neutral Lead Acetate (45%) solution.
- 5) Potassium oxalate (22%) solution
- 6) Standard invert sugar solution AR sucrose 9.5g and concentrated HCL 5 ml and volume made up to 100ml The solution was allowed to stand for further three days at 20-25°C for inversion to take place and could be used for 8.9 months during analysis.

25 ml of invert sugar solution was taken into flask and 50 ml of distilled water added and then neutralized with 20% of NaOH in the presence of phenolphthalein as an indicator, until the solution turned pink then acidified with IN HCL till pink color disappears. The volume was made up to the mark with distilled water. (1ml - mg of invert sugar)

3.8.6.2 Reducing sugar

Reducing sugar was estimated by taking 25 ml of ready sample and diluted with 100 ml of distilled water, 1 to 2 drop of phenolphthalein was added and titrated with 1 N

NaOH till pink color appears. 2ml of Lead acetate solution was added it was shaken and allowed to stand for 10 minutes. Then 2ml of Potassium Oxalate solution was added and volume make upto 250 ml was made and then filtered with filter paper.

In 250 ml of conical flask, Fehlings solution A and B were Taken. Burette was filled with clarified sugar solution In an open flame the conical flask was heated in hot condition only, 2-4 ml sugar solution solution was poured and 1-2 drops of methylene blue indicator was added immediately. The end point Appeared with d brick color. The reducing sugar was expressed as percent and calculated by following formula

$$\text{Reducing sugar (\%)} = \frac{\text{Invert sugar (mg)} \times \text{Dilution} \times 100}{\text{Titre} \times \text{Weight or volume of sample} \times 1000}$$

3.8.6.3 Total sugar (%)

The total sugar was estimated by taking 50 ml aliquot of clarified and deleadaded solution. In it 5 ml HCL added and allowed to stand for 24 hours at room tempreature. This has been neutralized with concentrated NaOH solution and volume made up to 250 ml. An aliquot was taken and total sugar were determined as invert sugars.

$$\text{Total as invert sugar (\%)} = \frac{\text{Invert sugar (mg)} \times \text{Dilution} \times 100}{\text{Titre} \times \text{wt or volume of sample taken} \times 100}$$

Sucrose (%) = (% Total Invert sugar- % Reducing sugar originally present x 0.95)

Total sugar (%) = Reducing sugar (%) + Sucrose (%)

3.8.6.4 Non-Reducing Sugar

Non-Reducing sugar is determined by subtracting the value of reducing sugar from the total sugar.

Non-Reducing sugar (%) = Total sugar (%) - Reducing sugar (%)

3.9 Microbial Analysis

3.9.1 Total Plate Count

Microbial analysis was done to determine the total plate count (TPC) of the samples on the nutrient agar media for bacterial count by the method recommended by

Harrigan and McCance (1966). Nutrient agar media was prepared and the samples were serially diluted up to 10^{-6} dilution factor. 1 ml of the samples, suspended in 9ml saline solution, was transferred to the respective petridishes of nutrient agar media. Three replicates were taken for each dilution. The inoculated petridishes were incubated in a BOD incubator for 48 hours at 37°C and bacterial colonies were calculated by the following formula:

$$\text{TPC (cfu/ml)} = \text{No. of colonies} \times \text{dilution factor} / \text{volume of the culture plate}$$

3.9.2 Coliform and Yeast Mould Count

The microbiological analysis of the whey based guava beverage was carried out as per the standard method for coliforms, yeast and molds using the violet red bile agar (VRBA) and potato dextrose agar (PDA), respectively. A lg of sample was diluted in 9ml of buffer blanks and subsequent dilutions were prepared up to 6 dilutions.

The number of microbial counts was calculated using the following formula:

Calculations:

$$\text{No. of Microorganisms/g} = \text{No. of colonies} \times \text{dilution factor} / \text{Weight of aliquot taken(ml)}$$

3.9.2.1 Enumeration of coliforms: The fourth and sixth dilutions of beverage samples which were prepared, has been used for enumeration of coliforms. The 1 ml from fourth and sixth dilution were taken in duplicate into Petri plates and the violet red bile agar was added and mixed well. The plates were allowed to solidify. The plates were again over layered with the same agar and allowed to solidify. These plates were incubated at 37°C for 24 h and numbers of coliform colonies developed were counted as colony forming units (c. f. u.) per gram.

3.9.2.2 Enumeration of yeast and mould (YMC): The fourth and sixth dilutions of all beverage samples were taken in duplicate into Petri plates and then potato dextrose agar was added by adjusting pH 3.5 by adding 5 ml of 10% sterilized tartaric acid. The plates were allowed to solidify and incubated at 37°C. Numbers of yeast and mould colonies developed were counted as colony forming units/gm.

3.10 Sensory analysis

The sensory properties of the Whey Guava beverage were assessed by a semi-trained sensory panel made up of 7 judges recruited from the centre's personnel and doctorate students. Semi-trained panelists were asked to assign a score based on a 9-point hedonic sensory scale given. Color and appearance, flavor, consistency, sweetness, and overall acceptability are all factors in the sensory evaluation of beverage.

The experimental trials were conducted and responses were fitted in the design. After that each individual experiment's responses were analyzed to assess the effect of independent variables on them. The desired goals for each factor and response were chosen. The goal may be applicable to either factor or responses. The possible goals or constraints are maximize minimize, target, within range, none (for response only) and set to an exact value (for factor only). In order to search a solution maximizing multiple responses, the goal were combined into an overall composite function called the desirability function.

Hedonic Scale

Organoleptic Score	Scale (Rating)
9	Liked extremely
8	Liked very much
7	Liked moderately
6	Liked slightly
5	Neither liked nor dislike
4	Disliked slightly
3	Disliked moderately
2	Disliked very much
1	Disliked extremely

3.11 Storage Study:

The samples were stored at refrigeration temperature (4-7 °C) and were monitored at 7 days interval upto 28 days. The samples were analyzed for change in pH, % TA

sensory evaluation scores, Total Phenolic content and % DPPH during storage. Microbiological analysis was also carried out at different time intervals.

3.12 Cost Estimation

The cost economics of whey based guava beverage incorporated with PLE extract and coconut sugar will work out taking into account the cost of raw materials, processing and labour cost, i.e fixed and variable cost.

3.13 Statistical Analysis

Data obtained during the investigation were suitably analysed by employing statistical tests such as Analysis of Variance (ANOVA) to know the significance difference between different combinations.



Fig 1: Guava



Fig 2: Chopped Guava Pieces



Fig 3: Screw Type Juicer



Fig 4: Guava Pulp Obtained

Fig 3.1 Guava Pulp Obtained

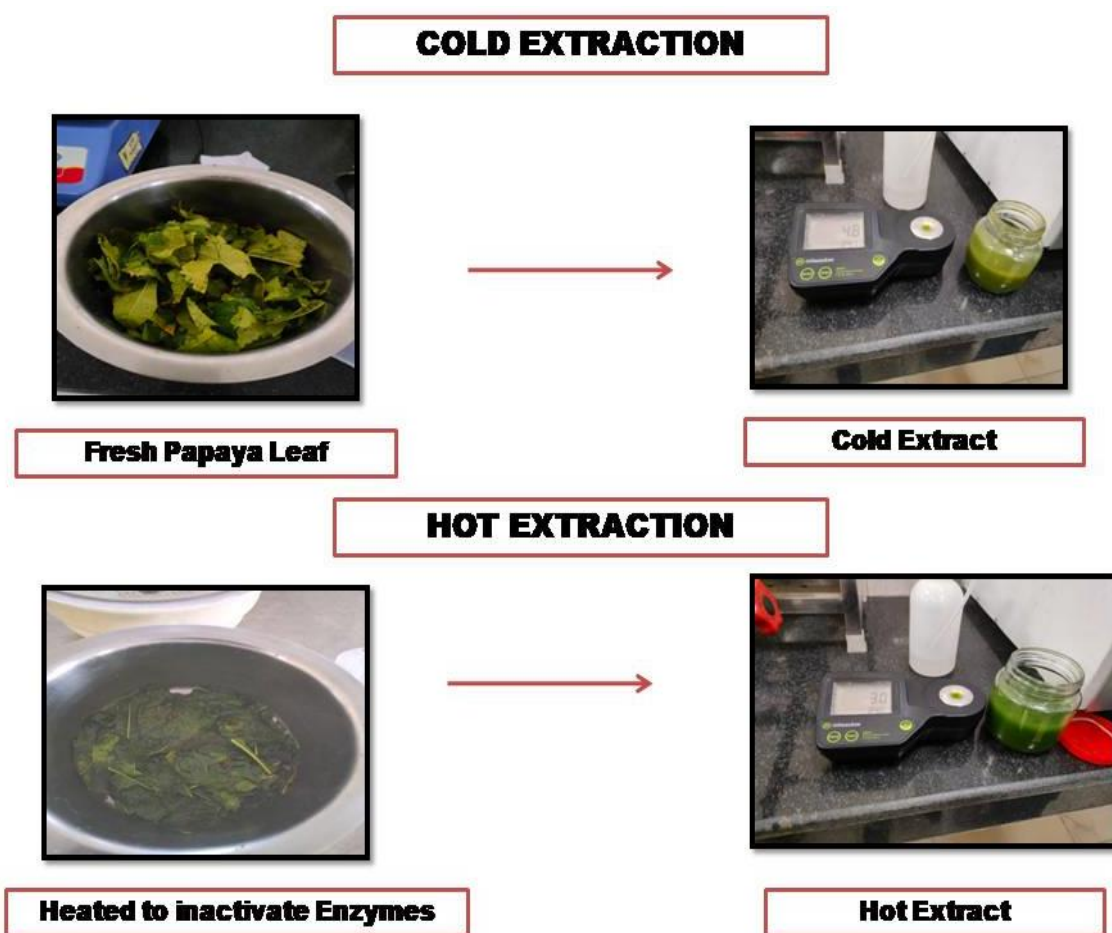


Fig 3.2 Method for Hot & Cold Extraction

RESULTS & DISCUSSION

The present investigation were carried out on the “ **Development of Whey based Guava Beverage incorporated with Papaya Leaf Extract**”. To fulfill the objectives of the study, experiments were conducted at department of Dairy Science and Food Technology laboratory, Institute of Agricultural Science located at the main campus of Banaras Hindu University, Varanasi (Uttar Pradesh). The results obtained along with relevant discussion pertaining to the study are presented in this chapter along with appropriate tables and illustrations.

4.1 Papaya herbal leaf juice extraction

The extraction of papaya herbal leaf juice extraction was done based on two extraction techniques viz. Hot Extraction Method and Cold Extraction Method. Based on the comparison between total phenolic content and antioxidant property of both the extracts, the hot extract was found to have more phenolic content and antioxidant property as compared to cold extract. Hence hot extract was chosen ideal for further process to carry on.

