PREPARATION & QUALITY EVALUATION OF WHEY BASED DRAGON FRUIT BEVERAGE AND STUDY ON ITS STORAGE STABILITY

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Preparation & Quality Evaluation of Whey Based Dragon Fruit Beverage and Study on its Storage Stability

A dissertation submitted to the Department of Food Technology, Central Campus of Technology, Tribhuvan University, in partial fulfillment of the requirements for the degree of B. Tech. in Food Technology

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Approval Letter

This ***dissertation*** entitled ***Preparation & Quality Evaluation of Whey Based Dragon Fruit Beverage and Study of its Storage Stability*** presented by **Puran Thakulla** has been accepted as the partial fulfillment of the requirement for the **B. Tech. degree in Food Technology**

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Abstract

The main aim of this study was to prepare whey based dragon fruit beverage and study of its storage stability. Whey-based dragon fruit beverage was prepared by blending paneer whey along with dragon fruit juice and required amount of sugar utilizing design expert® (v 13) under mixed condition. By varying whey (50-100%) and dragon fruit juice (0-50%) seven formulations were obtained (A, B, C, D, E, F and G). Samples were pasteurized at 90°C for 10 min and stored in pre-sterilized bottles. The best sample F was selected and it's chemical and phytochemical compositions were analyzed. It was stored in pre-sterilized bottles at ambient (25±5°C) and refrigerated (7±1°C) conditions for 25 days. The effect of storage time and temperature on physicochemical and microbial properties were evaluated.

Formulated samples were analyzed by sensory evaluation and the beverage sample F with 62.5% whey and 37.5% dragon fruit juice was found to be superior. The best sample F was selected and it's chemical and phytochemical compositions were analyzed. During storage analysis, TSS, acidity and reducing sugar increased while pH and ascorbic acid content decreased with progressing storage time in both storage condition. Similarly, during 25 days storage, total plate count & yeast and mold count were also increased under both storage condition. These changes in refrigerated storage condition were found to be minor in comparison to normal storage condition. From all the analysis, it can be observed that the beverage can be kept unspoiled for about 25 days without preservatives.

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List of Abbreviations

Abbreviation	Full form
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
BOD	Biochemical oxygen demand
CCT	Central Campus of Technology
DDC	Dairy Development Corporation
DPPH	2,2-diphenyl-1-picrylhydrazyl
FW	Fresh weight
GAE	Gallic acid equivalent
kDA	Kilodalton
LSD	Least significant difference
PCA	Plate count agar
PDA	Potato dextrose agar
QE	Quercetin equivalent
RTS	Ready to serve
TPC	Total plate count
WP	Whey protein
WPC	Whey protein concentrate
WPI	Whey protein isolate

Part I

Introduction

1.1 General introduction

Consumer's increased focus on health has resulted in an increase in the awareness of functional foods (Kohli *et al.*, 2019). A wide range of novel functional food products have been developed in recent years; more than 40% of these are dairy products, and the most popular functional beverages are those derived from milk whey. Fruit and milk-based drinks are becoming more and more popular these days due to their nutritional value in addition to their deliciousness (Ahmed *et al.*, 2023).

The valuable liquid by-product known as whey is produced during the precipitation of milk proteins in the cheese industry. It is the main by-product, left after the manufacture of paneer, channa, chakka, cheese, casein, etc. Whey constitutes 45-50% of total milk solids, 70% of milk sugar (lactose), 20% of milk proteins and 70-90% of milk minerals and most importantly, almost all the water soluble vitamins originally present in the milk (Yadav *et al.*, 2010). Furthermore, a variety of methods have been studied over time to convert large quantities of whey into products fit for human consumption. This is due to the fact that only around 60% of the whey produced worldwide is used to produce products; the remainder is either discarded or given to cattle (Arsić *et al.*, 2018).

Additionally, treating the whey before disposal is necessary owing to current environmental restrictions, which presents a difficult and expensive task for enterprises. In addition, it is adding Biochemical Oxygen Demand (BOD) load to effluent (approx 35000 to 45000 mg/L) (Yadav *et al.*, 2010). As an alternative, whey contains a variety of minerals, including vitamins (riboflavin and thiamine), calcium, magnesium, and phosphorus, as well as protein (serum albumin, immunoglobulins, β-lactoglobulin, and α-lactoalbumin), it can be successfully utilized in the preparation of the beverage. Whey proteins are also known as fast proteins because of their ability to quickly deliver nourishment to muscles. It also has the ability to prevent and treat a variety of illnesses, including gonorrhoea, anaemia, jaundice, arthritis, and liver problems (Begum *et al.*, 2019).

Dragon fruit, otherwise called pitaya or pitahaya, is an edible fruit of the *Hylocereus* genus. Dragon fruit is a good source of vitamins, minerals, dietary fiber, glucose, and fructose. Dragon fruit is low in carbohydrates, fat-free, high in fiber, minerals like calcium, iron, and phosphorus, and rich in vitamins B1 (thiamine), B2 (riboflavin), B3 (niacin), and C (ascorbic acid) as well as therapeutic compounds such as betacyanins (potent antioxidants). The flesh of dragon fruit contains a lot of polysaccharides and mixed oligosaccharides, which promote the growth of Bifidobacteria and Lactobacilli. Probiotics are these gastrointestinal microorganisms that inhibit the growth of gastrointestinal pathogens (Sonawane, 2017). Dragon fruit possesses anti-aging qualities, anti-cancer effects, positive effects on metabolism, digestion, and immune system, oxidative stress, diabetes, and cardiovascular disorders (Nurmahani *et al.*, 2012).

Ready to serve is a beverage which should have at least 10% fruit juice, 10% total dissolved solids, and about 0.3% acidity. The beverage is consumed without dilution, hence called ready to serve (Divyasree *et al.*, 2018). Fruit beverages are currently typically flavored with artificial ingredients, bottled, and sold in the market. The consumer, dairy and beverage businesses, and fruit growers would all benefit more if this could be replaced with fruit juice and dairy whey (Sakhale *et al.*, 2012).

1.2 Statement of the problem

The dairy industry generates substantial quantities of whey as a byproduct during the production of cheese, paneer and other dairy products. In Nepal, numbers of large and small-scale industries produce cheese and paneer products, and whey thus produced is dumped in streams. Production of whey by the different projects associated with DDC in the year 2071/2072 was estimated to be more than 3.8 million litres (Anon, 2015). Despite its nutritional value, whey is often considered a waste product, leading to significant environmental and economic challenges. The disposal of whey possess a major problem due to its high biochemical oxygen demand (BOD_5) of 30,000-50,000 ppm on waste water treatment plants or on the land which cause severe pollution if not managed properly (Hsu *et al.*, 1983). The preparation of beverages from whey may solve its disposal problem. Whey based fruit beverage can be made due to its broad range of solubility i.e. pH 3-8. The use of whey for the manufacture of whey-based beverage has been most traditional approach to whey utilization for human nutrition (Djurić *et al.*, 2004).

Red dragon fruit, known for its vibrant color and rich antioxidant content presents an opportunity to create innovative and nutritious drink to satisfy growing consumer demand for functional beverage. Product diversification is quite feasible using whey as water replacer without much change in the composition (Naik *et al.*, 2009). This study aims to address the dual challenging of whey disposal and development of health-promoting functional beverage by formulating whey-based dragon fruit beverage.

1.3 Objectives

1.3.1 General Objectives

The general objective of the dissertation work was to prepare and evaluate quality of whey-based dragon fruit beverage and to study its storage stability.

1.3.2 Specific objectives

The specific objectives of this dissertation work were to:

1. To carry out nutritive analysis of fresh whey and dragon fruit juice.
2. To formulate the proportion of whey and dragon fruit juice for sensory quality.
3. To carry out a sensory evaluation test to select the best product and carry out its nutritive analysis.
4. To carry out phytochemical analysis (antioxidants, total phenolic content, flavonoids & betacyanin content) of dragon fruit juice and the best product.
5. To study the chemical properties and the shelf-life with respect to microbial (total plate count & yeast and mold) of the best product.

1.4 Significance of the study

This study can be beneficial to identify the possible utilization of whey and dragon fruit as the raw materials in the preparation of highly nutritious and appetizing beverage. This study addresses several critical areas. Firstly, whey which is nutritionally rich in proteins, vitamins and minerals could enhance beverage nutritional profile, making beverage a source of essential nutrients like high-quality protein and bioactive compounds. Secondly, innovative use for whey in beverage production, can reduce waste and mitigate the environmental impact associated with its disposal.

This study could have economic significance by adding value to what is typically considered a waste product (whey), creating additional revenue streams for dairy producers and possibly reducing the costs associated with waste disposal and impact on promoting local agriculture of dragon fruit, thereby benefiting farmers and contributing to the local economy. The beverage could offer as a unique health-promoting product with potential benefits such as improved digestion, immune support, and muscle recovery. Thus, this drink based on whey and dragon fruit provides unique functionality in beverage systems due to its great nutritional features, bland flavor and ease of digestion.

1.5 Limitation of the study

The limitations of our study were:

1. Only the paneer whey was utilized.
2. Shelf life of the best sample was only studied for 25 days.
3. The detail nutritional analysis of raw materials and prepared product could not be done due to time and resource limitation.
4. The effect of storage time and temperature on Betacyanin content and antioxidant content of prepared beverage could not be studied due to time limitation.

Part II

Literature review

2.1 Whey

Whey may be defined, broadly, as the serum or watery part of the milk remaining after the separation of the curd that results from the coagulation of milk by acid or proteolytic enzymes (Zadow, 1994). It comprises almost 45-50% of the total solids in milk, 70% of the milk sugar (primarily lactose), 20% of the milk proteins, 70-90% of the milk minerals, and nearly all of the water-soluble vitamins originally present in milk (Dhamsaniya and Varshney, 2013). The protein fraction consists of approximately 50% β-lactoglobulin, 25% α-lactalbumin, and 25% other protein fractions, including immunoglobulins (Zadow, 1994). This led to uncovering the secrets of whey proteins and other components, providing a solid foundation for understanding their nutritional and functional values (Smithers, 2008). The two primary types of whey are sweet whey and acid whey. Acid whey is produced from cheese that has been directly acidified using a mineral acid or lactic acid, while sweet whey is derived from cheese that has been acidified by a starter culture (Bordenave-Juchereau *et al.*, 2005). Acid whey has a pH range of 3.8 to 4.6, whereas sweet whey has a pH range of 5.4 to 6.7 (Bordenave *et al.*, 2005).

2.2 Background of whey production

Dairy industries worldwide produce significant quantities of whey per liter of milk processed, varying based on the methods used and the products manufactured. The annual production of whey in 2023 as by-product reached 200 million tons. Although half of this whey is used in the food and pharmaceutical industries, substantial amounts are still discarded, leading to environmental issues due to its high biological oxygen demand (Mukherjee *et al.*, 2023).

Numerous efforts have been made to incorporate whey into various dairy products, but there remains significant potential to explore its use in beverages, especially in the production of health-based energy drinks (Singh *et al.*, 2009). The utilization of whey in beverage production is more effective and adds value than other methods of treatment and disposal. The majority of the whey produced is spray dried into whey powder, whey protein concentrates, and whey protein isolates (Smithers, 2008). These whey powders are used as ingredients in a wide range of food products, with protein-enriched goods being among the most commercially valuable product lines (Huffman, 1996).

2.3 Nutritional benefits of whey

Whey is a rich source of nutritious components like lactose, vitamins, protein and minerals, along with traces of fat. Whey protein is a complete protein because it contains all nine essential amino acid: isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Its biological components have proven their effects in the treatment of several chronic diseases like cancer, cardiovascular disease etc. As it is nutritionally to rich it can be used in infant athletic and geriatric food (Jelen, 1992; K. Patel, 2012). It has a potential as a functional food component to contribute to the regulation of body weight (Khamrui, 1998). Whey can aid in managing and lowering blood sugar spikes in individuals with type 2 diabetes by enhancing insulin secretion (Frid *et al.*, 2005). As whey contains lactose, it should be avoided by lactose intolerant individuals (Szilagyi and Ishayek, 2018). Other benefits include:

1. Aiding weight loss with significantly loss of body fat and greater preservation of lean muscle.
2. Anti-cancer properties and lowering cholesterol
3. Reduced blood pressure in patients with hypertension and their risk of developing heart disease or stroke and improves the immune response in children with asthma.