Table 4.1: TPC & DPPH of Hot PLE Extract and Cold PLE Extract

FORMULATIONS	DPPH (%)	TPC(mgGAE/100ml)
Cold Extract	65.030±0.006	6.000±006
Hot Extract	75.030±0.006	6.140±006

(The values are mean ± standard deviation of three set of values.)

4.2 Sensory Evaluation of Product Optimisation

The acceptability of the Whey based Guava beverage incorporated with different concentrations of PLE extract was measured in terms of color and appearance, flavor, consistency and overall acceptability on the basis of 9-point hedonic scale rating. The mean values assigned to all 12 treatment combinations by the 12 judges during evaluation were noted. T8 composed of 67.5ml whey, 20ml guava juice, 20% coconut sugar and 4% PLE extract got the highest acceptability with respect to color and appearance (8.567±0.087), taste (8.667±0.145), flavor (8.667±0.145), consistency

(8.567±0.120) and overall acceptability of (8.567±0.120). T1 was least accepted with overall acceptability score of (4.333±0.176). The result of the experiment is presented in Table 4.2.

Table 4.2: Sensory evaluation for the optimization of Product

Formulations	Color & appearance	Flavour	Taste	Consistency	Overall acceptability
T1	4.333± 0.176	4.233±0.145	4.333±0.176	5.167±0.088	4.333±0.176
T2	4.267±0.176	5.233±0.145	4.300±0.208	5.400±0.115	4.300±0.208
T3	4.100±0.058	4.567±0.285	3.333±0.176	5.500±0.115	4.600±0.115
T4	4.267±0.145	4.600±0.115	3.467±0.145	5.233±0.145	4.767±0.088
T5	5.167±0.088	4.767±0.088	4.600±0.115	6.467±0.088	5.400±0.115
T6	5.400±0.115	5.500±0.115	4.767±0.088	6.533±0.088	5.500±0.115
T7	6.467±0.088	6.400±0.115	5.500±0.115	6.467±0.120	7.267±0.186
T8	8.567±0.087	8.667±0.145	8.667±0.145	8.567±0.120	8.567±0.120
T9	7.433±0.088	6.533±0.088	6.533±0.088	7.333±0.088	6.533±0.088
T10	7.267±0.186	6.467±0.120	5.233±0.145	7.133±0.088	6.467±0.088
T11	6.700±0.115	7.333±0.088	5.167±0.088	7.433±0.088	6.700±0.115
T12	6.400±0.115	7.133±0.088	5.400±0.115	7.267±0.186	6.467±0.088
C.D.	0.378	0.406	0.408	0.337	0.386
SE(m)	0.129	0.138	0.139	0.115	0.132
SE(d)	0.182	0.196	0.197	0.162	0.186
C.V.	3.803	4.028	4.717	3.036	3.858

Above tabulated data is the mean value assigned to all 12 treatment combinations by 12 semi trained judges during evaluation.

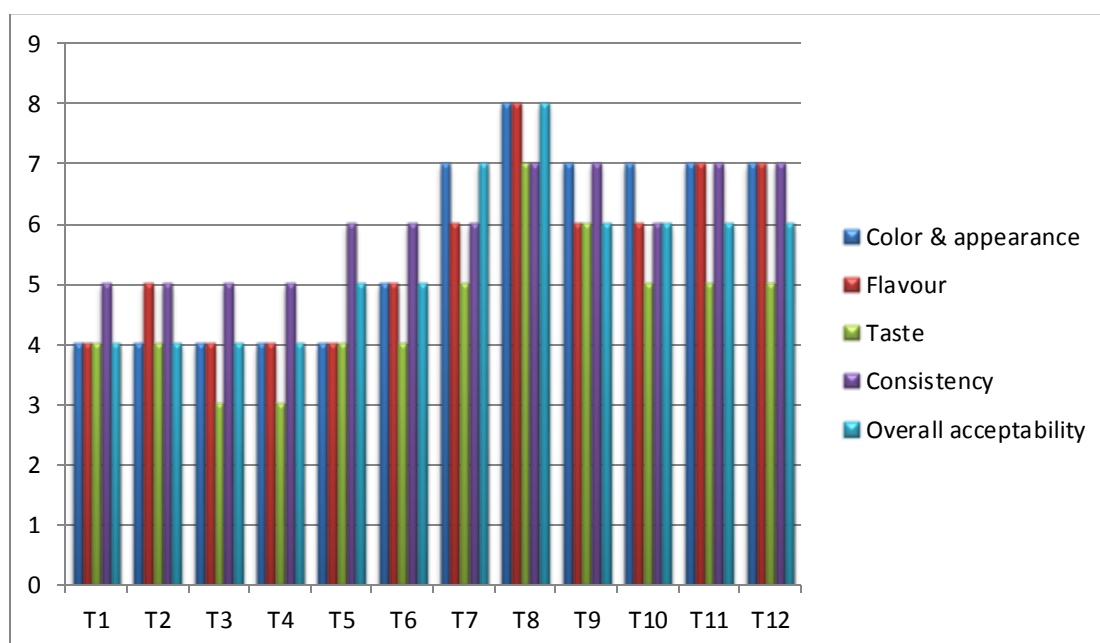


Fig 4.1: Sensory evaluation of different formulations

4.2.1 Color and Appearance

Table no.4.2 showed that T8 (67.5ml whey, 20ml guava juice, 20% coconut sugar and 4% PLE extract) treatment had highest value in sensory analysis for color and appearance which was 8.56 followed by T9 and T10 having score 7.4 and 7.2 and the lowest value was found in T3 having 4.1 score followed by T1, T2 and T4 which was 4.3, 4.2 and 4.2.

4.2.2 Flavour

Sensory score for this trait was highest in treatment T8 (67.5ml whey, 20ml guava juice, 20% coconut sugar and 4% PLE extract) treatment had highest value in sensory analysis for flavour which was 8.66 followed by T12 and T11 having values 7.13 and 7.33. Lowest values were found in T1, T4 and T2 having values 4.23, 4.56 and 5.23 respectively.

4.2.3 Taste

From the tabulated values of sensory score in table 4.1, it was considered that sensorial score of 8.66 value for the T8 (67.5ml whey, 20ml guava juice, 20% coconut sugar and 4% PLE extract) sample was highest for this parameter whereas, T3 with

sample had lowest value of sensory analysis which was 3.33. Sensory score for the other remaining samples fluctuated from 4.23 to 7.13.

4.2.4. Consistency

This parameter was used for an examination of viscosity of prepared samples. From obtained results it was observed that T8 (67.5ml whey, 20ml guava juice, 20% coconut sugar and 4% PLE extract) treatment had highest value in sensory analysis with score 8.66. Lowest values were found in T1 followed by T2 (5.16 and 5.4).

4.2.5 Overall Acceptability

Overall acceptability is the summary of all above mentioned parameters. It denoted the chances of acceptance of product by consumers. Highest overall acceptability shows that the product had highest consumer acceptance. So, from the whole analyzed data of sensory analysis of samples it was clearly shown that T8 (67.5ml whey, 20ml guava juice, 20% coconut sugar and 4% PLE extract) sample (8.56) of Whey Guava beverage was liked by most panelists and most disliked sample was T1, T2 & T3 with 4.33, 4.30 and 4.6 of sensorial analysis.

4.3 pH

In the analysis of pH at zero days recorded in table during chemical analysis, the pH of whey guava beverage incorporated with PLE extract varied from 3.39-4.15 and there was not much difference among the samples and pasteurization temperatures and timings did not affect the beverage. No significant changes were seen in the pH of the beverage upon the addition of PLE extract. But during the storage period the pH of the beverage was slightly decreased (**Divya and Archana, 2009**) reported the similar result of whey guava beverage, where the pH ranged from 3.83 to 4.20. (**Nirankar, 2004**) reported that pH of whey protein enriched beal fruit juice beverage ranged from 3.93 to 3.95 with the slight increase in acidity during storage.

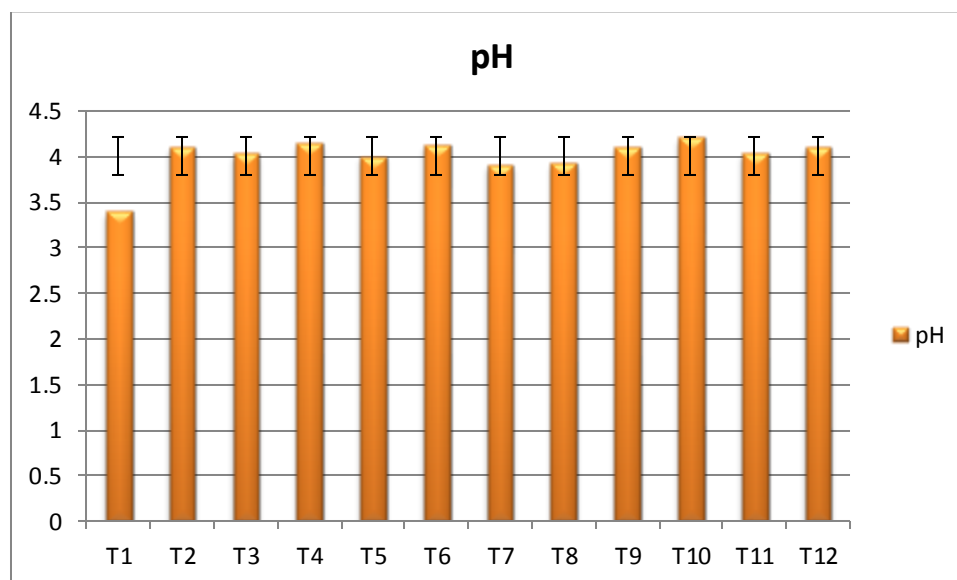


Fig 4.2 pH in different treatments

4.4 Titratable Acidity

The total acidity of whey guava beverage incorporated with PLE extract varied from 1.24 to 1.49 percent and the pasteurizing temperature and timing did not affect the acidity but during the storage period the acidity of whey guava beverage was increased slightly. Similar results were found in (Singh *et al.*, 2014). (Sarvana, 2005) found an increase in acidity of whey guava RTS beverage during the storage of four months. (Singh, 1985) found an increase in guava RTS during the storage of four months. (Singh *et al.*, 2014) also found an increase in acidity during the period of three months. In the experimental samples, the titratable acidity ranges from 1.24 to 1.4. The highest acidity was found in T8 and T11 with the value of 1.4 and 1.38 and the least value was recorded in T4 and T12 followed by T10.