The nutritional value of paneer whey is presented in Table 2.1.

Table 2.1 Nutritional value per 100 g of sweet whey

Components	Amount
Energy	112 kJ (27 kcal)
Carbohydrates (sugar)	5.14 g
Fat	0.36 g
Protein	0.85 g
Minerals (Calcium)	47 mg (5%)
Other constituents (water)	93.12 g

Source: Smithers (2008)

2.4 Whey components

2.4.1 Whey proteins

Whey protein consists of two categories: major and minor proteins. The major whey proteins include Beta-lactoglobulin (65%), Alpha-lactalbumin (25%), and serum albumin (8%). The minor proteins and peptides encompass Glycomacropeptide (GMP), Bovine serum albumin, lactoferrin, immunoglobulins, and phosphor-lipoproteins. Whey proteins have a Biological value of 110, which is higher than the value for casein, soy protein, beef, or wheat gluten and have a high content of sulfur-containing amino acids such as cysteine and methionine (Fox *et al.*, 1998). The components found in whey protein are presented in Table 2.2.

Table 2.2 Components found in whey protein

Whey components	% of whey protein	Benefits
Beta-lactoglobulin	50-55%	Source of essential and branched chain amino acids
Alpha-lactalbumin	20-25%	Primary protein found in human breast milk. Source of essential and branched chain amino acids
Immunoglobulins	10-15%	Primary protein found in colostrum Immune modulating benefits
Lactoferrin	1-2%	Antioxidant, Antibacterial, antiviral, and antifungal. Promotes growth of beneficial bacteria Naturally occurs in breast milk, tears, saliva, bile, blood, and mucus
Bovine Serum Albumin	5-10%	Source of essential amino acids Large protein
Glycomacropeptide	10-15%	Source of branched chain amino acids Lacks the aromatic amino acids phenylalanine, tryptophan, and tyrosine
Lactoperoxidase	0.50%	Inhibits growth of bacteria

Source: Marshall, (2004)

2.4.1.1 β-lactoglobulin

β-Lactoglobulin, a major protein making up about 10 to 15% of the total proteins in milk, is a globular protein composed of 162 amino acids and has a molecular weight of 18.4 kDa (Liu *et al.*, 2007). β-Lactoglobulin can bind to fatty acids and retinol (vitamin A), which gives it excellent foaming and gelation properties (Heino, 2010).

2.4.1.2 α-lactalbumin

α-lactalbumin constitutes about 25% of whey protein, is a small whey protein with molecular weight of 14.2 kDa. It is acidic, with an isoelectric pH between 4 and 5 and has ability to bind calcium ions (Whitney *et al.*, 1976). It is rich in tryptophan (Trp), which is crucial for maintaining healthy brain function. Additionally, it contains many amino acids necessary for muscle protein synthesis (Thompson *et al.*, 1988).

2.4.1.3 Bovine serum albumin

Another protein present in cow milk is bovine serum albumin (BSA), which has a molecular weight of 66 kDa. Despite its significant physiological effects on human health, its interactions with food and milk are not well understood. Due to its low concentration in milk, BSA has minimal impact on the physicochemical properties of whey protein (Heino, 2010).

2.4.1.4 Lactoferrin

Lactoferrin is a multifunctional protein with a molecular weight of 76.5 kDa that has been studied broadly the past decades. It is well known for its iron binding ability, which eventually led to the discovery of its antibacterial properties. Furthermore, lactoferrin has shown strong antiviral, antifungal and antiparasitic properties against a variety of species (Jenssen and Hancock, 2009).

2.4.1.5 Glycomacropeptide

Casein macro peptide (CMP), also known as glycomacropeptide (GMP), is the C-terminal segment of kappa casein. It has a molecular weight ranging from 6 to 10 kDa and features a unique amino acid profile. GMP possesses various chemical properties, such as strong emulsifying capabilities and stability across a wide pH range (Neelima *et al.*, 2013).

2.4.1.6 Immunoglobulin

The immune component of milk is known as immunoglobulin. Antibodies called immunoglobulins can shield people from a variety of germs and viruses. Cow's milk contains a low level of immunoglobulin, whereas human milk has the highest level. The molecular weight of immunoglobulin ranges from 150 to 1000 kDa. Because these proteins contain immune-active peptides, their inclusion in a whey product is advantageous (Tovar *et al.*, 2012).

2.4.2 Lactose

Lactose, which makes up about 70% of the total solids in whey, is likely the least valuable and most challenging component to utilize (Macwan *et al.*, 2016).

2.4.3 Minerals

Whey is a valuable source of electrolytes, such as sodium and potassium, which are essential for recovery from diarrhea. It also contains minerals like calcium, magnesium, and phosphorus, both in solution and partially bound to proteins. Additionally, zinc is present in trace amounts (Zadow, 2012).

2.4.4 Vitamins

During the manufacturing process, varying amounts of water-soluble vitamins are transferred into whey: 40-70% of vitamin B12, 55-75% of vitamin B6 and pantothenic acid, 70-80% of riboflavin and biotin, and 80-90% of thiamine, niacin, folic acid, and vitamin C. Notably, more vitamin B12 is transferred into whey when rennet coagulation is used compared to acid coagulation (Zadow, 2012).

2.5 Valorization of whey

The dairy industry generates significant amounts of whey as a by-product, which possess serious environmental challenges due to its high organic content. In recent decades, research has focused on finding more environmentally and economically efficient ways to utilize whey, aiming to transform this by-product into a valuable raw material. Sustainable whey management mainly focuses on biotechnological and food-related applications, leading to the creation of value-added products like whey powders, whey proteins, functional foods and beverages, edible films and coatings, lactic acid, various biochemicals, bioplastics, biofuels, and other similar high-value bioproducts (Zandona *et al.*, 2021).

A substantial portion of whey powder, whey concentrate, reduced lactose/ reduced mineral whey, whey protein concentrates and lactose are used in human food product applications, whereas whey blends are used mainly in animal feed products. Whey-based blends are formulated to provide a composition similar to that of non-fat dry milk. Most of the whey concentrate for human food is used in dairy, bakery and confectionery products. Most of the acid whey used in human food product production is produced as dried and modified whey (Darade and Ghodake, 2012).

2.6. Composition of whey

The composition of whey is primarily determined by its source (type of cheese produced) and methods used in whey processing. The composition of whey is illustrated in Table 2.3.

Table 2.3 Composition of whey

Parameters	Acid whey	Rennet whey
Total solids (%)	6.3-7.0	6.3-7.0
pH	3.8-4.6	5.6-6.0
Lactose (%)	5.03	5.01
Protein (%)	0.38	0.98
Fat (%)	0.13	0.34
Ash (%)	0.60	0.54
Lactic acid (%)	0.21	0.14
Calcium (ppm)	710.65	501.50
Phosphorous (ppm)	560.50	441.50
Potassium (g/l)	1.4-1.6	1.4-1.6
Chloride (g/l)	1.0-1.2	1.0-1.2
Magnesium (g/l)	0.11	0.08

Source: Darade and Ghodake (2012)

2.7 Therapeutic and functional value of whey

2.7.1 Therapeutic value of whey

Whey based beverages target a large scale of consumers - from old people to little children. Because of its health benefits, it was used to treat some illnesses, such as tuberculosis and skin and digestive tract diseases, since the time of Ancient Greece. Whey was also successfully applied for treatments of diarrhea, bile illness, skin problems, scales in the urinary tract and some intoxication. Due to high amount of whey proteins with nutritional value these beverages are ideal source of energy and nutrients for athletes. Whey proteins are a rich source of branched chain amino acids (BCAA) like isoleucine, leucine and valine. BCAAs unlike other essential amino acids are metabolized directly into the muscle tissue and are first amino acids used during periods of exercise and resistance trainings (Shukla *et al.*, 2000)

Whey protein fractions include also lactoferrin which is best known for its ability to bind iron, Glycomacropeptide (GMP), which is naturally free of phenylalanine and alpha-lactalbumin after rennet coagulation, is a calcium binding protein. The presence of lactoferrin in whey beverages improves iron absorption from food and/or assist to prevent pathogens from attaching to the intestinal walls which is essential for nutrition of little children and babies. In addition, these beverages might enhance calcium absorption for the elderly population suffering from osteoporosis (Miller, 2005).

The strong antioxidant action in whey is mostly attributed to cystine-rich proteins that facilitate the synthesis of glutathione, a strong intracellular antioxidant, also investigated as an anti-aging agent. Glutathione peroxidase, generated from selenium and cysteine, contributes a detoxifying property by converting lipid peroxides into less toxic hydroxyl acids, while α-lactalbumin, due to its iron-chelating characteristics, chelates heavy metals and lowers oxidative stress. Whey's immunoglobulin and lysozyme confer passive immunity to infants and others due to their antimicrobial effect. Blood pressure is lowered by antihypertensive peptides derived from bovine β-lactoglobulin (Miller, 2005).

Many minerals, such as potassium, which is involved in the transmission of nerve impulses and muscular contractions; magnesium, which depolarizes the nerve or muscle and lowers blood pressure while maintaining an alkaline tissue pH; and calcium, which the body uses to maintain bone density, cell wall integrity, and nerve impulses. Lactoperoxidase inhibits the growth of iron dependent bacteria. Whey is a good source of vitamin like A, B_1, B_2, B_3, B_5, B_6, C, D, and E (Onwulata and Huth, 2009).

2.7.2 Whey proteins as functional ingredient

In addition to their nutritional and therapeutic advantages, whey proteins offer a number of advantageous properties, such as excellent solubility, foaming, emulsifying, gelling, and water binding capacities (Patel *et al.*, 1990). In the alternative medicine community, whey protein is promoted extensively and consumed as a dietary supplement, with numerous health benefits (Marshall, 2004). Numerous functional qualities of whey contribute to the flavor, color, texture, and overall acceptability of a wide range of foods. Moreover, post prandial insulin stimulation and increased plasma amino acid levels have been linked to branched-chain amino acids from whey, including isoleucine, leucine, and valine (Matthews, 2005). Table 2.4 shows the whey protein as a functional ingredient.

Table 2.4 Whey protein as a functional ingredient

Function	Benefits	Uses
Emulsification	Creates stable emulsions and prevents fat globules from forming clumps.	Baked products, beverages, Ice-cream mixes, mayonnaise type dressings.
Flavor Enhancement	Brings out already present flavors or adds flavor.	Baked products, beverages, confectionery, snacks.
Gelling and heating setting	Maintains moistness and improves texture and mouth feel.	Baked products, beverages, dairy products, yoghurts.
Solubility	Easily dispersed in most systems. Prevents sedimentation in beverages, soups and sauces.	Beverages, confectionery, frozen desserts, infant formula, soups and sauces.
Water binding and building viscosity	Provides fat-like attributes in products allowing reduction in fat content, improved texture and moistness.	Baked products, beverages, dairy products, coffee creamers, soups and sauces.
Whipping, foaming and aeration	Maintains foam properties, enhancing appearance, taste and texture.	Baked products such as meringues and cakes, confectionery, ice-cream, frozen desserts.

Source: Bohara (2018)

2.8 Application of whey products

Due to the fact that lactose makes up the majority of whey solids, along with soluble proteins, vitamins, and minerals, a number of biotechnological and physicochemical techniques have been used to use whey as a substrate to create valuable and essential industrial products (Prazeres *et al.*, 2012). From an industrial perspective, whey products, such WPIs and WPCs, are well-known functional groups of dairy ingredients that are widely used in a variety of food applications (Foegeding *et al.*, 2002).

They are widely used as food additives because of its unique functional qualities, which include thickening, emulsification, gelation, and the ability to bind fat and flavor (Larsen *et al.*, 2010). The food system's hydration, flavor/texture, surfactant, visual, textural, structural, and rheological qualities can all be altered by WPC. Bakery goods, meat and fish products, dairy products, confections, fruit beverages, medical and nutritional applications, food for special needs (like baby formula, dietetic foods), and nutraceutical foods are just a few of the food categories that use whey-based ingredients (Onwulata and Huth, 2009). Due to the heterogeneous mixture of lactose, proteins, fats, vitamins, minerals etc., whey can be used as a fermentation media for the production of various bio-products. Some of the fermentation derived products include bio-ethanol (Siso, 1996), single cell protein (Raj *et al.*, 2014), glycerol (Rapin *et al.*, 1994) etc.

2.8.1 Whey products

2.8.1.1 Whey protein isolate

Whey protein isolate is a highly refined dairy-derived protein supplement containing over 90% protein, with minimal lactose and fat, making it suitable for individuals with lactose intolerance (Gomaa *et al.*, 2022). Branch-chain amino acids (BCAAs), especially leucine, which is abundant in WPI, are essential for promoting muscle protein synthesis and improving recovery after exercise. Due to its high digestibility and rapid absorption, WPI elicits a strong postprandial plasma amino acid response, contributing to improved metabolic health and physical performance. WPI has demonstrated functional properties such as emulsifying, gelling, and antioxidant activity, making it valuable in food formulation and biomedical applications.

2.8.1.2 Whey protein concentrate

Whey protein concentrate (WPC) is a dairy-derived protein supplement typically containing 35–80% protein, along with lactose, fat, and bioactive compounds that contribute to its nutritional and functional properties. Due to its techno-functional properties such as emulsification, gelling, and foaming, WPC is widely used in food formulations including beverages, bakery products, and dairy-based snacks (Kumar et al.,2018). The cost-effectiveness and nutrient density of WPC make it a preferred choice over whey protein isolate in many commercial formulations, despite its higher lactose and fat content (Pan *et al.*, 2025).

2.8.1.3 Whey protein concentrate

Whey protein hydrolysate (WPH) is produced through enzymatic cleavage of intact whey proteins, resulting in low-molecular-weight peptides with enhanced digestibility and rapid amino acid absorption (Nakayama *et al.*, 2019). WPH contains bioactive peptides that exhibit antioxidant, antihypertensive, and immunomodulatory properties, making it a promising ingredient in functional foods and nutraceuticals. The degree of hydrolysis and drying method (e.g., spray vs. freeze drying) influence WPH's physicochemical characteristics and its antioxidative potential (Kleekayai *et al.*, 2022).

2.8.1.4 Whey protein bar

Whey protein bars are formulated as convenient, nutrient-dense snacks that deliver high-quality protein, often exceeding 20 g per serving, making them suitable for athletes and health-conscious consumers. These bars typically incorporate whey protein concentrate (WPC) or isolate (WPI), along with functional ingredients like inulin, dried fruits, and nuts to enhance fiber content, antioxidant activity, and sensory appeal. Studies have shown that whey protein bars can support lean body mass gain and post-exercise recovery when consumed regularly alongside resistance training. (Szydłowska *et al.*, 2020).

2.9.2 Bakery and confectionery Products

2.9.2.1 Bakery products

Whey is commonly used to make breads, cakes, cookies, biscuits, crackers, muffins, and icing in the pastry, confectionery, and baking industries. Cake structure and taste development are contributed by eggs. Whey proteins can also be used to replace eggs to reduce production costs because the resulting product crumbles less when sliced and packaged, saving cost. Additionally, egg yolks include cholesterol, there is increased interest in replacing WPCs for this component due to nutritional concerns (Stoliar, 2009).

WPC34 can be used as a partial replacement for both egg and fat in items such as spice cookies or chocolate chip cookies. WPC80, on the other hand, is an excellent egg substitute in bread, cakes and biscuits (both dry and soft), and muffins. Lactose (from whey) is frequently used as a sucrose alternative in baking, confectionery, and pastry because it increases the Maillard reaction, improves emulsification and crumb structure, and boosts flavor. Furthermore, whey proteins are high in essential amino acids and are regarded as a high-quality protein source. Furthermore, they are 18 distinguished by a high level of calcium and other minerals such as potassium and zinc, making whey protein a great component to bread item (Stoliar, 2009).

2.9.2.2 Confectionary products

Whey products such as demineralized whey powders, low-lactose whey powders, WPCs and isolates, and lactose have been used in confectionary such as chocolates and chocolate chips, sweets, jellies, and chewing gums. Lactose, or milk sugar, can be used as a bulking agent. It is somewhat sweeter than sucrose, less soluble, and has a low hygroscopicity; yet, it effects the color, taste, and texture of the completed product and participates in the Maillard reaction. For these reasons, the use of lactose can be reduced to a greater or lesser extent,18 depending on a variety of confectionary features. Another application for WPC derivatives is the manufacture of aerated confectionery and chocolate. Concentrate foaming properties are utilized in this circumstance. Furthermore, WPI and high protein content concentrates (WPC80) can be successfully used in the creation of protein bars for athletes (Barry, 2008).

2.9.3 Whey Beverage

2.9.3.1 Whey beverages based on fruit juices

The most widely produced whey beverages are blends of fruit juices and unprocessed or deproteinated whey or UF permeates. These products typically serve a purpose comparable to that of traditional fruit juices, such as a breakfast beverage, healthy fruit juice, snack-type beverage, or beverage with a healthy image as a vitamin source. The two most common basic ingredients are liquid whey and liquid fruit juice or, most commonly, fruit juice concentrate. Citrus fruits (primarily orange, followed by lemon, and rarely grapefruit), as well as mango, passion fruit, pear, apple, strawberry, raspberry, or fruit juice combinations with exotic descriptive terms ('tropic mix', 'multifruit,' etc.) are commonly used in these beverages because they have proven to be very effective in masking the undesirable odor of cooked milk and the salty-sour flavor of fresh whey. Berries, which are high in iron and antioxidants, have been added and have proven to be highly beneficial (Djurić *et al.*, 2004).

Whey based mango RTS beverage prepared with whey and mango pulp in the ratio 69.9: 20 (with 10% sugar and 0.1% CMC at 0.3% acidity level), was found to give the beverage with best sensory score in terms of taste, body and overall acceptance (Pandey and Ojha, 2020). Sakhale *et al.* (2012) revealed that the RTS beverage prepared with 70% whey and 30% mango juice had the highest ascorbic acid content (9.80 mg/100 g) and scored maximum for almost all sensory quality attributes (appearance, color, flavor, and overall acceptability). Custard apple whey beverage made with a 90:10, 85:15, and 80:20 pulp to whey ratio. The blend that achieved the highest sensory score among these was 85:15 (whey: pulp) with 10% sugar (Ingale *et al.*, 2009). Organoleptic assessment revealed that the whey-based pineapple fruit juice beverage, made with whey and water (70:30) and 20% fruit juice, was more acceptable than the samples made with a different ratio of whey to water and fruit juice concentration because it had a good flavor and taste and overall acceptance (R. Chandra *et al.*, 2017b)

2.9.3.2 Nutritious beverages

There are already fruit-flavored beverages with 75–90% whey content (Nelson et al., 1996). Peach puree (20%), strawberry (10%), and red raspberry (10%) were acceptable flavorings. The flavored whey drinks were made by concentrating them to 18-20° Brix and adding citric acid to lower the pH to 3.6. Proteins in these whole whey drinks may precipitate and form deposits in the container at low pH. If the market is sensitive to residues, drinks made from deproteinized whey or whey permeate avoid this issue (Krishnamurthy *et al.*, 1990).

For drinks containing highly resorbable lactose, vitamins, and important mineral salts, deproteinized whey or whey permeate are preferable bases. Lactose is fermented to lactic acid after permeate has been acidified with organic acids to create whey lemonades and still whey beverages (Beucler *et al.*, 2005).

Calcium (Ca) is frequently added to whey-based nutritional drinks to meet the recommended Ca intake. However, whey protein-based nutritional solutions that fortify with calcium often encounter technical and product quality issues, such as low heat stability, high viscosity, colloidal instability, and reduced heat transfer (Barone *et al.*, 2022).

2.9.3.3 Alcoholic whey beverages

Whey is an excellent raw material for making alcoholic beverages because lactose accounts up 70% of its dry matter. These beverages include whey beer, whey wine, whey champagne and beverage with low alcohol content 1.5% alcohol (Jeličić *et al.*, 2008).

2.9.3.3.1 Whey beverages with low alcohol content

Deproteinizing whey, concentrating whey, fermenting lactose (often by yeast strains *Kluyveromyces fragilis* and *Saccharomyces lactis*) or adding sucrose until the desired alcohol content (0.5–1%) are all steps in the production of low-alcohol whey beverages. Flavoring, sweetening, and bottling are additional steps. As a result, part of the lactose is converted to lactic acid, giving the finished product a refreshingly sour flavor, and the remaining portion ferments to produce alcohol (Jeličić *et al.*, 2008).

Koumiss-like drinks can be made by fermenting whey and buttermilk mixes or whey and milk mixtures. Guan and Brunner created a koumiss-type product using a skim milk/whey blend that was inoculated with 2.5-10% of a culture including lactobacilli and yeast and cultured at 78°F for 12-15 hours to a lactic acid level of 1%. The fermented result is mixed, homogenized, and packaged in glass bottles. The bottles are kept at 68-77°F for 2 hours to create CO2 and alcohol before being cooled to less than 40°F. The koumiss-like product has a four-week shelf life at 40°F (Guan and Brunner, 1987).

2.9.3.3.2 Whey beer

It is possible to manufacture whey beer without adding malt, or it can be enriched with vitamins and minerals or starch hydrolyzates. Some issues that may arise include the presence of milk fat, which can result in the loss of beer foam, an off-odor and flavor due to low solubility whey proteins and the inability of beer yeasts to metabolize lactose. (Jeličić *et al.*, 2008).

2.9.3.3.3 Whey wine

Whey wine contains relatively low alcohol amount (10-11%) and is mostly flavored with fruit aromas. Production of whey wine includes clearing, deproteinazation, lactose hydrolysis by ß-galactosidase, decanting and cooling, addition of yeasts and fermentation, decanting, aging, filtering and bottling (Jeličić *et al.*, 2008).

2.10 Need for development of whey based beverage

The use of cheese whey in human nutrition, particularly for therapeutic purposes, can be traced back to the ancient Greeks; Hippocrates recommended whey for a variety of human maladies in 460 B.C. Five important driver groups are driving market dynamics: growing concentration in the global beverages market; divergent functional beverage trends globally; flavor developments; product differentiation; and cross-category innovations. Whey beverages are made and prepared with nutritional, biological, and functional qualities in mind (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 flavor 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.

2.11 Use of fruits in preparation of whey beverage

The whey-guava beverage prepared by (Bhatt *et al.*, 2014) in the ratio of 67.5:20 was found to be more palatable and of good quality in terms of protein and vitamin C content.

Dhamsaniya and Varshney (2013) used milk whey and ripe banana juice to make a delicious and healthful RTS beverage. Mentha arvensis (mint) extract was added as a natural flavoring ingredient. Within 100 mL of the produced beverage, the amounts of milk whey, M. arvensis extract, and banana juice were 5–15 mL, 1–5 mL, and 72–86 mL, respectively. Samples of beverages were screened according to their physicochemical and sensory characteristics. It was concluded that the whey-banana-RTS beverage, which contained 77 mL of milk whey, 15 mL of banana juice, 3 mL of M. arvensis extract, and 8 g of sugar powder per 100 mL, was acceptable and suggested for large-scale manufacture. The additional researches in whey beverages are listed in Table 2.5.