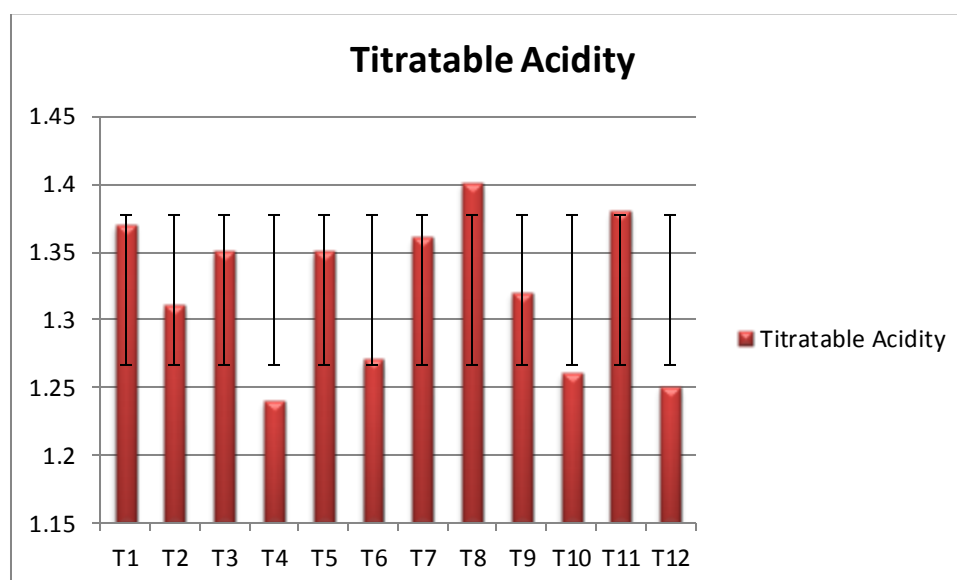


Fig 4.3 Titratable acidity for different treatments

4.5 Moisture Content

According to the results obtained, the most abundant constituent in whey guava beverage incorporated with PLE was moisture. The moisture content of the whey guava beverage was 80% in the study conducted by (Singh *et al.*, 2014). Also (Gad *et al.*, 2013) has recorded the moisture percentage of a whey mango beverage as 79%.

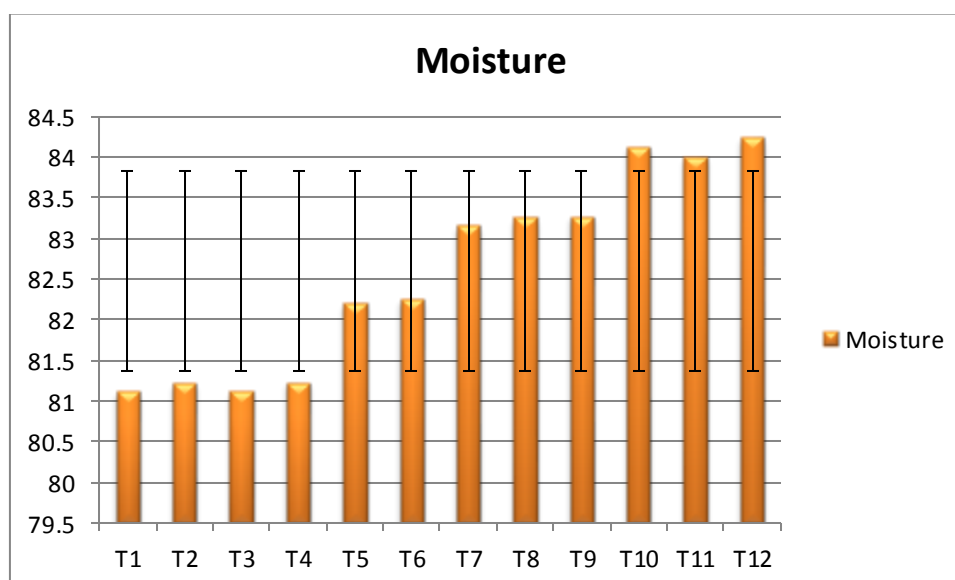


Fig 4.4 Moisture of different treatment

4.6 Ash Content

The data pertaining to the different treatments of Whey Guava Beverage is present in Table 4.5 and Fig.4.4. The maximum ash content was found in T12 and lowest ash content was found in T1 and T2 with values 1.5% and 1.54% for both the treatments. There is an increase in the level of ash content due to the addition of coconut sugar concentration in treatment T5 and T9 having values 1.67 and 1.7 as the concentration of guava remains the same in all treatments.

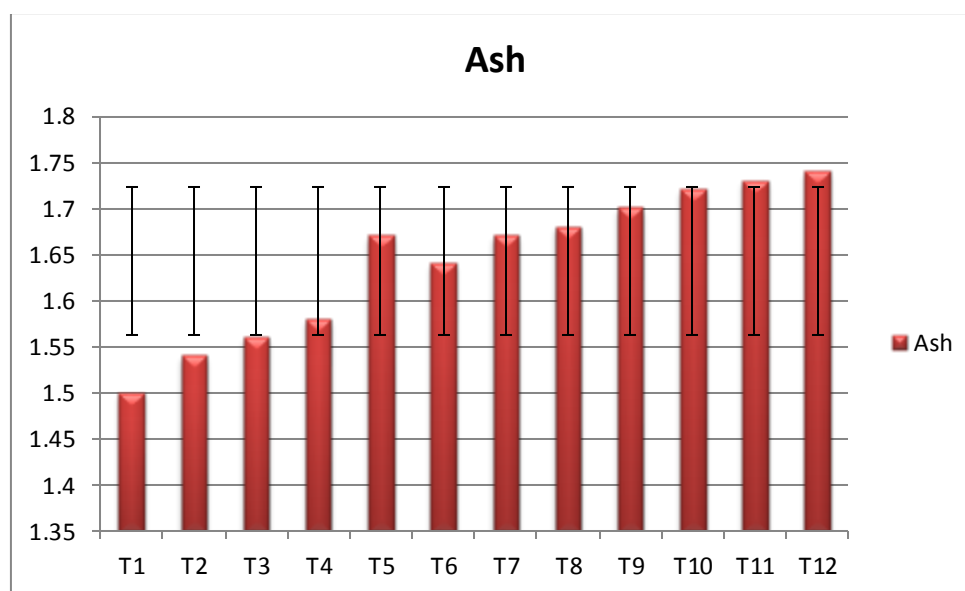


Fig 4.5 Ash Content in different treatments

4.7 Ascorbic Acid

In the experiment of chemical analysis of whey guava beverage it was found that there was no significant change in the ascorbic acid in the various treatments. The ascorbic acid recorded was 55.085(mg/100g). No significant changes were found in the treatments due to the addition of PLE. (Singh et al.,2014) reported the Ascorbic acid range of whey guava beverage from 23.28 to 28.67. The fluctuations in the ascorbic acid content may be due to the environmental changes of the selection of fruits (Ghani et al.,2016).

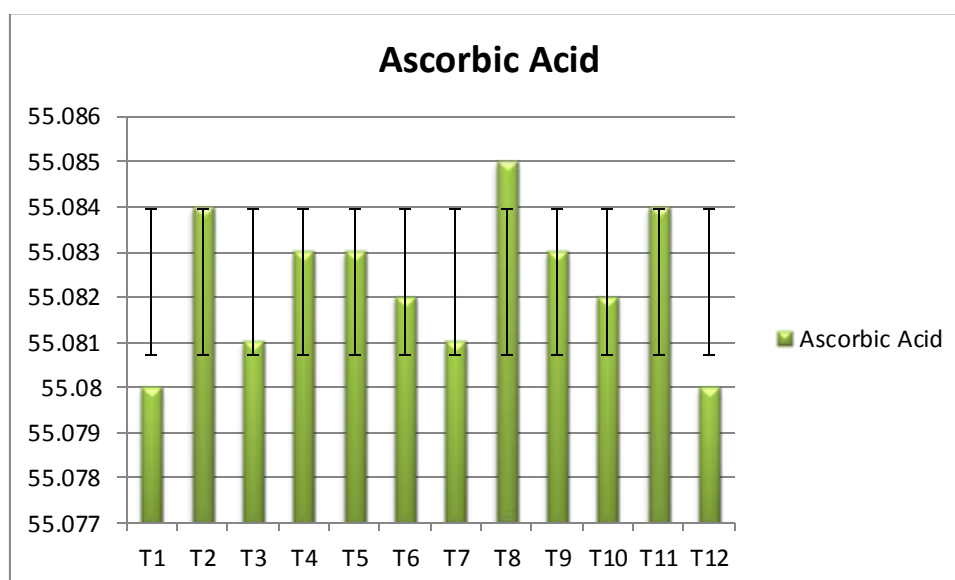


Fig 4.6 Ascorbic Acid in different treatments

4.8 Reducing Sugar

There is also an increase in reducing sugar from 49.46mg/ml to 68.03mg/ml. It was recorded that treatment T12 has the highest score of 68.03mg/ml followed by T10 and T11 having values 67.12 and 67.78mg/ml. The increase in sugar might be due to the hydrolysis of polysaccharides that could have resulted in increased soluble sugar content (Attri *et al.*, 1991). Similar results were found in (Sharma *et al.*, 2020).

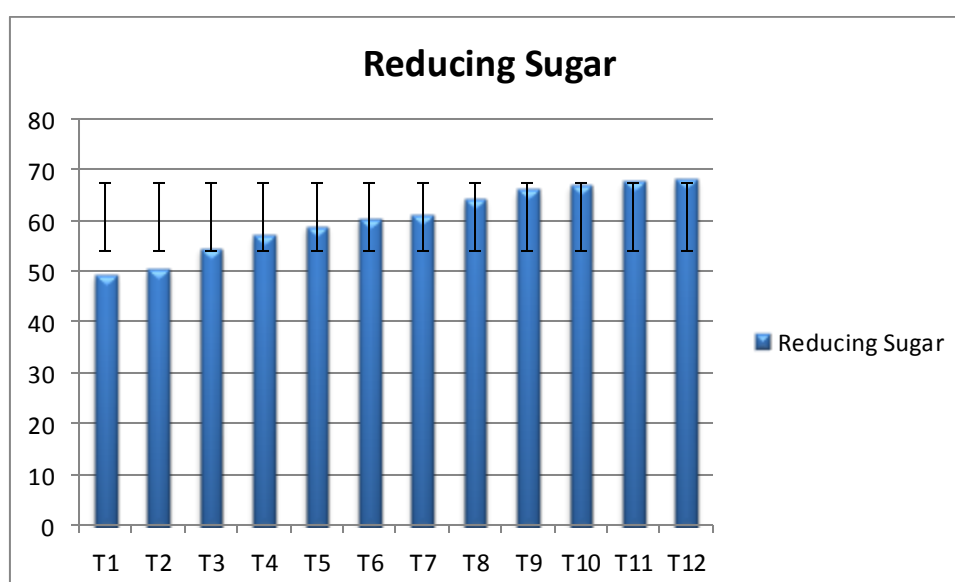


Fig 4.7 Reducing Sugar in different treatments

4.9 Non- Reducing Sugar

In the analysis of non-reducing sugar at zero days recorded during chemical analysis, T5 and T6 was observed as most significantly different treatment with non-reducing sugar values of 88.06% and 86.5% respectively. The least non reducing sugar recorded was T3 and T4 with values 78.32% and 75.83%. Similar trends were found in (Sharma *et al.*, 2020).