Table 2.5 Other examples of fruits use in whey beverage

S.N.	Type of whey	Products	Reference
1.	Channa whey	Whey kiwi fruit extract beverage	(Rohit *et al.*, 2019)
2.	Paneer whey	Whey banana beverage	(Shrestha and Dahal, 2021)
		Whey beetroot mint beverage	(Satpute *et al.*, 2018)
3.	Cheese whey	Whey pineapple herbal beverage	(Kumar *et al.*, 2017)
		Sport drink based on passion fruit	(Silva *et al.*, 2023)
4.	Chakka whey	Whey watermelon beverage	(Borkar *et al.*, 2020)
		Whey pomegranate beverage	(Babar *et al.*, 2008)

2.12 Use of dragon fruit in beverage

2.12.1 Dragon fruit

Grown on a veining epiphytic cactus (*Hylocereus sp.*), pitaya, pitahaya, also known as dragon fruit, are native to tropical forest regions of Mexico, Central America, and South America. The plants are supported by aerial roots and climb up tree trunks. The juicy flesh can be white or magenta, and the fruits have thornless skins that are pink or red in color. The seeds are small and are consumed with the fruit. The fruit can weigh up to 900 grams, but the average weight is between 350 and 450 grams. The fruit weight depends on variety selection as well as pollination (Merten, 2003). Because of its stunning beauty, the dragon fruit flower has earned the nicknames "Noble Woman" and "Queen of the Night." The huge, creamy white flowers (25 cm in diameter) that bloom at night give it its beautiful importance (Kakade *et al.*, 2020). The main benefit of this crop is that, once planted, it will continue to grow for over 20 years, and 800 dragon fruit plants may be grown on one hectare. It produces fruit in the second year after planting and attain in full production within five years. Commercial cultivation of it is practiced in united states, Australia, Vietnam, Taiwan, Nicaragua, and Israel (Merten, 2003).

Pitaya, also known as dragon fruit, is a member of the Cactaceae family and is found primarily in the genera *Hylocereus* and *Selenicereus*. The Hylocereus genus, which includes about 16 different species, produces the most widely grown commercially farmed variants (Barthlott and Hunt, 1993). The genus *Selenicereus* comprises 20 species distributed through tropical America and the Caribbean region.

The majority of Hylocereus species are native to Latin America, mostly Mexico and Colombia, with some others possibly hailing from the West Indies (Britton and Rose, 1963). The white skinned dragon fruit (*Hylocereus undatus*), which originated in Vietnam, was introduced to Nepal in the year 2000 AD by an American engineer who was stationed there. Only around 2014 AD its commercial plantations began in the Kabhre district (Atreya *et al.*, 2020).

The semi-epiphytic plant known as dragon fruit prefers a dry tropical and subtropical climate conditions that range in temperature from 21 to 29 °C on average, but can sustain temperatures as high as 40 °C and as low as 0 °C for brief periods. It needs plenty of sunshine and 600-1300 mm of rain every year, with alternate dry and rainy seasons. It can reach heights of 1.5 to 2.5 meters, branching like a delicate vine. It is a three-winged, succulent terrestrial or epiphytic cactus (Ishnava and Patel, 2019). The stem has several branching segments and is mushy and vine-like. Each segment bears three wavy wings, one to three spines, or occasionally none at all (Merten, 2003). The plant's aerial roots take up water, grow on the stem's undersides and hold the stems firmly in place on vertical surfaces. Typically white in color, dragon fruit is 25 to 30 cm in length and 15 to 17 cm in width.

A single pole of a dragon fruit tree with five to six branches can yield 150 to 200 kg of fruit throughout its productive season, which runs from September to May. The dragon fruit has remarkable features, including bright red, purple, or yellow skin variations and noticeable scales. The fruit is characterized by oval, elliptical or pear shape. The flesh has a mildly sweet or occasionally slightly sour flavor. The flesh is either white or red and is covered with edible tiny black seeds all over (Blancke, 2016).

2.12.3 Taxonomy and botanical description of dragon fruit

The genus Hylocereus is a small genus that contains 18 tropical American species (Barthlott and Hunt, 1993; Maberley, 1993). The nomenclature of dragon fruit is as shown in table 2.6.

Table 2.6 Nomenclature of dragon fruit

Kingdom	Plantae (Plants)
Sub kingdom	Trachebionta (vascular plants)
Super division	Spermatophyta (seed plants)
Division	Magnoliophyata (flowering plants)
Class	Magnoliopsida (dicotyledons)
Order	Caryophyllales
Family	Cactaceae (cactus family)
Genus	Hylocereus
Species	Hylocereus polyrhizus

Source: Britton and Rose (1963)

Gunasena *et al.* (2006) have classified different edible cacti species based on nature of stem habit, fruit skin and pulp color. Some of the vine cacti of Hylocereus species with their fruit skin and pulp color are given in Table 2.7

Table 2.7 Dragon fruit species

Species	Fruit peel color	Pulp color
Hylocereus undatus	White	Red
H. undatus, *H. costaricensis*, *H. polyrhizus*	Red	Red
H. ocamponis	Red	Yellow
H. megalanthus	White	Yellow

Source: Britton and Rose (1963)

2.12.2 Origin, history and distribution and production

H. undatus spread over the Americas and the Caribbean in pre-Columbian times, by dispersal through birds and people who cultivated the plant for fruits. Native to tropical and subtropical forest regions of Mexico, Central America, and Northern South America, the dragon fruit, also known as pitaya, is widely distributed and occurs naturally in these areas (Barthlott and Hunt, 1993; Britton and Rose, 1963). In the sixteenth century, this fruit was introduced into the Philippines by the Spanish and into Indochina by a French priest in the mid nineteenth century (earlier it was Vietnam, Laos and Cambodia) and later it is acclimatized and became a major fruit crop of region due to conducive climatic conditions. Later, it became an important fruit crop throughout South-East Asia and now it is cultivated widely in the tropics and subtropics (Nerd *et al.*, 2002; Nobel, 2002).

From the center of origin, it has been spread to several countries. The cultivations of dragon fruit have been taken up successfully in many countries viz. Australia, Cambodia, China, Colombia, Ecuador, Guatemala, Hawaii, Indonesia, Israel, Malaysia, New Zealand, Peru, Philippines, Taiwan, Thailand, Spain, Sri Lanka, and Vietnam etc. In India, this fruit crop has been recently introduced and the cultivation has been taken by few growers in parts of Maharashtra, Karnataka, Gujarat, West Bengal etc. At present, Vietnam is the major producer and exporter of dragon fruit in the world. Presently, India's import basket receives pitaya fruits from Vietnam and Thailand (Kakade *et al.*, 2022).

Production data for most new and expanding tropical fruit is rarely available. Available evidence from individual countries suggests that dragon fruit production is expanding rapidly in the past and current decade. It has proliferated from where it began to several other countries. Dragon fruit is being grown successfully in many countries. Australia, Cambodia, Ecuador, Guatemala, Hawaii, Indonesia, Israel, Malaysia, New Zealand, Peru, Philippines, Taiwan, Spain, Sri Lanka, and Vietnam and others countries. In southeast Asia, especially in Thailand and Vietnam, dragon fruit is extensively cultivated as an export commodity and Vietnam is the largest producer of dragon fruit in the world today (Kakade *et al.*, 2022). The major countries, Vietnam, China and Indonesia, provide over 93% of the world dragon fruit production. Vietnam alone provides more than half (51.1%) of the world production. It has almost 55,419 ha devoted to dragon fruit farming with a volume of production more than 1 million metric tons/hector/year valued at US $895.70 million (2018). The average productivity is 22-35 metric tons/hector/yr (Sharma *et al.*, 2021). It is the fifth most famous tropical crop fruit from Asia behind lychee, longan, banana and mango (Chen and Paull, 2019).

In the year 2000 AD, an American engineer introduced the white dragon fruit to Nepal from Vietnam. Its commercial farming started only after 2014 AD in Kabhre district. With the satisfactory results of these commercial farms, quite a number of farmers from various corners of the country have started plantations of white fleshed as well as red fleshed cultivars on trial basis in small scale or even on commercial scale (a total of about 8-10 ha) by a few enthusiastic persons including ex Minister Mr. Lokendra Bist in Dang district. This way, now dragon fruit is becoming an exciting new fruit crop for warmer region of the country (Atreya *et al.*, 2020).

2.12.3 Nutritional value

The nutritional value of dragon fruit varies depending on its species, place of origin, and harvest season (Elmarzugi *et al.*, 2016). The phytochemical composition and nutritional composition of red dragon fruit are significantly influenced by their growth environment (Liaotrakoon, 2013). More superior essential fatty acids. Dragon fruit pulp and peel extract have antibacterial effect and can be used as a natural antioxidant. Additionally, the red pulp has a high content of betalains, which satisfies to the growing market demand for natural food colorant and antioxidant-containing products (Perween *et al.*, 2018). Table 2.8 shows nutritional composition of red dragon fruit juice.

Table 2.8 Nutritional composition of red dragon fruit juice (fresh weight) per 100 g

Components	Amount (in g)
Energy	263.5 kJ
Moisture	85.5
Ash	0.54
Carbohydrate	12.97
Protein	1.45
Fat	-
Total dietary fiber	2.65
Iron	0.30 mg
Magnesium	26.40 mg
Potassium	158.29 mg
Sodium	35.63 mg
Zinc	0.40 mg
Calcium	6.27 mg
Vitamin A	85.22 µg
Vitamin C	24.66 µg

Source: Nurul and Asmah (2014)

According to Jeronimo *et al.* (2017), mature dragon fruit is characterized by a notable amount of total soluble solids, a substantial amount of organic acids, protein, and a wide range of minerals, such as calcium, magnesium, and potassium. Dragon fruit is a good source of vitamins, minerals, dietary fiber, glucose, and fructose. Dragon fruit is low in carbohydrates, fat-free, high in fiber, minerals like calcium, iron, and phosphorus, and rich in vitamins B1 (thiamine), B2 (riboflavin), B3 (niacin), and C (ascorbic acid). On the other hand, 50% of the essential fatty acids found in seeds are linoleic and linolenic acid (Sonawane, 2017). Many researchers have come to the conclusion that the vitamin C content varies according to the fruit's maturity level, species, origin, crop, and extraction technique. While red-flesh dragon fruit differs from white-flesh dragon fruit in terms of moisture content, fat content, and other characteristics (Luu *et al.*, 2021). Certain studies have determined that the peel of dragon fruit has higher quantities of organic acids and amino acids than the pulp. Tartaric acid, malic acid, fumaric acid, citramalic acid, propanedioic acid, talonic acid, and mannonic acid are the organic acids. It also includes essential amino acids such as aspartic acid, valine, alanine, serine, tyrosine, glutamic acid, leucine, lysine, and tryptophan (Jiang *et al.*, 2021).

2.12.4 Health benefits of dragon fruit

Dragon fruit is rich source of nutrients such as vitamins, minerals, complex carbohydrates, dietary fibers and antioxidants. According to studies, dragon fruit stimulates the growth of beneficial intestinal flora and a red or purple pigment called betacyanin has anti-oxidant effects (Liaotrakoon, 2013). Betacyanin’s, phenolic compounds, polysaccharides, and terpenoids, which function naturally as antioxidants and anti-inflammatory agents, are among the phytochemicals with bioactive qualities found in the pulp and peel of dragon fruit. Regular consumption of dragon fruit can help prevent cough and asthma due to its high vitamin C content. It can also speed up the healing of cuts and wounds (Rao and Sasanka, 2015).