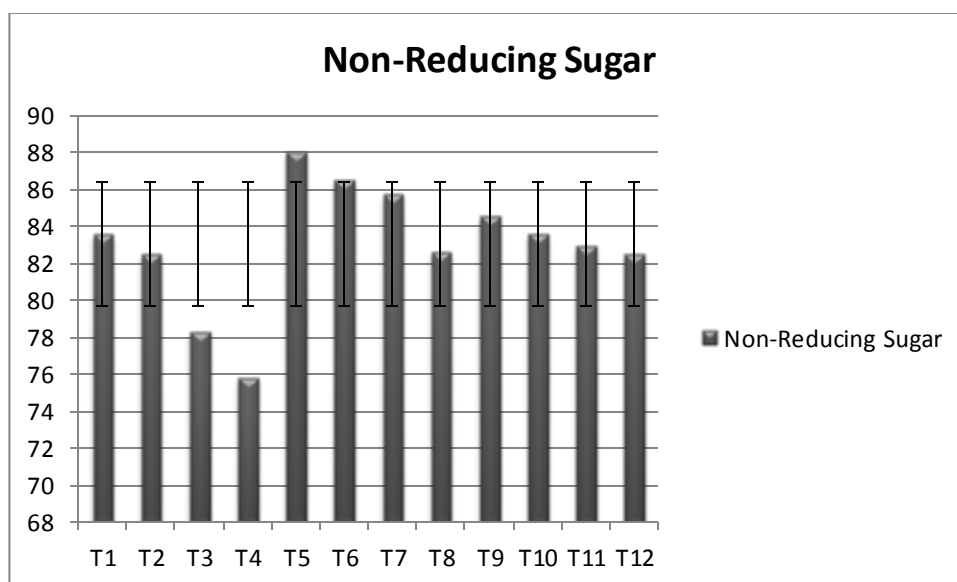


Fig 4.8 Non-Reducing Sugar in different treatment

4.10 Total Sugar

The effect of adding coconut sugar to whey guava beverage incorporated with PLE extract on total sugar was shown to be significant ($P < 0.05$). All of the experimental samples were significantly different from one another. Total sugar levels in the experimental samples ranged from 133.06 (T1) to 150.75 (T12). The overall sugar content in the experimental whey guava beverage samples increased dramatically as the percentage of coconut sugar increased. It is most likely owing to the coconut sugar's sugar content. Similarly, **Handle (2016)** the influence of addition of various levels of kiwi pulp on total sugar in whey beverage. It was noticed that as the level of addition of kiwi fruit pulp increased the total sugar content in the experimental whey beverage. **(Ismail *et al.*, 2011)** reported the total sugar content 16.28% in the cheese

whey-based mango beverage while studying microbial and chemical evaluation of whey based mango beverage.

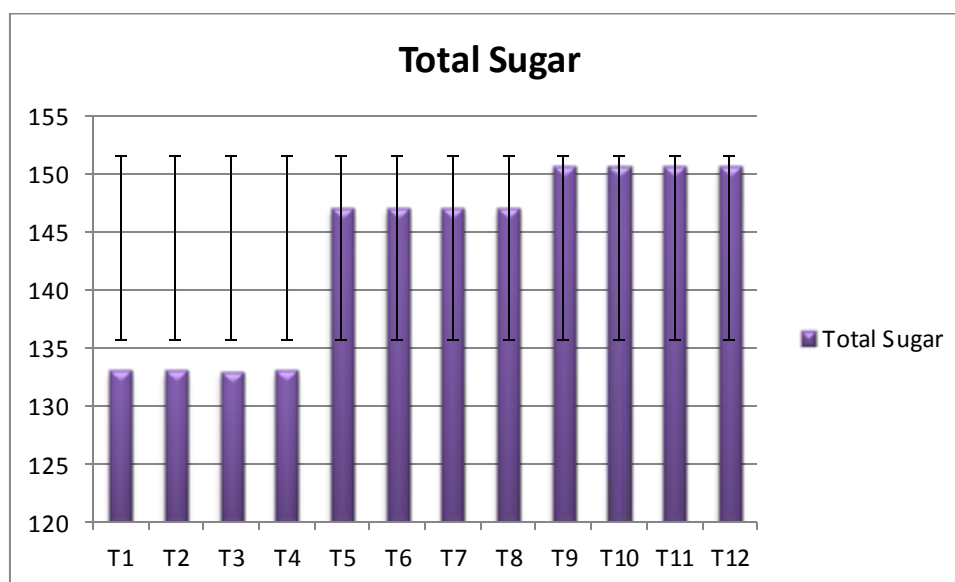


Fig 4.9 Total Sugar in different treatments

4.11 Protein

The influence of experimental treatments on protein content of whey guava beverage incorporated with PLE extract was significant ($P < 0.05$). The mean values for protein content under various experimental samples ranged from 0.67 to 1.02. The highest value for protein content was 1.02 in the treatment of T1 and T8. (Handle, 2016) studied the influence of experimental treatments on protein content in Channa whey beverage. The mean values for protein content under various experimental samples ranged from 0.71 to 0.89. (Bhavsagar *et al.*, 2010) reported the values for protein content from 0.57% to 0.76% in the pineapple flavoured beverage.

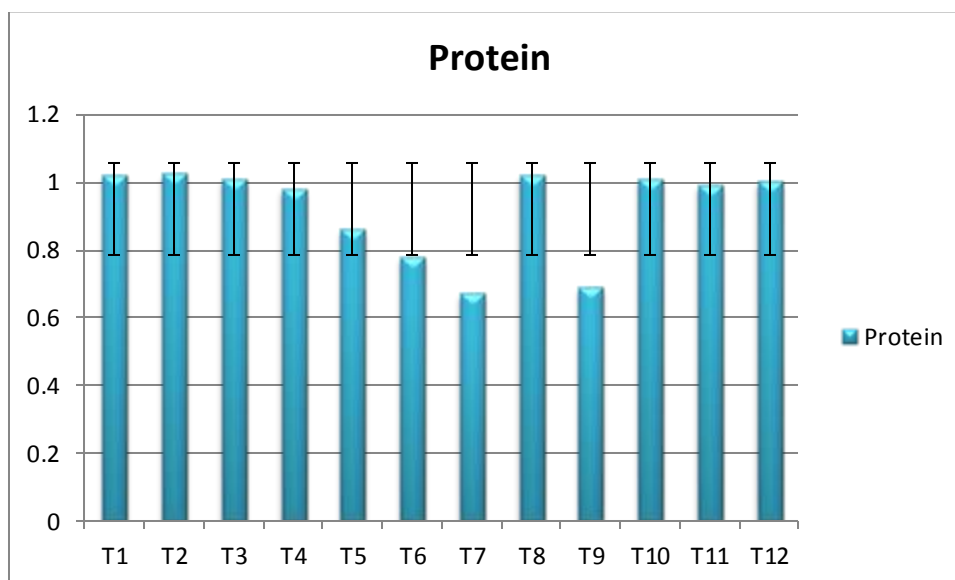


Fig 4.10 Protein Concentration in different treatments

4.12 % DPPH Inhibition

The DPPH concentration of whey guava beverage ranged from 78.03% (T1) to 89.04% (T8). The percentage of DPPH gradually increased upon the increase in concentration of the PLE extract. It may be possible due to the addition of PLE extract from hot extraction method which significant antioxidant property of 75.03%. (**Silva et al., 2007**) reported increasing processing temperature could result in increasing solubility of resultant solute, which ultimately is responsible to increase in antioxidant efficacy. As a rule, higher DPPH of the extracts can be obtained with continuous increasing extracting temperature (**Wagh and Chatli 2017**).

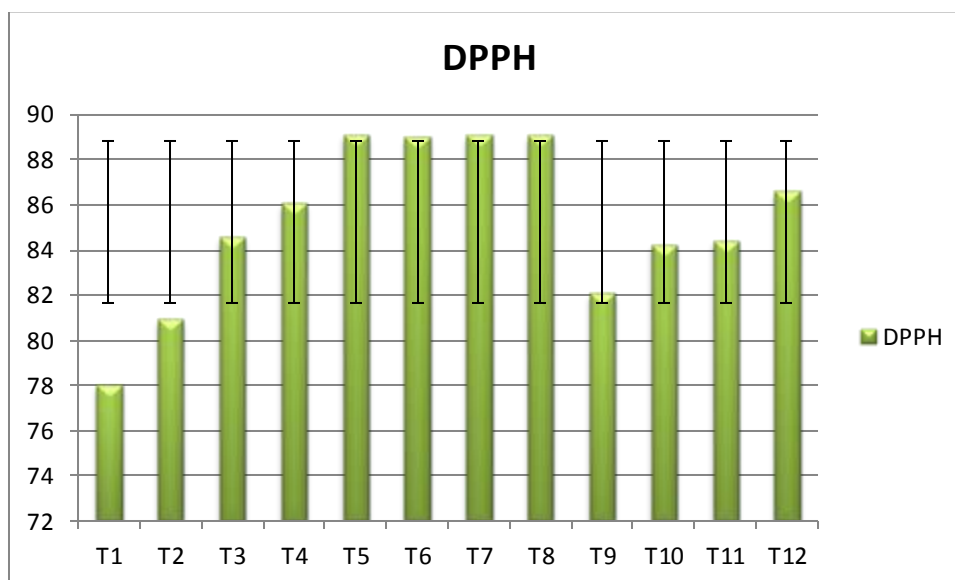


Fig 4.11 DPPH in different treatments

4.13 Total Phenolic Content

Using the standard curve the phenolic content of the extracts were determined. The total phenolic content of the whey guava beverage was highest in the treatment T8 with value of 9.08mgGAE/100ml and lowest count recorded was in the treatment T10 with the value of 8.01mgGAE/100ml. It was observed that the phenolic content gradually increased upon the increasing the concentration of PLE extract. (**Runnie *et al.*, 2004**) earlier reported the higher phenolic content of 4.24 ± 0.22 mgGAE/100ml in dry leaves of papaya which might be the reason for increase in phenolic content of the beverage.

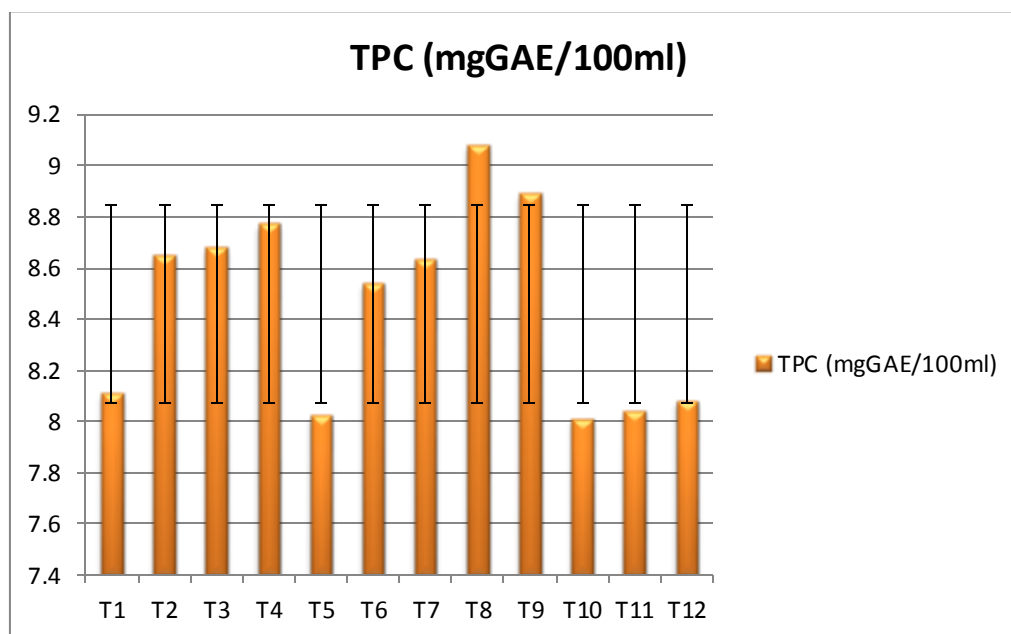


Fig 4.12 TPC in different treatments

4.14 Proximate and Functional analysis of Whey Guava Beverage with different treatments of PLE extract

According to the proximate analysis and physiochemical analysis of all the 12 treatments, it was concluded that the treatment T8 was found best among all the treatments. It was concluded that the treatment T8 having (67.5ml whey, 20ml guava juice, 20% coconut sugar and 4% PLE extract) had higher antioxidant efficacy and total phenolic content. Therefore treatment T8 was selected for shelf life study along with Control (WGB without PLE extract).



Fig 4.13: Optimization of PLE extract at different percentages



Fig 4.14: Control; optimized sample; guava pulp; Hot extraction of PLE extract

Table 4.3: Proximate Analysis for optimization of Product

Treatment	TSS(Brix)	pH	Acidity(%)	Moisture(%)
T1	20.007±0.000	3.407±0.009	1.370±0.006	81.187±0.038
T2	20.017±0.005	4.133±0.088	1.337±0.015	81.517±0.348
T3	20.020±0.000	4.020±0.006	1.363±0.009	81.533±0.360
T4	20.050±0.000	4.140±0.006	1.260±0.012	82.197±0.555
T5	20.027±0.017	4.023±0.012	1.363±0.007	82.570±0.340
T6	20.063±0.017	4.140±0.006	1.297±0.015	82.890±0.323
T7	20.043±0.017	3.927±0.009	1.377±0.009	83.507±0.309
T8	20.063±0.017	3.940±0.006	1.423±0.015	83.507±0.248
T9	20.060±0.011	4.117±0.009	1.337±0.012	83.877±0.305
T10	20.100±0.015	4.217±0.012	1.260±0.006	83.757±0.306
T11	20.103±0.018	4.040±0.006	1.367±0.009	83.830±0.299
T12	20.123±0.007	4.127±0.006	1.253±0.009	83.553±0.344
CD	0.036	0.079	0.031	0.978
SE(m)	0.012	0.027	0.011	0.333
SE(d)	0.017	0.038	0.015	0.471
C.V.	0.107	1.159	1.369	0.696

(The values are mean ± standard deviation of three set of values.)

Table 4.4: Physiochemical Analysis for optimization of Product

Treatment	Reducing Sugar (%)	Total Sugar(%)	DPPH	Ascorbic acid (mg/100g)	TPC	Protein
T1	51.553±1.593	133.037±0.012	80.323±1.191	55.082±0.001	8.080±0.030	1.022±0.001
T2	55.500±3.139	133.060±0.027	83.233±1.165	55.082±0.001	8.613±0.037	1.014±0.004
T3	58.793±2.924	133.033±0.000	84.990±0.517	55.083±0.015	8.663±0.017	1.004±0.012
T4	60.793±2.724	133.060±0.012	85.710±0.595	55.083±0.014	8.730±0.040	0.940±0.040
T5	62.220±2.492	147.023±0.009	89.073±0.022	55.082±0.000	8.240±0.220	0.833±0.027
T6	63.213±2.293	147.040±0.030	89.024±0.022	55.082±0.000	8.617±0.077	0.743±0.037
T7	64.683±2.024	147.057±0.040	89.034±0.003	55.082±0.002	8.600±0.030	0.787±0.117
T8	62.617±2.728	147.103±0.024	89.037±0.021	55.083±0.008	9.017±0.063	0.800±0.110
T9	63.507±3.141	150.717±0.039	82.763±0.733	55.083±0.015	8.597±0.293	0.903±0.107
T10	64.627±2.830	150.730±0.028	83.497±0.733	55.082±0.000	8.020±0.010	0.997±0.007
T11	65.547±2.498	150.740±0.000	85.110±0.720	55.082±0.000	8.053±0.013	0.997±0.003
T12	62.283±3.228	150.733±0.000	85.830±0.720	55.082±0.012	8.413±0.333	1.010±0.006
CD	7.856	0.033	1.990	N/A	0.433	0.172
SE(m)	2.675	0.011	0.678	0.001	0.147	0.058
SE(d)	3.784	0.016	0.959	0.001	0.208	0.083
C.V.	7.562	0.014	1.371	0.003	3.014	10.995

(The values are mean ± standard deviation of three set of values.)

4.15 Shelf-Life Evaluation

The formulated beverage was sealed, packed in glass bottles and kept at 4-5°C throughout the storage period for the purpose to study the effect of storage on the product. pH, % titratable acidity, total phenolic content and overall acceptability of the product were taken into consideration for this purpose. A decreasing trend and an increasing trend was obtained for pH and titratable acidity on storage period of 28 days. This was due to slight increase in acidity of the product which made pH of the product to show slight increase. A gradual decrease in total phenolic content of the beverage was estimated and this is mainly due to loss of phenols on storage. This study was found to be in accordance with a study conducted on peach beverages (Sattar *et al.*, 2020).

The beverage was scored for overall acceptability for 0 days, 7 days, 21 days, 28 days. The acceptability in terms of overall had decreased from 8.83+0.516 to 5.89 0.342. Fresh prepared beverage was liked very much till 21days of storage and decrease in acceptance was there with slight liking of the beverage persisted till 28day of storage. It can be extracted from the study that the beverage had shelf life of 21 days when stored at 4°C without use of any preservative. It was also observed that the antioxidant property of the product increased upon the addition of PLE extract when compared with the control having no added PLE extract.

Results & Discussion

Table 4.5:Physiochemical changes during Storage of Control and Optimized Sample

TREATMENT	0 DAYS		7 th DAY		14 th DAY		21 st DAY		28 th DAY	
	Control	Optimized Product	Control	Optimized Product	Control	Optimized Product	Control	Optimized Product	Control	Optimized Product
pH	3.92±0.001	3.93±0.001	3.92±0.009	3.93±0.003	3.92±0.002	3.9±0.001	3.42±0.002	2.86±0.003	3.04±0.002	2.04±0.005
Acidity	1.4±0.002	1.4±0.005	1.4±0.211	1.4±0.006	1.37±0.223	1.38±0.006	1.33±0.345	1.34±0.004	1.02±0.201	1.00±0.003
TSS (%)	20±0.334	20±0.000	19.85±0.212	19.73±0.001	17.45±0.221	17.35±0.002	10.85±0.453	10.76±0.030	9.08±0.002	9.05±0.005
Ascorbic Acid (mg/100g)	55.084±0.445	55.085±0.005	54.063±0.765	54.064±0.003	48.56±0.334	50.08±0.020	40.02±0.345	40.02±0.030	38.02±0.231	36.05±0.023
Reducing Sugar (%)	62.617±0.556	62.617±0.320	64.04±0.675	64.03±0.022	66.05±0.229	68.08±0.005	68.56±0.221	68.87±0.001	70.02±0.245	70.00±0.165
Non-Reducing Sugar (%)	82.64±0.210	82.64±0.320	70.04±0.301	70.03±0.224	60.05±0.543	60.08±0.010	55.56±0.439	56.64±0.000	50.05±0.432	50.02±0.421
Total Sugar (%)	147.103±0.458	147.103±0.420	145.125±0.564	145.25±0.245	142.54±0.992	142.66±0.223	138.45±0.756	137.82±0.021	134.24±0.754	134.50±0.755
DPPH (%)	56.53±0.765	89.04±0.022	50.34±0.435	80.05±0.310	40.52±0.005	72.34±0.221	36.45±0.452	54.35±0.001	24.65 ±0.002	52.02±0.004
TPC (mgGAE/100ml)	5.4±0.332	9.08±0.000	5.52±0.631	9.1±0.048	5.64±0.923	9.24±0.223	5.76±0.548	9.48±0.202	5.25±0.542	8.24±0.521
Overall Acceptability	8.883±0.416	8.03±0.002	7.81±0.48	7.05±0.425	6.25±0.31	5.26±0.332	3.76±0.631	3.63±0.547	3.02±0.432	3.05±0.342
C.D.	0.433	0.184	0.027	0.008	0.011	0.164	0.333	0.184	0.124	0.032
SE(m)	0.75	0.058	1.990	0.003	0.033	0.053	0.433	0.058	0.011	0.024
SE(d)	0.83	0.082	1.994	0.004	0.125	0.083	0.632	0.082	0.045	0.064
C.V.	0.954	3.425	1.371	2.429	0.68	0.68	0.755	3.281	2.431	1.824

(The values are mean ± standard deviation of three set of values.)