Research indicates that the consumption of dragon fruit aided in the growth of beneficial gut flora and the antioxidant pigment betacyanin, which is a reddish-purple pigment (Liaotrakoon, 2013). It is rich in antioxidants, low in calories, and cholesterol-free. It also helps to manage blood pressure and reduce the risk of cardiovascular heart disease (Ishnava and Patel, 2019). The flesh of dragon fruit contains a lot of polysaccharides and mixed oligosaccharides, which promote the growth of Bifidobacteria and Lactobacilli. Probiotics are these gastrointestinal microorganisms that inhibit the growth of gastrointestinal pathogens. Another application for dragon fruit is as a natural probiotic (Sonawane, 2017).

It possesses anti-aging qualities, anti-cancer effects, positive effects on metabolism, digestion, and immune system, oxidative stress, diabetes, and cardiovascular disorders (Nurmahani *et al.*, 2012). It is used to treat diabetes and boost immune system function. Blood circulation is improved by medicines derived from its bloom and stem. The fruit provides a wealth of nutrients, such as fiber, antioxidants, calcium, phosphorus, and vitamin C. The nutrients included in dragon fruits can help prevent asthma and arthritis, decrease cholesterol level, and control diabetes. High iron content in red dragon fruit raises levels of erythrocytes and haemoglobin in pregnant women (MR, 2010). The high level of calcium and phosphorus contained in dragon fruit plays an important role in the formation of tissue and healthy teeth and helps to reinforce bones (Choo and Yong, 2011).

Despite having a high fibre content, dragon fruits aid in the digestion of food. Dragon fruit is also a great source of the B vitamin family (B_1, B_2, and B_3), which is important for health benefits. In addition to serving as a multivitamin, vitamin B2 in dragon fruit also helps to improve and restore appetite loss. Vitamin B_1 facilitates improved glucose metabolism and energy generation. In addition, vitamin B3 from dragon fruit lowers harmful cholesterol levels while also moisturising and smoothing skin. It also reduces blood pressure and improves vision. Dragon fruit is used as food colouring and can also be eaten as a fresh fruit to quench thirst because of its high water content in relation to other nutrients (Jeronimo *et al.*, 2017)

Table 2.9 Functions of some of the main components contained in dragon fruit

Components	Amount	Functions
Flavonoids	Red fleshed 46.29 ± 2.47 mg RE/100 g FW & white fleshed 26.71 ± 4.46 mg RE/100 g FW	Flavonoids are acts on brain cells and blood vessels to reduce the risk of heart diseases. It minimizes heart diseases and maintains blood pressure.
Betalains	42.71 ± 2.48 mg/100 g fresh pulp	Betalains can combat oxidative stress and may have the ability to suppress cancer cells. It has the ability to aid in weight loss, improves digestion, reduce LDL cholesterol in the blood and strengthen the immune system.
Hydroxycinnamates	Minor amounts of hydroxycinnamic acids	Hydroxycinnamates helps to prevent cancer.
Carotenoids (Beta-carotene)	1.4 mg/100 g	Reduced risk of cancer and cardio-vascular disease.
Linoleic acid and linolenic acid	Seeds rich with 50% of essential fatty acids	The seeds of Dragon fruits are high in polyunsaturated fats (omega-3 and omega-6 fatty acids) that reduce triglycerides and lower the risk of cardiovascular disorders
Vitamin C	White-fleshed 31.11 ± 3.85 mg /100g FW & Red fleshed 20.00 ± 1.33 mg / 100g FW	Regular consumption of Dragon fruit that contains a high amount of Vitamin C would help in fighting against cough and asthma; increases the wound healing properties and quickly heals the cuts areas; moreover, enhances the immune system and also stimulate the activity of other antioxidants in the body.
Phosphorus (P) and calcium (Ca)	P 22.5 mg/100g and Ca 8.5 mg/100g	Dragon fruit contains high levels of phosphorus and calcium; which helps to reinforce bones and plays an important role in tissue formation and forms healthy teeth.
Iron	1.9 mg/100 g	Red dragon fruit have so much iron which increases haemoglobin and erythrocyte levels in pregnant women.

Source: Hossain *et al.* (2021)

Part III

Materials and method

3.1 Materials

3.1.1 Whey

Paneer whey was collected from Kamdhenu Dairy Development Coop Ltd, Itahari-1, Sunsari. It was stored under refrigerated (7±1°C) condition for further use.

3.1.2 Sugar

Table sugar was purchased from local store of Dharan. Sugar was grinded for further use.

3.1.3 Dragon fruit

Dragon fruits (*Hylocereus polyrhizus)* brought from dragon fruit farm Panmara, Sunsari. They were sorted, graded and washed thoroughly in water to remove foreign materials. They were peeled cut into small pieces before grinding. Thereafter juice was extracted by passing through three folds of muslin cloth.

3.1.4 Bottle

Glass bottles of 250 ml capacity were obtained from dairy laboratory of CCT.

3.1.5 Equipment and chemicals

Equipment and chemicals required were utilized from Central Campus of Technology laboratory which are given in Appendix D.1 and D.2.

3.2 Methods

3.2.1 Preparation of whey

Paneer whey was collected from Kamdhenu Dairy Development Coop Ltd, Itahari-1, Sunsari. According to them, standardized cow milk or received milk was heated to 90°C for 5 minutes and when temperature decreased to 72°C, 2% citric acid solution gradually added followed by continuous stirring which resulted in complete coagulation of milk protein (casein). The paneer whey was separated from the curd by filtration through muslin cloth and was collected in the bottles which were stored in refrigerated condition. The process flowchart for the preparation of Paneer whey is presented in Fig 3.1.

Milk
↓
Standardization
↓
Heating (90°C)
↓
Cooling (70°C)
↓
Addition of coagulant (2% citric acid)
↓
Coagulation (70°C)
↓
Draining
↓
Hoopping and pressing
↓
Whey

Fig 3.1 Flowchart for preparation of paneer whey

Source: Srivastava *et al.* (2018)

3.2.2 Experimental design

Different recipe formulations were formulated using Design Expert® (v13) under mixed condition and are presented in Table 3.1.

Table 3.1 Different recipe samples

S.N.	Code	Whey (ml)	Dragon fruit juice (ml)
1.	A	100	0
2.	B	87.5	12.5
3.	C	83.33	16.67
4.	D	75	25
5.	E	66.67	33.33
6.	F	62.5	37.5
7.	G	50	50

3.2.3 Preparation of dragon fruit juice

The process flow diagram for the preparation of natural dragon fruit juice was shown in following Fig 3.2. The ripe dragon fruits were washed with water and peeled manually with the help of knife. Peeled dragon fruit was cut into small pieces for the convenience of grinding. The extracted juice was filtered with two fold of muslin cloth to get juice of less in soluble residues. The prepared juice was stored at 7±1°C temperature in the refrigerator for further use in the RTS beverage preparation.

Ripen harvested dragon fruit

↓

Washing and peeling of outer skin

↓

Cutting and scooping

↓

Grinding of pulps

↓

Filtration with muslin cloth or strainer

↓

Dragon fruit juice

↓

Storage (at 4-8 ℃ refrigerated temperature)

Fig.3.2 Process flow chart for preparation of dragon fruit juice

Source: Wakchaure *et al.* (2023)

3.2.4 Preparation of whey-based dragon fruit beverage

Clarified filtered whey

(100%, 87.5%, 83.33%, 75%, 66.67%, 62.5%, 50%)

Heated to 45°C

Dragon fruit juice (0%,12.5%,16.67%,25 %,33.33%,37.5%,50%) → Mixing ← Ground sugar

(13% TSS maintained)

Filtration through clean muslin cloth

Whey dragon fruit beverage collection

Bottling (in 250 ml glass bottle)

In bottle pasteurization (90°C for 10 min)

Cool to room temperature

Sensory evaluation

Selection of best formulation

Storage at refrigeration (7±1°C)

Analysis of beverage at every 5 days interval up to 25 days

Fig.3.3 Flowchart for preparation of whey-based dragon fruit beverage

Source: Revathi and Singh (2014) with modifications

Different formulation of whey-based dragon fruit beverage was prepared by heating whey at 45°C with addition of sugar, dragon fruit juice as shown in the flow chart. After proper mixing it was filtered through clean muslin cloth to obtain whey-dragon fruit beverage, filled into glass bottle of 250 ml capacity and crown corked. After crown corking it was heated at 85-90°C for 10-15 min and immediately cooled and subjected to sensory evaluation to find out best formulation which was stored in refrigeration and ambient temperature for further study of TPC, yeast & mold count, pH, acidity, TSS, reducing sugar, ascorbic acid at 5 days interval for 25 days. The complete process flowchart for the preparation of beverage is presented in Fig.3.3.

3.3 Analytical procedure

3.3.1 Physicochemical analysis of paneer whey, dragon fruit and prepared whey beverage

Different parameter like TSS, fat content, acidity, pH, protein, moisture content, reducing sugar, total sugar, ash content, ascorbic acid, antioxidant activity, total flavonoids, total phenolic content and betacyanin content were analyzed for paneer whey, dragon fruit juice and prepared beverage.

3.3.1.1 Determination of total soluble solids (TSS)

Total soluble solids were determined with hand refractometer (0-30 °Bx) and values were expressed as °Brix according to (Ranganna, 1986).

3.3.1.2 Determination of titratable acidity

It was measured by titrating 10 ml of clear juice with standard N/10 NaOH and result was expressed as percentage citric acid according to (Ranganna, 1986).

3.3.1.3 Determination of pH

It was directly measured by using pH meter. pH meter was standardized by using buffer solution of pH 7 and 4 at the temperature required.

3.3.1.4 Determination of ascorbic acid

Ascorbic acid content in the sample was determined by the visual titration method using the dye 2, 6-dichlorophenolindophenol as described in (Ranganna, 1986).

3.3.1.5 Determination of total sugar and reducing sugar

It was determined by Lane and Enyon's method according to (Ranganna, 1986)

3.3.1.6 Determination of moisture

Moisture content of raw material and product was determined as per the methods described by (Ranganna, 1986).

3.3.1.7 Determination of protein content

Protein content in the sample was determined by kjeldahl method (by estimating nitrogen content) according to (Ranganna, 1986).

3.3.1.8 Determination of ash content

Total ash content of the samples was determined by using dry ashing according to (Ranganna, 1986).

3.3.1.9 Determination of fat content

Fat content was determined by Gerber method according to (Kharel, 1999).

3.3.1.10 Determination of total solids

It was determined by subtracting the moisture from the 100 according to (Ranganna, 1986).

3.3.1.11 Determination of total phenolic content

The determination of total phenol content was conducted following the procedure described by Sadasivam (1996) using Folin-Ciocalteau reagent. Phenols present in the sample react with phosphomolybdic acid in the Folin-Ciocalteau reagent under alkaline conditions, resulting in the formation of a blue colored complex known as molybdenum blue. To perform the assay, 0.5 ml of the extract and 1 ml of Folin-Ciocalteau reagent were mixed and allowed to incubate at room temperature for 30 minutes. Subsequently, 2.5 ml of saturated sodium carbonate was added, and the mixture was further incubated for 1 hour at room temperature. The absorbance of the resulting solution was measured at 760 nm to determine the total phenol content. The concentration of total phenols was quantified and expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight of the liquid. A standard curve was constructed using gallic acid as a reference compound.

3.2.1.12 Determination of flavonoid content

The total flavonoid content was determined using a modified aluminum chloride assay method, following the procedure described in the study by (Barek *et al.*, 2015). 2 ml of each extract solution was transferred into a volumetric flask with a capacity of 10 ml. To the flask, 0.2 ml of 5% $NaNO_3$ solution was added and allowed to stand for 5 minutes. Afterward, 0.2 ml of 5% $AlCl_3$ solution was added and allowed to stand for 5 minutes. Then, 2 ml of 1N NaOH was added to the flask, and the volume was adjusted to 5 ml with distilled water. The absorbance of the solution was measured at 510 nm against a reagent blank. The test results were compared to a standard curve of Quercetin with concentration of 20, 40, 60, 80 and 100 µg/ml. The total flavonoid content was then calculated and expressed as milligrams of Quercetin Equivalents.