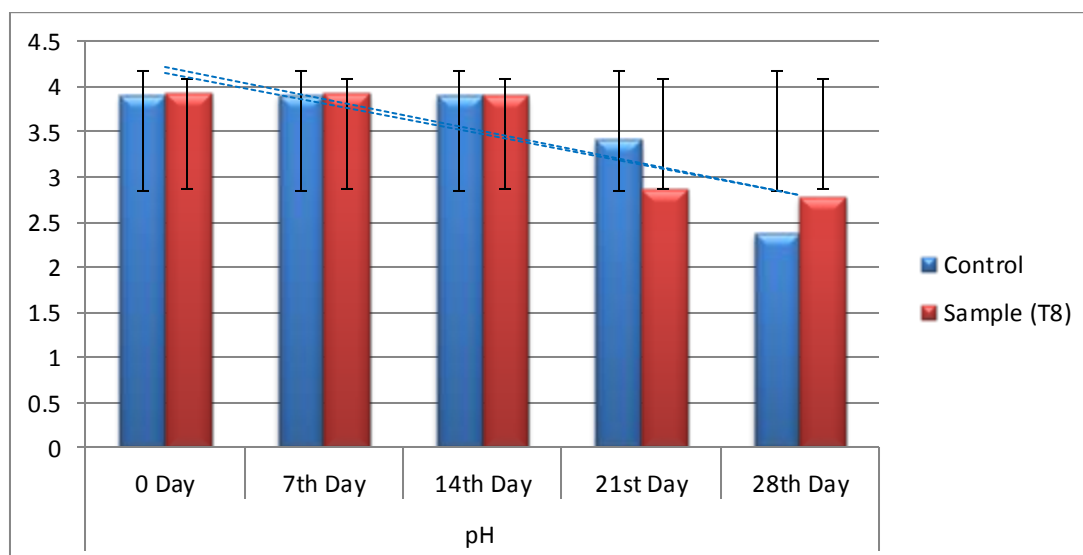


Fig 4.15 Effect of storage period on the pH of the formulated product

4.16 Acidity

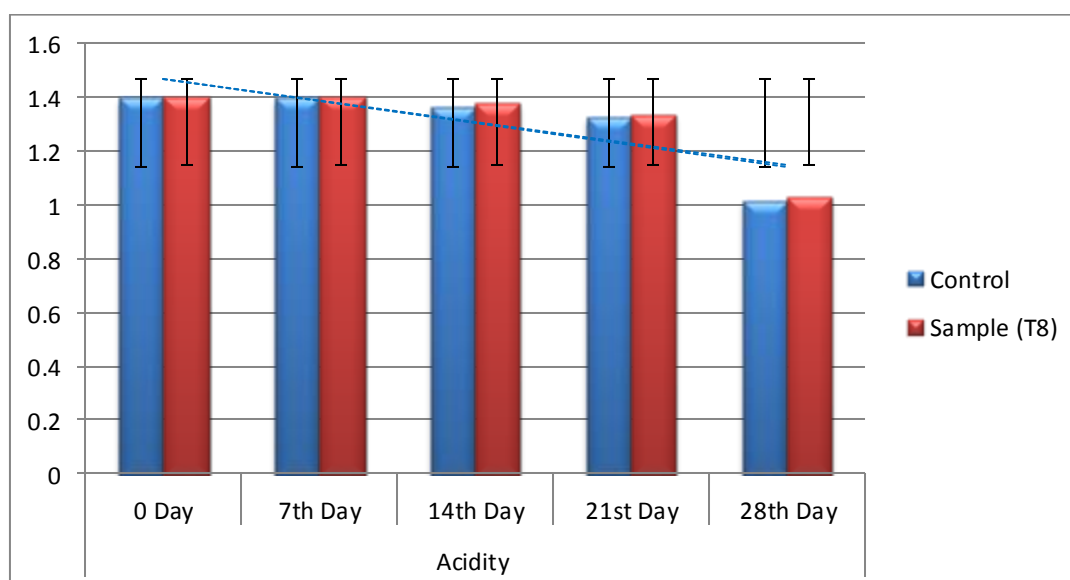


Fig 4.16 Effect of storage period on the acidity of the formulated product

4.17 TSS

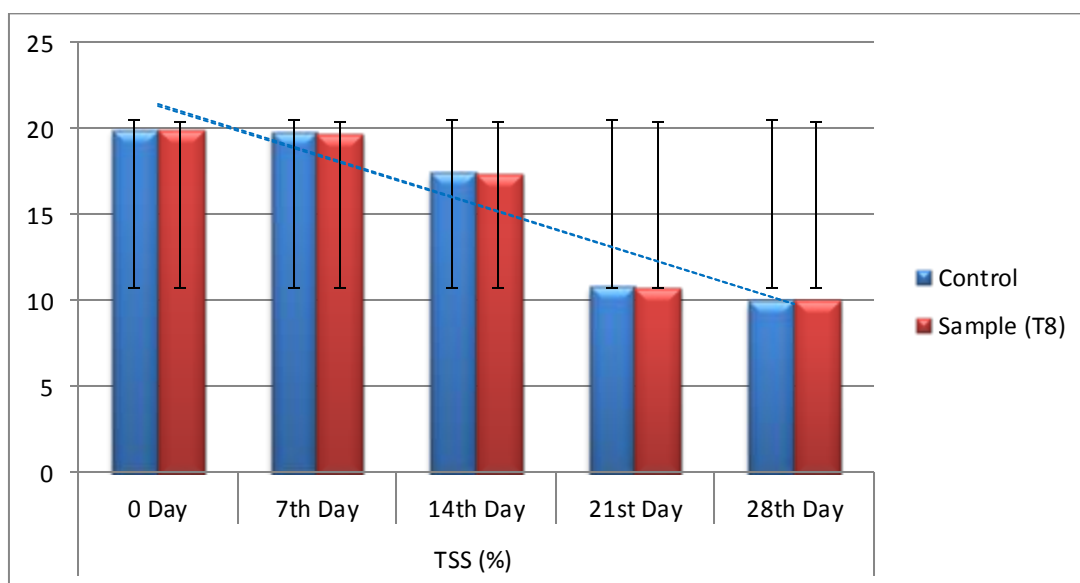


Fig 4.17 Effect of storage period on the TSS of the formulated product

4.18 Ascorbic Acid

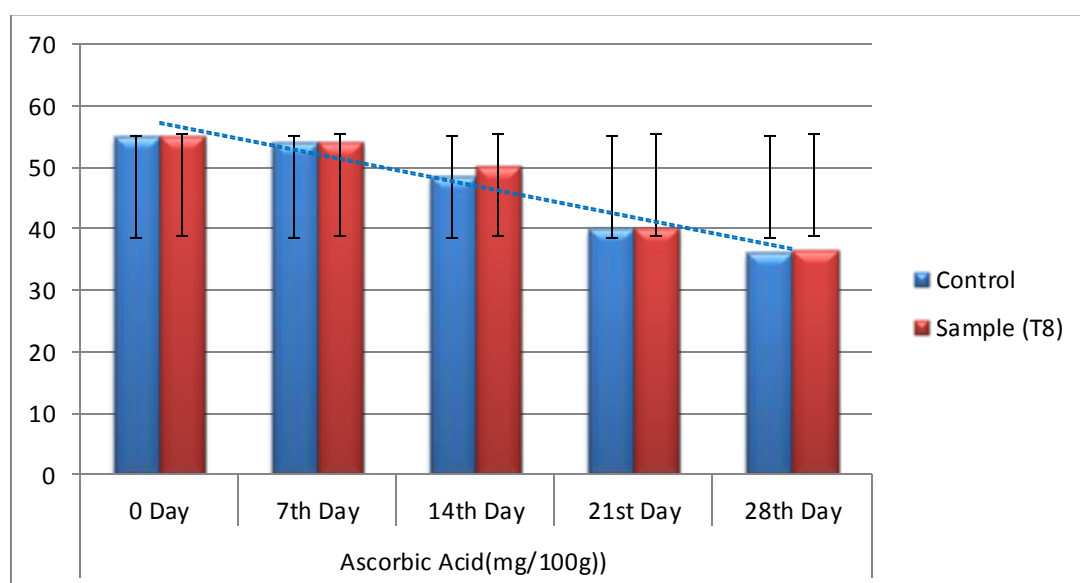


Fig 4.18 Effect of storage period on the Ascorbic Acid of the formulated product

4.19 Reducing Sugar

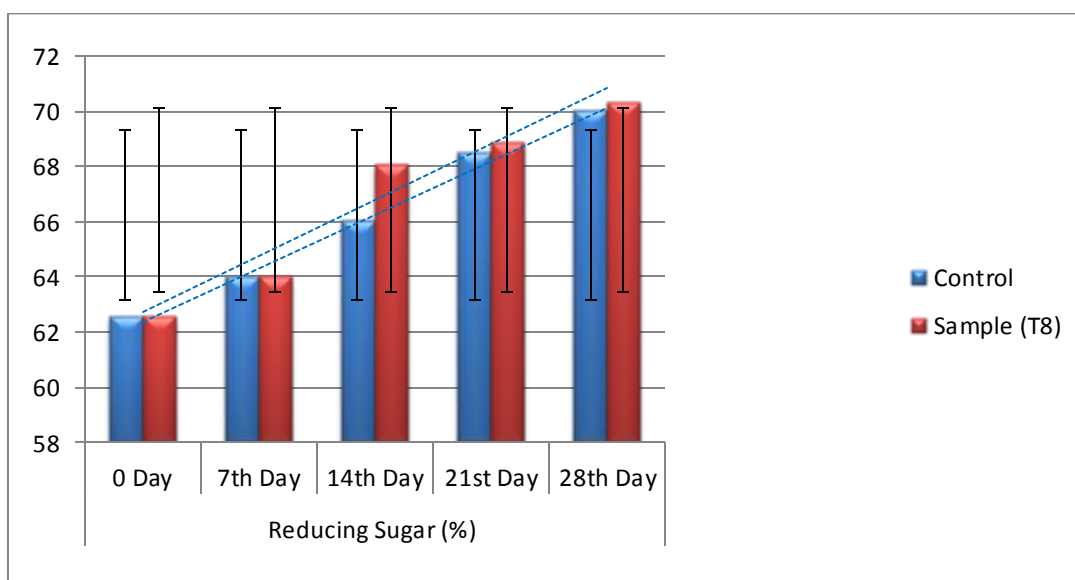


Fig 4.19 Effect of storage period on the Reducing Sugar of the formulated product

4.20 Non-Reducing Sugar

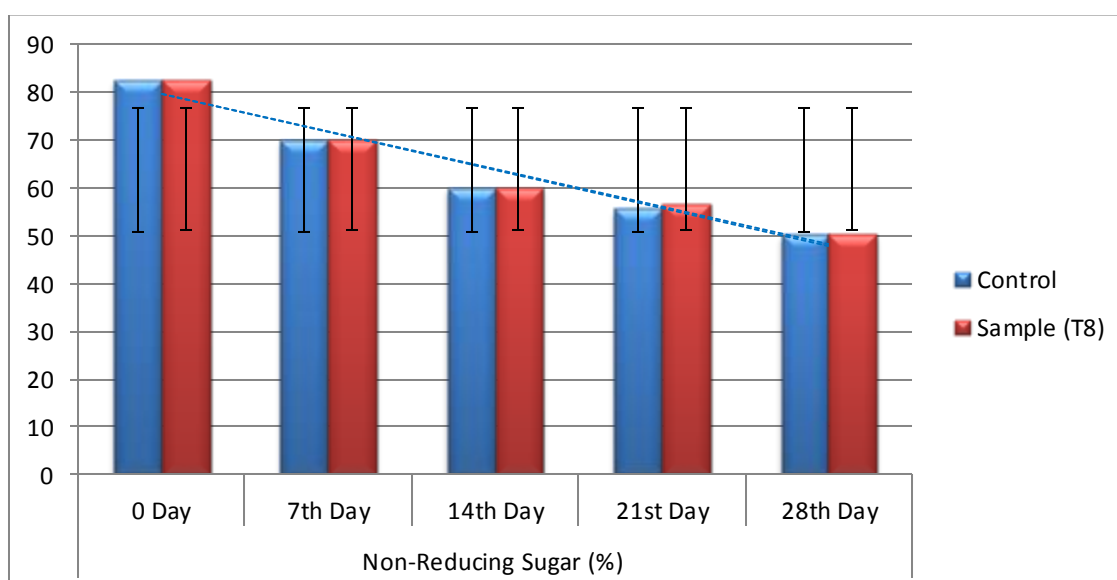


Fig 4.20: Effect of storage period on the Non-Reducing Sugar of the formulated product