3.2.1.13 Preparation of betacyanin extract

The juice was mixed with distilled water in the ratio of 1:2 for 5 min. Juice mixture was shaken using volumetric shaker at 200 rpm for 120 min and then filtered through Whattman no. 41 filter paper. After centrifugation at 5000g for 15 min, the supernatant collected was used for determination of betacyanin.

3.2.1.14 Determination of betacyanin content

For total betacyanin determination, the absorbance of aqueous extract was measured at 538 nm using a spectrophotometer described by Lim *et al.* (2011), and the concentration was calculated using the formula:

$$\text{Betacyanin content (mg/100g)} = \frac{A(MW)\times V\times(DF)\times 1000}{ELW} \times 100$$

Where A= absorbance at 538nm, L (path length) =1.0cm, DF= dilution factor, V= volume extract (ml), W= fresh weight of extracting material (g). For betanin, E (mean molar absorptivity) = 6.5×10^4 L/mol cm in H2O and MW (molecular weight) = 550 g/mol.

3.2.1.16 Determination of antioxidant activity

The antioxidant activity was determined as per Panico *et al.* (2009) with slight modifications. The control sample (A control) was made by adding 0.28 ml of DPPH solution (0.1 mM, in 95% methanol) to a 10 ml conical flask, and then diluting it with methanol to the necessary volume. 0.28 ml of the DPPH solution and 0.28 ml of the test sample (A sample) were used in the preparation and poured into a 10 ml conical flask. The mixture was then diluted with methanol to the necessary level. Following repeated inversions, the mixture was incubated for 30 min at ambient temperature in a darkened area. The absorbance was calculated with the aid of a spectrophotometer set at 517 nm, in comparison to the control sample. The radical scavenging activity was estimated as a decrease in DPPH absorbance and was calculated using the following equation:

$$\%\ \text{Radical scavenging activity (RSA)} = [(A_0 - A_1)/A_0] \times 100$$

Where, A_0 is the absorbance value of the DPPH blank solution and A_1 is the absorbance value of the sample solution.

3.3.3 Sensory evaluation

Sensory evaluation of prepared beverage was carried out using 9-point hedonic scale described by Ranganna (1986). Sensory panelists were semi trained panelists from Central Campus of Technology, Dharan. The parameters for sensory evaluation were: color, flavor, taste and overall acceptance. The panelists were given instruction to give 9 points to extremely liked and 1 points to the extremely disliked point sample. The specimen of the evaluation of card is shown in Appendix A.

3.3.4 Statistical analysis

ANOVA (Analysis of variance) was used to analyze the data from the sensory evaluation. The Genstat release 12.1 software program developed by VSN International Ltd. was used to analyze the significant differences between them using LSD at the 5 % level of significance, and Microsoft Excel 19 was used to perform statistical calculations of triplicate data.

3.3.5 Microbiological analysis

Total Plate Count (TPC) was determined using the pour plate technique on Plate Count Agar (PCA) medium (incubated at 30°C/48 h). Yeast and mold count was determined using the pour plate technique on Potato Dextrose Agar (PDA) medium (incubated at 25-27°C/48-72 h) (AOAC, 2005).

3.3.6 Storage studies

Glass bottles were aseptically filled with whey-based dragon fruit beverage and crown corked. For 25 days, the bottles containing beverages were kept at refrigerated (7±1°C) and room temperature (25±5°C). Samples were taken every 5 days and tested for physicochemical properties (TSS, acidity, pH, reducing sugar, and ascorbic acid) as well as microbiological qualities (total plate count & yeast and mold count).

Part IV

Results and discussions

Whey based dragon fruit beverage was prepared in the laboratory of Central Campus of Technology for the study. Dragon fruit juice and paneer whey were the major ingredients. Dragon fruit juice was prepared as shown in the Fig.3.3. while paneer whey was obtained from Kamdhenu dairy. The extracted raw materials were filtered and different formulated samples were prepared and heat treated for pasteurization at 90°C for about 10 min. The samples were analyzed through sensory evaluation and best scored sample was stored for 25 days at refrigerated (7±1°C) and ambient (25±1°C) temperature for storage stability.

4.1 Analysis of raw materials

In the preparation of whey-based dragon fruit beverage, paneer whey and dragon fruit pulp were the major ingredient which were analyzed for their chemical composition presented in Table 4.1

Table 4.1 Chemical composition of raw materials (db)

S.N.	Parameters	Whey	Dragon fruit
1.	T.S.S (°Bx)	5.41±0.05	11±0
2.	Moisture (%)	93.64±0.03	87.90±0.03
3.	pH	5.10±0.05	4.50±0.05
4.	Acidity (%)	0.25±0.1	0.20±0.01
5.	Reducing sugar (%)	4.50±0.37	4.53±0.37
6.	Non-reducing sugar (%)	4.41±0.31	3.47±0.21
7.	Total sugar (%)	8.91±0.46	8.00±0.33
8.	Protein (%)	0.53±0.03	0.66±0.36
9.	Ash (%)	0.45±0.03	0.42±0.01
10.	Fat (%)	0.58±0.01	-
11.	Ascorbic acid (mg/100g)	-	7.2±0.14
12.	Total solids (%)	6.36±0.01	12.1±0.01

*Values in the table are the means of three determination ± standard deviations.

The phytochemical composition of dragon fruit juice is shown in Table 4.2.

Table 4.2 Phytochemical composition of dragon fruit juice

S.N.	Parameters	Values
1.	Total phenolic content (mg GAE/100g)	41.60±0.72
2.	Total flavonoids (mg QE/100 g of FS)	4.56±0.16
3.	Radical scavenging activity (%)	74.54±0.4
4.	Betacyanin content (mg/100 g)	20.46±0.6

*Values in the table are the means of three determination ± standard deviations.

The TSS, moisture, pH, acidity, reducing sugar, total sugar, protein, ash and fat content of paneer whey was found to be 5.41°Bx, 93.64%, 5.10%, 0.15%, 4.67%, 8.99%, 0.53%, 0.45% and 0.58% respectively. The TSS, moisture, pH, acidity, reducing sugar, total sugar, protein, ash and fat of paneer whey were in the range in accordance with (Bohora, 2018).

The TSS, moisture, pH, acidity, reducing sugar, total sugar, protein, ash and total solids of dragon fruit juice were found to be 11°Bx, 87.90%, 4.53%, 8.00%, 0.66%, 0.42% and 12.1% respectively. The ascorbic acid was found to be 7.2 (mg/100g). The total phenolic content, total flavonoids, betacyanin content and radical scavenging activity of dragon fruit juice were found to be 41.60 mg GAE/100g, 4.56 mg QE/100g, 20.46 mg/100g and 74.54% respectively. The TSS, moisture, pH, acidity, reducing sugar, non-reducing sugar, total sugar, ash, and protein of the dragon fruit were found in accordance with (Budhathoki *et al.*, 2023; Mishra, 2024). According to Arivalagan *et al.* (2021), dragon fruit showed total polyphenol content and betacyanin content in the range 35.0-48.3 mg GAE/100g and 20.4-23.0 mg/100g respectively. Findings obtained during this study were in range. Total flavonoid content and radical scavenging activity were slightly higher than the findings stated by Manan *et al.,* (2019). This may be due to the variation of species and also the tropical regions where it is grown (Atreya *et al.*, 2020).

4.2 Effect of formulations on sensory characteristics

Seven different samples of varying proportion of whey and dragon fruit juice were taken and coded as A, B, C, D, E, F and G respectively. The TSS of the samples were kept constant

13±1°Bx. Then the formulated samples were subjected to sensory evaluation by a group of 9 semi-trained panelists. The Analysis of Variance (ANOVA) was carried out using least significant difference (LSD) at 5 % level of significance.

4.2.1 Color

From the sensory evaluation for the color of the seven different samples A, B, C, D, E, F and G, the mean sensory scores were found to be 4.0, 5.2, 5.6, 6.3, 7.29, 8.5 and 8.1 respectively (Fig. 4.1). LSD showed that sample A and E were significantly different ($p<0.05$) from other samples. Sample B&C and C&D were not significantly different ($p<0.05$) to each other. Also, sample F and G were not significantly different from each other ($p>0.05$). The color of sample F was found to be superior with the highest mean score i.e. 8.5.

There was increase in the score given by the panelists with increase in the dragon fruit juice. As per findings by Chatterjee *et al.* (2015) for whey-orange beverage where increase in the juice percentage increased the score of the product was in accordance with the findings. Similar results were also reported by Nagadevi and Puraikalan (2015).

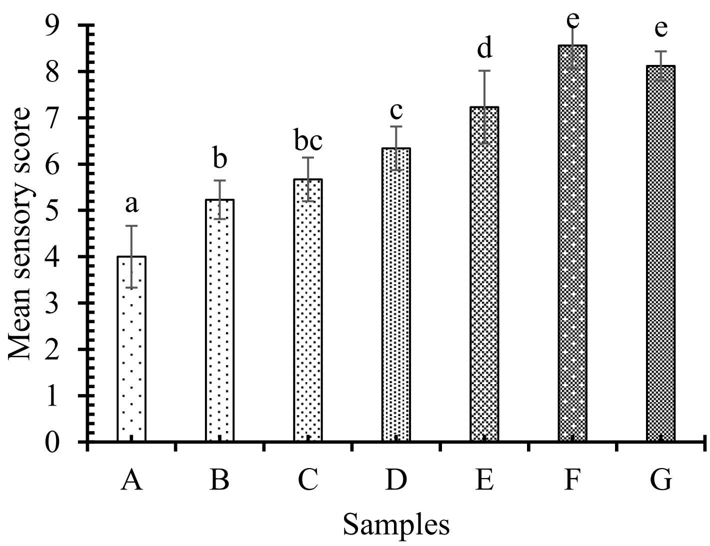


Fig.4.1 Mean sensory score for color

The mean sensory scores for color of whey-dragon fruit beverage is represented in Fig 4.1 Values on top of the bars bearing similar superscript were not significantly different at 5% level of significance. Vertical error bars represent ± standard deviation of scores given by panelists.

4.2.2 Flavor

Seven samples A, B, C, D, E, F and G of different proportion of juice were subjected to sensory evaluation and mean sensory score were observed to be 4.4, 4.5, 5.2, 6.3, 6.6, 8.6 and 8.1 respectively (Fig.4.2). LSD showed that sample A&B and B&C were not significantly different from each other at 5% level of significance. Sample D and E were not significantly different ($p>0.05$) with each other. Similarly, sample F and G were not significantly different ($p>0.05$) from each other. The flavor of sample F was found to be superior to other at 5% level of significance with highest mean sensory score of 8.6.

The highest score to sample F might be because of the appropriate combination of whey and dragon fruit juice. The results obtained were supported by (Chandra *et al.*, 2017a) for the preparation of whey pineapple RTS. The present findings were in conformity with the results reported by Naik *et al.* (2009) for the sensory characteristics of whey based watermelon beverage.

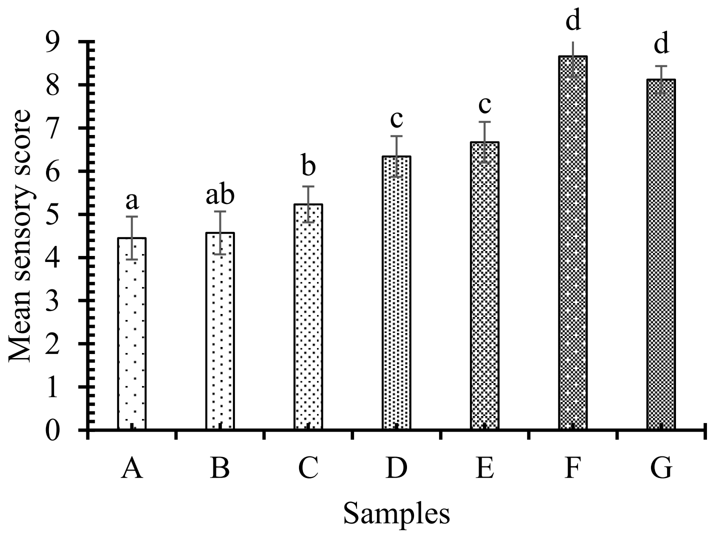


Fig. 4.2 Mean sensory score for flavor

Fig 4.2 represents the mean sensory scores for flavor of whey-dragon fruit beverage. Values on top of the bars bearing similar superscript were not significantly different at 5% level of significance. Vertical error bars represent ± standard deviation of scores given by panelists.

4.2.3 Taste

Like other parameters, juice variation has significant effect on the taste at 5% level of significance. The mean sensory score for taste were found to be 5.0, 5.3, 6.1, 7.1, 7.5, 8.5 and 7.8 for sample A, B, C, D, E, F and G respectively (Fig.4.3). LSD showed that sample A and B were not significantly different from each other ($p>0.05$). Similarly, sample D and E were also not significantly different from each other. Likewise, sample F and G were also not significantly different from each other ($p>0.05$). Sample C and F were significantly different from other samples at 5% level of significance. Among the seven different samples, sample F got the highest mean sensory score i.e. 8.5 because of the high acceptance by the panelists.

The increase in the score may be due to increase in flavor and sweetness due to higher amount of juice as well as sugar in the beverage samples. Moreover, result obtained by Chatterjee *et al.* (2015) showed similar pattern of scores with alternating increase and decrease in scores.

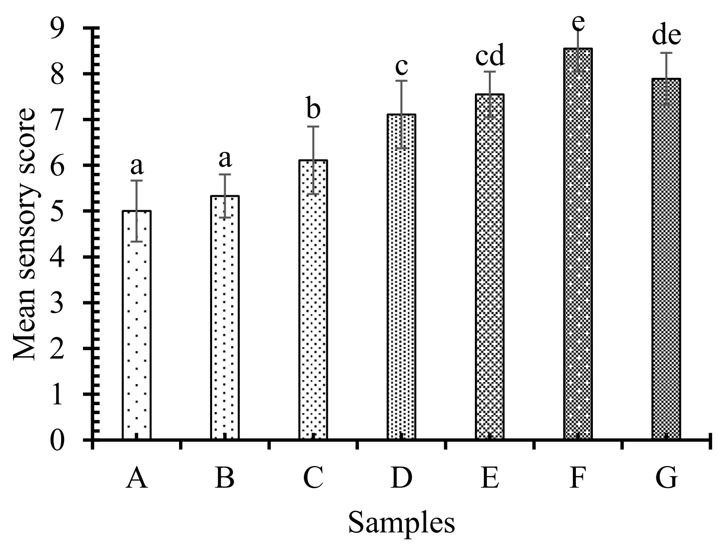


Fig.4.3 Mean sensory score for taste

Fig 4.3 represents the mean sensory scores for flavor of whey-dragon fruit beverage. Values on top of the bars bearing similar superscript were not significantly different at 5% level of significance. Vertical error bars represent ± standard deviation of scores given by panelists.

4.2.5 Overall acceptance

Overall acceptance is the main factor for accepting the sample. The mean sensory score from the sensory evaluation of the seven different samples A, B, C, D, E, F and G were found to be 4.3, 5.1, 5.5, 6.6, 7.2, 8.5 and 7.8 respectively (Fig 4.4). Sample A, sample F and sample G were found to be significantly different from other samples ($p<0.05$). Sample B and C were not significantly different from each other ($p>0.05$). Similarly, sample D and E were also not significantly different from each other ($p>0.05$). Therefore, based on overall acceptance sample F was found to be best among all with highest score i.e. 8.5.

Chandra *et al.* (2017a) reported increase in the sensory score for the overall acceptance of beverage while increasing the orange juice content in the whey-based fruit juice beverage. According to Nagadevi and Puraikalan (2015) and Zaman *et al.* (2023), the general acceptability of the product with enhanced fruit juice content scored best. These above findings support the results obtained.

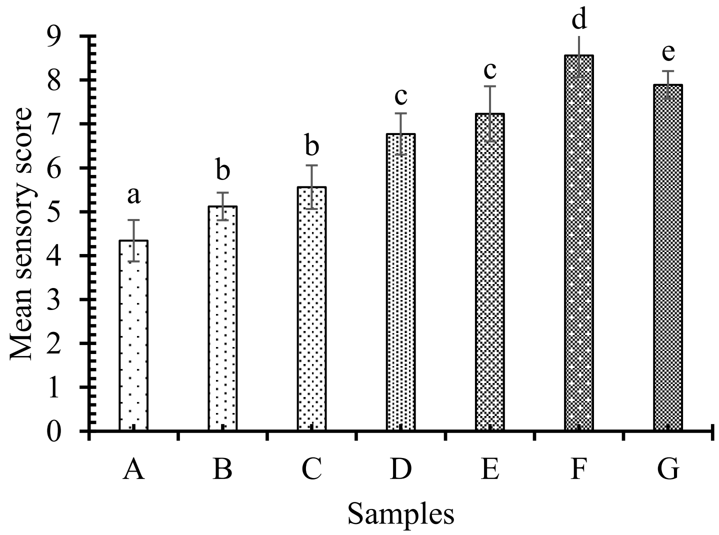


Fig.4.4 Mean sensory score for overall acceptance

Fig 4.4 represents the mean sensory scores for overall acceptance of whey-dragon fruit beverage. Values on top of the bars bearing similar superscript were not significantly different at 5% level of significance. Vertical error bars represent ± standard deviation of scores given by panelists.

4.3 Chemical and phytochemical composition of best sample (F)

The sample F with whey 62.5% (V/V) and Dragon fruit juice 37.5% (V/V) was rated superior in the sensory evaluation. Dragon fruit's color, flavor and taste on the beverage was acceptable. The chemical and phytochemical composition of the best sample is presented in Table 4.3 and Table 4.4 respectively.

Table 4.3 Chemical composition of best sample (F) (db)

S.N.	Parameters	Values
1.	T.S.S (°Bx)	13.1±0.05
2.	Moisture (%)	86.68±0.08
3.	pH	5.47±0.005
4.	Acidity (%)	0.32±0.01
5.	Reducing sugar (%)	4.51±0.03
6.	Non-reducing sugar (%)	6.42±0.01
7.	Total sugar (%)	10.93±0.22
8.	Protein (%)	0.55±0.03
9.	Ash (%)	0.74±0.02
10.	Fat (%)	0.24±0.02
11.	Ascorbic acid (mg/100g)	4.50±0.17
12.	Total solids (%)	13.32±0.06

*Values in the table are the means of three determination ± standard deviations.

Table 4.4 Phytochemical composition of best sample (F)

S.N.	Parameters	Values
1.	Total phenolic content (mg GAE/100ml)	24.84±0.67
2.	Total flavonoids (mg QE/100ml)	2.47±0.44
3.	Radical scavenging activity (%)	31.82±0.15
4.	Betacyanin content (mg/100 g)	8.23±0.37

*Values in the table are the means of three determination ± standard deviations.

Ascorbic acid, total phenolic content, and antioxidant activity are all abundant in dragon fruit. The total soluble solids, moisture content, pH and total solids in the best sample F were found to be 13.1°Bx, 86.68%, 5.47 and 13.32% respectively. The average titratable acidity in terms of lactic acid was found to be 0.32%. The reducing sugar, non-reducing sugar and total sugar of the final product were reported as 4.51%, 6.42%, and 10.93% respectively. The protein, ash, and fat were found to be 0.55%, 0.74%, and 0.24% respectively. The ascorbic acid content was found to be 4.50 mg/100g. Total phenolic content, total flavonoid content, radical scavenging activity and betacyanin content were found to be 24.84 mg GAE/100g, 2.47 mg QE/100g, 31.82% and 8.23 mg/100g respectively.

The TSS was similar to that reported by Choudhary *et al.* (2020) which was 13.10°Bx. The moisture content, acidity and total solids were similar to the findings of Shrestha and Dahal (2021) which were 87.6%, 0.302%, and 14.85% respectively. The pH of best sample was similar to the finding of Devi *et al.* (2017). Total sugar, and fat content were found to be similar to the Begum *et al.* (2019) which were 10.95% and 0.24% respectively but ash content was very low which was 0.19%. According to the results obtained from Choudhary *et al.* (2020) the ascorbic acid content (10.57 mg/100g) was higher than the results obtained (4.50 mg/100g). The protein content obtained was in accordance to the finding reported by (Shrestha and Dahal, 2021).

The phytochemical composition of beverage like total phenolic content, total flavonoids and antioxidant activities were higher than the results obtained by Zaman *et al.* (2023) which was done for whey based sugarcane beverage. This is due to the fact that dragon fruit has higher phytochemical composition in comparison to sugarcane.

4.4 Storage study

Prepared whey dragon fruit beverage was stored for 25 days time period at refrigerated condition (7±1°C) and ambient temperature (25±5°C). On every 5 days interval microbiological and chemical compositions were determined.

4.4.1 Microbiological analysis

In microbiological analysis TPC, yeast and mold count were done. There was no any colony observed during initial storage periods. During preparation of beverage heat treatment at

90°C for 10 min was done. At this temperature treatment destruction or elimination of all viable organisms along with enzymes inactivation occurs in/on a food product. Due to storage condition in air-tight caped product, growth of microorganism was found to be slow Further, whey contains lactose sugar and proteins which favor growth of microorganism during storage. The change in TPC and yeast & mold count with respect to number days of storage and temperature are presented in Fig.4.5 and Fig.4.6 respectively.

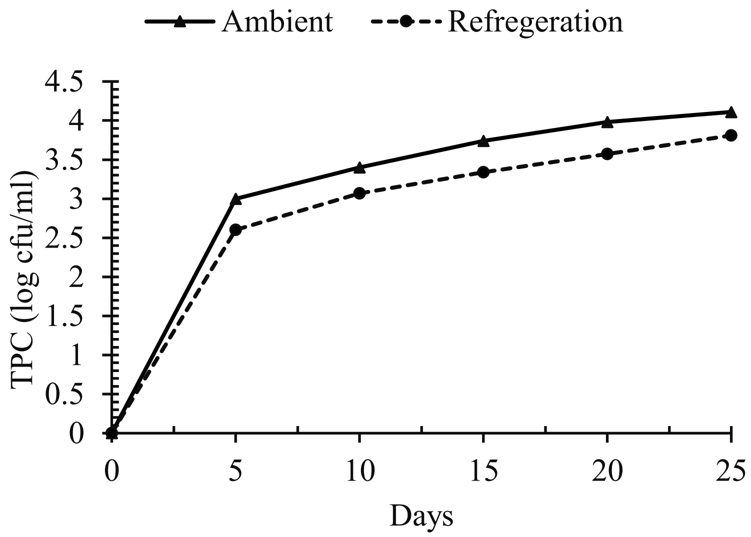


Fig.4.5 Change in TPC with respect to number of days of storage and temperature

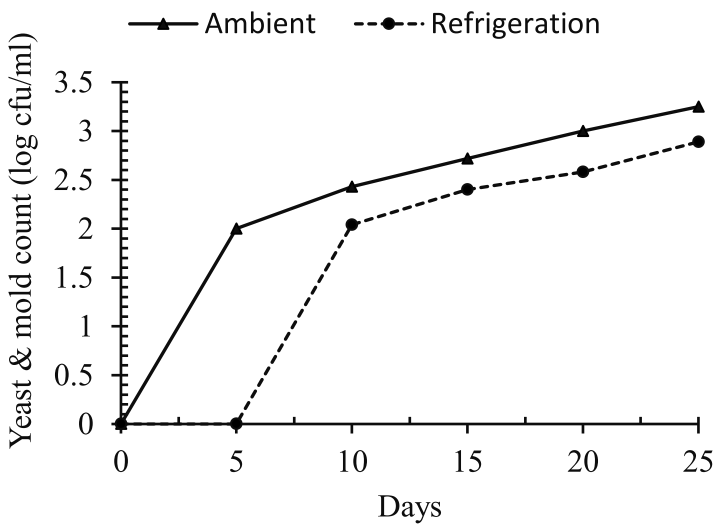


Fig.4.6 Change in yeast & mold count with respect to number of days of storage and temperature

The microbial load, which consists of bacteria, mold, and yeast, increased over time, although the growth of microorganisms differed depending on whether they were stored at ambient or refrigerated temperatures. On the 0, and first day, the TPC, yeast & mold count were all zero. However, over time, both at room temperature and in the refrigerated temperature, these counts rose. Notably, there was a substantial variation in the microbial development under these conditions. On day 25, the TPC was found to be 1.3×10^4 and 6.5×10^3 cfu/ml at room and refrigerated temperatures respectively. However, these microbial counts were in well within the acceptable range suggested by FSSAI for micro flora in any milk-based beverage (maximum 50×10^3 cfu/mL). Similarly, yeast and mold count were found to be 1.8×10^3 and 7.5×10^2 cfu/ml at room and refrigerated conditions respectively on 25th day. Similar results have also been reported by (Bohara, 2018; Sakhale *et al.*, 2012) in whey based pineapple and whey- mango RTS beverage.