4.21 Total Sugar

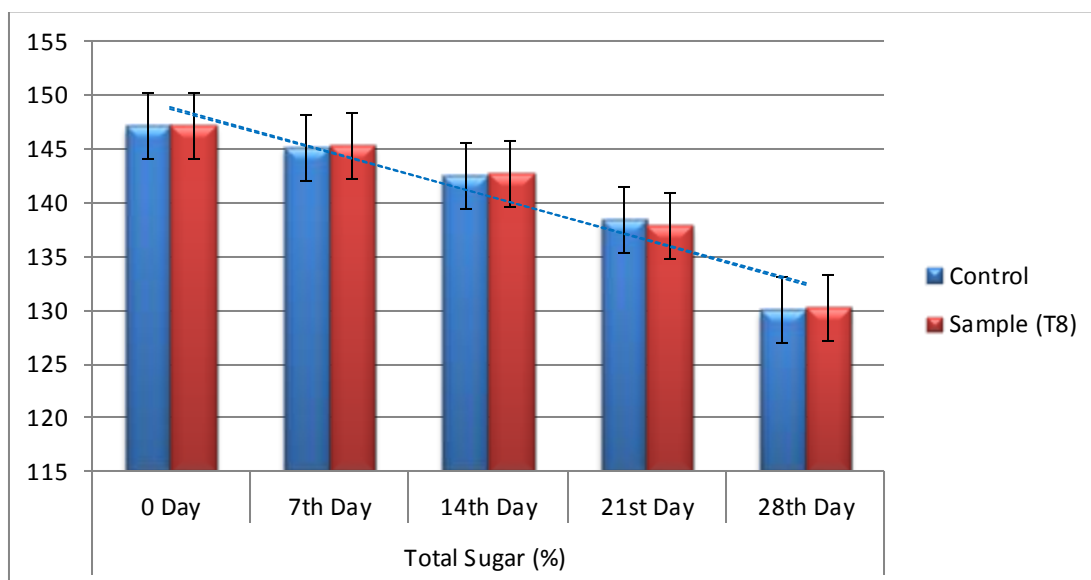


Fig 4.21: Effect of storage period on the Total Sugar of the formulated product

4.22 %DPPH Inhibition

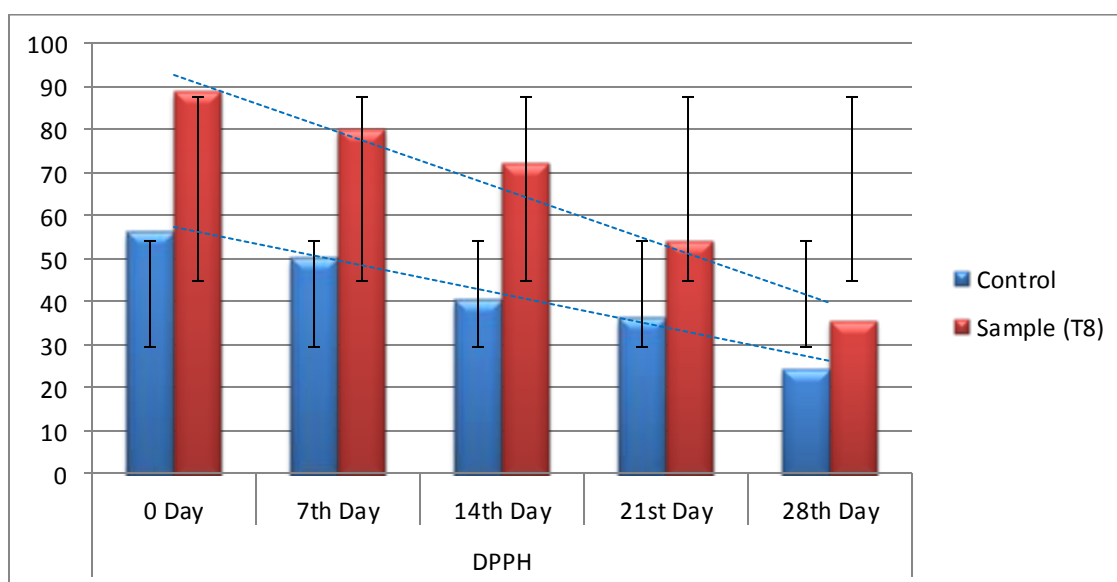


Fig 4.22: Effect of storage period on the DPPH of the formulated product

4.23 TPC

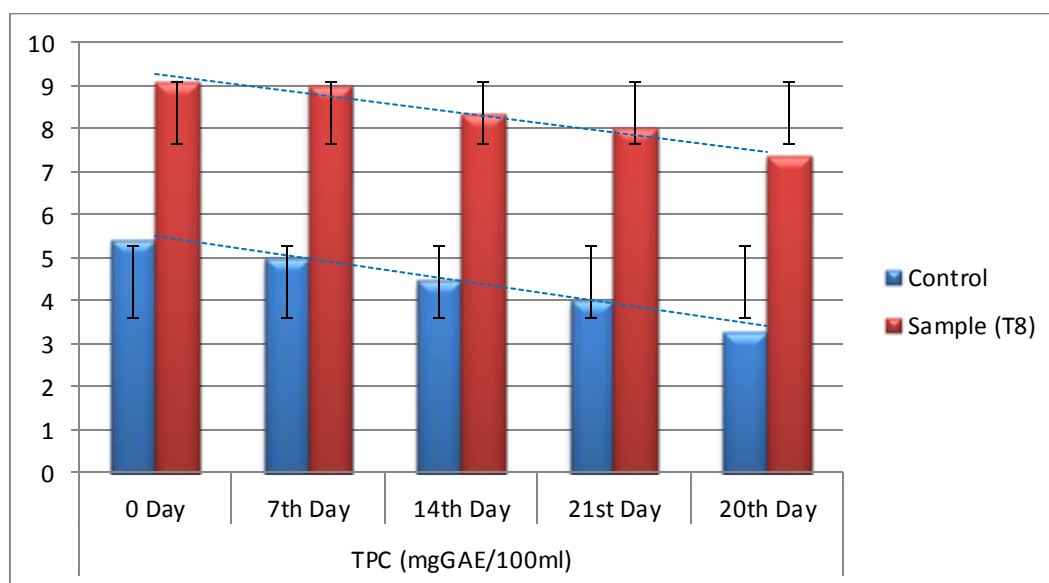


Fig 4.23: Effect of storage period on the TPC of the formulated product

4.24 Microbial Analysis

The whey guava beverage was analyzed for Total Plate Count, Coliform Count and Yeast and Mould count periodically upto 28 days of shelf life. The data obtained with respect to microbial count (CFU/ml) are tabulated in Table 4.8. The Total Plate Count increases and after the completion of 21 days it reaches 7.2×10^5 at 10^{-4} dilution and 6.5×10^7 at 10^{-6} dilution for control and 7.8×10^5 at 10^{-4} and 6.2×10^7 at 10^{-6} dilution for the optimized sample. After 28 days the count was TNTC (too numerous to count). (Ismail *et al.*, 2011) studied the microbial and chemical evaluation of whey based mango beverage and reported that the Total Plate Count significantly increased with increase of storage of whey based mango beverage. (Yonis *et al.*, 2014) also reported similar results when studied the utilization of sweet whey in production of whey guava beverages and observed significant increase in the total plate count with increase of storage period.

The Coliform count was not observed upto 14 days of storage period of both control and optimized sample. The count increases from 21st day to 28th day from 0.3×10^5 at 10^{-4} dilution and 0.1×10^7 at 10^{-6} dilution for control and 0.2×10^5 at 10^{-4} dilution and 0.1×10^7 at 10^{-6} for sample to 0.5×10^5 at 10^{-4} dilution and 0.2×10^7 at 10^{-6} dilution for control and 0.6×10^5 at 10^{-4} dilution and 0.3×10^7 at 10^{-6} dilution for sample. (Ismail *et al.*, 2011) studied the microbial and chemical evaluation of whey

based mango beverage and reported that the Coliform Count was significantly increased with increase of storage of whey based mango beverage. (Yonis *et al.*, 2014) also reported similar results when studied the utilization of sweet whey in production of whey guava beverages and observed significant increase in the Coliform count with increase of storage period.

Yeast and Mould count was not detected in both the Control and the Optimized sample for 7 days. The growth was observed upto 0.2×10^5 at 10^{-4} dilution and 0.1×10^7 at 10^{-6} dilution for control and 0.2×10^5 at 10^{-4} dilution and 0.2×10^5 at 10^{-6} dilution for sample on 14th day of storage period . On 21st day the growth was 0.4×10^5 at 10^{-4} dilution and 0.2×10^7 at 10^{-6} dilution for control and 0.4×10^5 at 10^{-4} dilution and 0.2×10^7 at 10^{-6} dilution for sample. On 28th day of storage period the growth increased to 0.7×10^5 at 10^{-4} dilution and 0.4×10^7 at 10^{-6} dilution for control and 0.8×10^5 at 10^{-4} dilution and 0.5×10^7 at 10^{-6} dilution for sample. (Yonis *et al.*, 2014) also reported similar results when studied the utilization of sweet whey in production of whey guava beverages and observed significant increase in the Yeast and Mould count with increase of storage period.

Table 4.6 Microbial Analysis of Control & Optimized sample during Storage Period

Parameter	Storage Period (Days)	Samples			
		Control		Optimized Product	
Total Plate Count (CFU/ml)		Dilutions		Dilutions	
		10^{-4}	10^{-6}	10^{-4}	10^{-6}
	0	1.2×10^5	0.7×10^7	1×10^5	0.5×10^7
	7	2.2×10^5	1.5×10^7	2×10^5	1.6×10^7
	14	3.0×10^5	2.5×10^7	2.4×10^5	1.8×10^7
	21	7.2×10^5	6.5×10^7	7.8×10^5	6.2×10^7
	28	TNTC	TNTC	TNTC	TNTC
Coliform Count (CFU/ml)	0	NIL	NIL	NIL	NIL
	7	NIL	NIL	NIL	NIL
	14	NIL	NIL	NIL	NIL
	21	0.3×10^5	0.1×10^7	0.2×10^5	0.1×10^7
	28	0.5×10^5	0.2×10^7	0.6×10^5	0.3×10^7
Yeast and Mould Count (CFU/ml)	0	NIL	NIL	NIL	NIL
	7	NIL	NIL	NIL	NIL
	14	0.2×10^5	0.1×10^7	0.2×10^5	0.1×10^7
	21	0.4×10^5	0.2×10^7	0.4×10^5	0.2×10^7
	28	0.7×10^5	0.4×10^7	0.8×10^5	0.5×10^7

CFU colony forming unit; TNTC too numerous to count

4.25 Cost estimation

Table 4.7 Cost Analysis of the Optimized Sample

S.No.	Component	Quantity	Rate (per kg)	Total Cost
A.	Variable Cost			
a.	Direct Material Cost			
1.	Whey	1litre	12/L	12
2.	Coconut Sugar	200g	900/ kg	180
3.	Guava	200g	80/kg	16
4.	Papaya Leaf	50g	30/kg	1.5
5.	Pakaging Material	6 bottles	@ Rs. 5 per bottle	30
	Sub-total (a)			Rs. 239.5
b.	Processing Expenses			
S.N.	Component	Rate	Total Cost (	
1.	Electricity	Rs.9/ unit	9	
2.	LPG Cost	Rs. 1000/ 14.2kg	10	
3.	Labour cost	Rs. 300/8 hrs.	40	
4.	Misc. Cost	-	40	
	Sub-Total (b)		Rs. 99	

*Excluding storage, shipping and transportation

Total Cost= Material Cost+ Processing Cost

= Rs.338

Total production of Beverage from 1L Whey +200g Guava +200g Coconut Sugar+56 ml PLE Extract from 50g Papaya leaf = 1450ml or 1.45 L

Per Unit Cost= Total cost incurred/Total quantity Produced

Therefore, per unit cost would be Rs.233 /L

Hence, the cost for each bottle containing 250ml of WGB incorporated with PLE extract would be Rs.58.27.

The cost of development of Whey based Guava beverage incorporated with PLE extract was worked by taking into consideration the prevailing market prices for various items of raw materials. The labour charges were considered on the basis of actual hours required to prepare the optimized product. The data on cost analysis of whey based guava beverage incorporated with PLE extract are tabulated in Table 4.9.

The increase in cost was mainly due to the cost of coconut sugar which is quite expensive due to its high nutritive value and also having low glycemic index making it fit to consume even for diabetic patients. This cost did not include rent, storage and transport, sales commission and local taxes etc.

In cost of production all raw material cost and other cost like processing cost, labour cost were included **(Koli, 2000)**.

SUMMARY & CONCLUSION

Present research work on “Development of Whey Based Guava Beverage fortified with PLE extract” was carried out to develop an optimized product with the high antioxidant property and low glycemic index making it fit to consume even for diabetic patients. The project was undertaken with the following objectives :

- To carry out comparative analysis of two different methods of papaya leaf extraction.
- Preparation of whey based guava beverage incorporated with papaya leaf extract (PLE).
- To carry out physiochemical analysis of whey based guava beverage incorporated with papaya leaf extract.
- To study the shelf –life analysis and cost estimation of the beverage.

In this study, we have optimized the Whey based Guava beverage on the basis of sensory analysis

and all physiochemical attributes including DPPH and Total Phenolic Content.

- Sensory Analysis was carried out in three segments viz. one for the optimization of whey guava beverage, second for the optimization of coconut sugar to be added at different percentages and third for the incorporation of Hot Extract of PLE at different percentages.

Treatment T8 was given the highest score of sensorial score of 8.66 value for the T8 (67.5ml whey, 20ml guava juice, 20% coconut sugar and 4% PLE extract) sample was highest for this parameter whereas, T3 with sample had lowest value of sensory analysis which was 3.33. Sensory score for the other remaining samples fluctuated from 4.23 to 7.13. So, from the whole analyzed data of sensory analysis of samples it was clearly shown that T8 (67.5ml whey, 20ml guava juice, 20% coconut sugar and 4% PLE extract) sample

(8.56) of Whey Guava beverage was liked by most panelists and most disliked sample was T1, T2 & T3 with 4.33, 4.30 and 4.6 of sensorial analysis.

- Based on all the Proximate and Physiochemical analysis it was concluded that the treatment T8 was best suited and was selected as an optimized product for shelf life study against the Control having no PLE extract added. The DPPH concentration of whey guava beverage ranged from 78.03% (T1) to 89.04% (T8). The percentage of DPPH gradually increased upon the increase in concentration of the PLE extract.
- Using the standard curve the total phenolic content of the extracts were determined. The total phenolic content of the whey guava beverage was highest in the treatment T8 with value of 9.08mgGAE/100ml and lowest count recorded was in the treatment T10 with the value of 8.01mgGAE/100ml. It was observed that the phenolic content gradually increased upon the increasing the concentration of PLE extract.
- Shelf life study was also carried out for 28 days for monitoring all the physiochemical and microbial changes undergoing during these periods. It was found that the Antioxidant property, Total Phenolic Content, Vitamin C gradually decreased with increase in days. It was concluded that the whey guava beverage incorporated with PLE extract was fit to consume upto 14 days of storage as there was no coliform, yeast and mould count during these days and total plate count was negligible upto 15 days of storage and increased after 15 days.
- The cost estimation of the optimized product was done and was calculated as Rs. 58.27 for one bottle containing 250ml of WGB. The increase in cost was mainly due to the cost of coconut sugar which is quite expensive due to its high nutritive value and also having low glycemic index making it fit to consume even for diabetic patients.

Conclusion

It can be concluded that whey can be found successful for the development of whey based guava beverage incorporated with PLE extract. The beverage possesses high color, flavor and stability properties. It contains high antioxidant property due to the addition of PLE extract . The coconut sugar present in the beverage makes it ideal for diabetic patients because of low glycemic index, along with the goodness of Guava making it rich in Vitamin C. The beverage can be kept refrigerated upto 14 days of shelf life without the addition of any preservatives. The product may be proven to be a nutritionally and organoleptically appealing beverage with a pleasant flavor, several vital minerals, nutrients and energy.

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APPENDIX I

SENSORY EVALUATION CARD**Name:****Date:****Product: Whey Guava Beverage****Time:**

Instructions: The given sample is whey guava beverage and it has been prepared in the laboratory. You are requested to assess the product in terms of general acceptability with special reference to color & appearance, aroma, texture and overall acceptability on the basis of nine-point hedonic scale given below:

Liked Extremely	9	Disliked Slightly	4
Liked Very Much	8	Disliked Moderately	3
Liked Moderately	7	Disliked Very Much	2
Liked Slightly	6	Disliked Extremely	1
Neither like nor dislike	5		

CHARACTERISTICS	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉
COLOUR & APPEARANCE									
AROMA									
BODY									
TEXTURE									
OVERALL ACCEPTABILITY									

SUGGESTIONS/REMARKS:**SIGNATURE:**

APPENDIX II

SENSORY EVALUATION CARD

Name:

Date:

Product: Whey Guava Beverage with Coconut Sugar

Time:

Instructions: The given sample is whey guava beverage with different percentages of coconut sugar and it has been prepared in the laboratory. You are requested to assess the product in terms of general acceptability with special reference to color & appearance, aroma, texture and overall acceptability on the basis of nine-point hedonic scale given below:

Liked Extremely	9	Disliked Slightly	4
Liked Very Much	8	Disliked Moderately	3
Liked Moderately	7	Disliked Very Much	2
Liked Slightly	6	Disliked Extremely	1
Neither like nor dislike	5		

CHARACTERISTICS	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆
COLOUR & APPEARANCE						
AROMA						
BODY						
TEXTURE						
OVERALL ACCEPTABILITY						

SUGGESTIONS/REMARKS:

SIGNATURE:

APPENDIX III

SENSORY EVALUATION CARD

Name:

Date:

Product: Whey Guava Beverage with PLE extract

Time:

Instructions: The given sample is whey guava beverage incorporated with different percentages of PLE extract and it has been prepared in the laboratory. You are requested to assess the product in terms of general acceptability with special reference to color & appearance, aroma, texture and overall acceptability on the basis of nine-point hedonic scale given below:

Liked Extremely	9	Disliked Slightly	4
Liked Very Much	8	Disliked Moderately	3
Liked Moderately	7	Disliked Very Much	2
Liked Slightly	6	Disliked Extremely	1
Neither like nor dislike	5		

CHARACTERISTICS	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉	T ₁₀	T ₁₁	T ₁₂
COLOUR & APPEARANCE												
AROMA												
BODY												
TEXTURE												
OVERALL ACCEPTABILITY												

SUGGESTIONS/REMARKS:

SIGNATURE:

APPENDIX IV

ANOVA TABLE FOR SENSORY EVALUATIONS (ONE FACTOR ANALYSIS)

Anova for Color and Appearance

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	11	73.030	6.639	133.528	-0.00000
Error	24	1.193	0.050		
Total	35	74.223			

Anova for Taste

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	11	67.274	6.116	105.342	0.00000
Error	24	1.393	0.058		
Total	35	68.668			

Anova for Consistency

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	11	37.201	3.382	85.739	-0.00000
Error	24	0.947	0.039		
Total	35	38.148			

Anova for Flavour

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	11	60.910	5.537	96.299	-0.00000
Error	24	1.380	0.058		
Total	35	62.290			

Anova for Overall Acceptability

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	11	57.181	5.198	100.072	0.00000
Error	24	1.247	0.052		
Total	35	58.428			

APPENDIX V

ANOVA FOR PROXIMATE ANALYSIS (ONE FACTOR ANALYSIS)

Anova for pH

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	11	1.478	0.134	61.933	0.00000
Error	24	0.052	0.002		
Total	35	1.530			

Anova for Ash

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	11	0.167	0.015	1.218	0.32778
Error	24	0.300	0.013		
Total	35	0.467			

Anova for Titratable Acidity

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	11	0.098	0.009	26.803	0.00000
Error	24	0.008	0.000		
Total	35	0.106			

Anova for Moisture

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	11	32.901	2.991	8.989	0.00000
Error	24	7.986	0.333		
Total	35	40.887			

APPENDIX VI

ANOVA FOR PHYSIOCHEMICAL ANALYSIS (ONE FACTOR ANALYSIS)

Anova for Protein

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	11	0.353	0.032	3.134	0.00933
Error	24	0.246	0.010		
Total	35	0.599			

Anova for Reducing Sugar

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	11	563.362	51.215	2.385	0.03638
Error	24	515.392	21.475		
Total	35	1,078.754			

Anova for Non- Reducing Sugar

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	11	135.464	12.315	1.272	0.29763
Error	24	232.316	9.680		
Total	35	367.780			

Anova for Total Sugar

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	11	2,089.348	189.941	502,761.181	-0.00000
Error	24	0.009	0.000		
Total	35	2,089.357			

Anova for Ascorbic Acid

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	11	0.000	0.000	0.293	0.98097
Error	24	0.000	0.000		
Total	35	0.000			

Anova for DPPH

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	11	281.911	25.628	18.596	0.00000
Error	24	33.075	1.378		
Total	35	314.986			

Anova for Total Phenolic Content

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	11	0.047	0.004	9.267	0.00000
Error	24	0.011	0.000		
Total	35	0.058			