4.4.2 Chemical analysis

Effect of storage period on chemical compositions (TSS, pH, titrable acidity, reducing sugar and ascorbic acid content) of the best sample were analyzed. The change in chemical properties during storage is presented in Table 4.6.

Table 4.6 Physicochemical properties during storage of best sample

Parameters	Days/ condition	Day-0	Day-5	Day-10	Day-15	Day-20	Day-25
TSS (°Bx)	A	13.1 (0.04)	13.2 (0.04)	13.3 (0.04)	13.5 (0.04)	13.7 (0.04)	14.0 (0.04)
	R	13.1 (0.04)	13.1 (0.04)	13.2 (0.04)	13.3 (0.04)	13.4 (0.04)	13.5 (0.04)
pH	A	5.47 (0.01)	5.45 (0.01)	5.40 (0.01)	5.36 (0.01)	5.29 (0.02)	5.22 (0.01)
	R	5.47 (0.01)	5.46 (0.004)	5.43 (0.01)	5.4 (0.01)	5.37 (0.01)	5.35 (0.004)
Acidity (%)	A	0.32 (0.01)	0.35 (0.01)	0.39 (0.01)	0.47 (0.01)	0.59 (0.01)	0.66 (0.01)
	R	0.32 (0.01)	0.33 (0.01)	0.35 (0.01)	0.36 (0.01)	0.38 (0.02)	0.4 (0.02)
Reducing sugar (%)	A	4.51 (0.02)	4.68 (0.01)	4.83 (0.01)	4.96 (0.03)	5.12 (0.02)	5.29 (0.07)
	R	4.51 (0.02)	4.61 (0.01)	4.72 (0.01)	4.81 (0.03)	4.93 (0.02)	5.01 (0.04)
Ascorbic acid (mg/100g)	A	4.5 (0.02)	4.09 (0.08)	3.46 (0.02)	2.81 (0.01)	1.68 (0.02)	1.04 (0.01)
	R	4.5 (0.02)	4.5 (0.01)	4.17 (0.02)	3.75 (0.01)	3.22 (0.01)	2.98 (0.06)

*Values in the table are the means of three determination ± standard deviations.

4.4.2.1 Effect on TSS

The effect of storage days on the TSS of beverage is shown in Fig 4.7. During storage period, TSS was found to be slightly increased. TSS of the beverage increased from 13.1 to 14.0 °Bx (25±5°C) and 13.1 to 13.5 °Bx (7±1°C) during 30 days of storage.

The conversion of polysaccharides into monosaccharide and oligosaccharides caused an increase in TSS. For preservation of juice quality during storage, the TSS content of juice should either remain the same or minimally rise (Bhardwaj and Pandey, 2011). Similar results were reported in the preparation of whey-apple RTS beverage Choudhary *et al.* (2020) and whey-banana herbal beverage (Yadav *et al.*, 2010). LSD showed that there was significant difference on TSS at 5% level of significance.

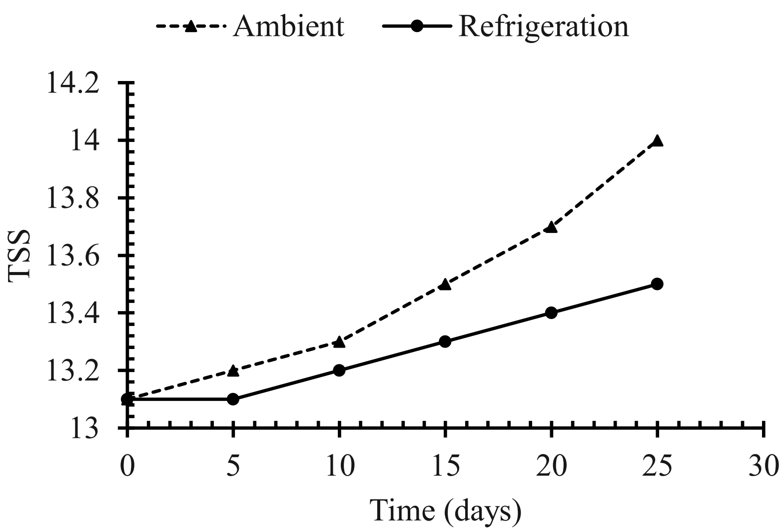


Fig.4.7 Effect of storage time and temperature on TSS of prepared beverage

Ahmed *et al.* (2023) also observed an increase in the TSS of the developed whey-mango mixed beverage during storage. One possible explanation for the observed rise in TSS in this study could be due to higher starch content of dragon fruit, which during storage could undergo hydrolysis in to formation of monosaccharides and other soluble sugars (Yadav *et al.*, 2010).

4.4.2.2 Effect on pH

The effect of storage on the pH of beverage is shown in Fig 4.8. During storage pH was found to be gradually decreased from 5.47 to 4.22 (25±5◦C) and 5.47 to 5.35 (7±1°C) that affects the organoleptic quality of juice.

This might be due to increase in titrable acidity, as acidity and pH are inversely proportional to each other. The possible reasons for decrease in pH might be due to ascorbic acid degradation over time, leading to formation of acidic by-products, which may lower pH and dissociation of organic acid of beverage, releasing more hydrogen ions. Majumdar *et al.* (2011) and Harshal *et al.* (2023) showed similar outcomes for the blended juice of bottle gourds and basil leaves and whey-orange beverage. LSD showed that there was significant difference on pH at 5% level of significance (Appendix D). The similar trend of pH was reported by Naik *et al.* (2009) in case of whey-based watermelon beverage.

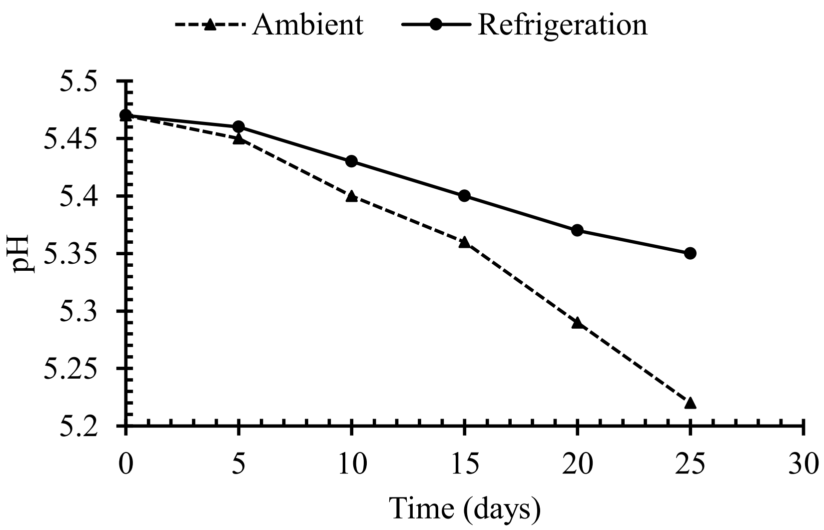


Fig.4.8 Effect of storage time and temperature on pH of prepared beverage

4.4.2.3 Effect on acidity

The increase in the titrable acidity of the beverage during 25 days of storage at two different temperatures were observed as shown in the Fig 4.9. Acidity of beverage was found to have gradually increased from 0.32 to 0.66 (25±5°C) and 0.32 to 0.40 (7±1°C).

The probable reason for increase in acidity may be due to the production of organic acids and amino acids by the action of ascorbic acid on the sugar and the protein content of the beverage. Lactose and proteins are converted into lactic acid and amino acids leading to increase in acidity and decrease in pH of beverage (Kalra *et al.*, 1991). This could also occur due to the conversion or breakdown of polysaccharides and oxidation of reducing sugars to acids (Divyasree *et al.*, 2018). The result are in agreement with the findings stated in whey based orange beverage Harshal *et al.* (2023) and also in apple-whey RTS beverage (Choudhary *et al.*, 2020). Also increase in acidity might be due to the slight growth of micro-organism in the beverage. LSD showed that there was significant difference on titrable acidity at 5% level of significance.

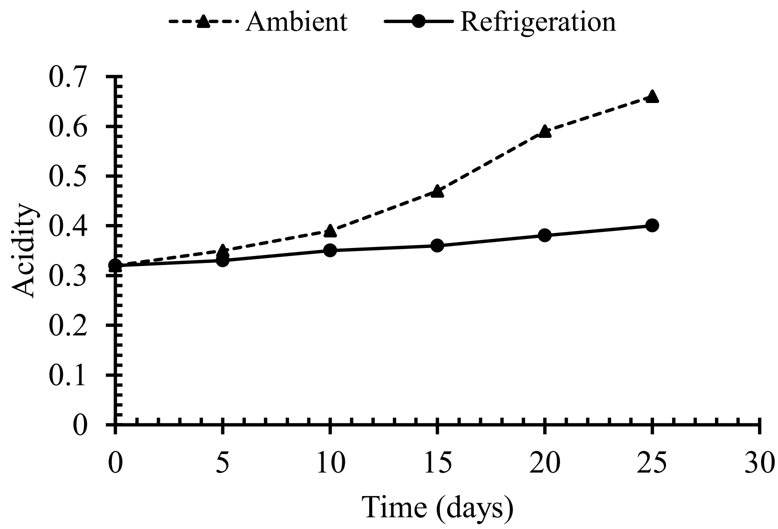


Fig.4.9 Effect of storage time and temperature on acidity of prepared beverage

4.4.2.4 Effect on reducing sugar

Gradual increase in the reducing sugar of the beverage during 25 days of storage at two different temperatures were observed as shown in Fig 4.10. Reducing sugar of the beverage was found to be 4.51, 4.68, 4.83, 4.96, 5.12, and 5.29 for ambient sample (25±5°C), 4.51, 4.61, 4.72, 4.81, 4.93 and 5.01 for refrigeration sample (7±1°C) during 0, 5, 10, 15, 20 and 25 days of storage respectively. Statistical analysis showed that storage had significant effect ($p<0.05$) on reducing sugar content of the beverage.

Fruit juices often have a increased sugar content the longer they are stored. The increase was probably due to the hydrolysis of polysaccharides like starch, pectin, etc and conversion into simple sugars (glucose and fructose) (Bhardwaj and Pandey, 2011). The reason for increase in reducing sugar of beverage with storage period might be due to the hydrolysis or inversion of non-reducing sugar to reducing sugar (Aruna *et al.*, 1997). The results are in agreement to the findings reported in storage under refrigeration of whey based banana herbal beverage Yadav *et al.* (2010) and herbal apple-whey RTS beverage (Choudhary *et al.*, 2020) . LSD showed that there was significant difference on reducing sugar at 5% level of significance.

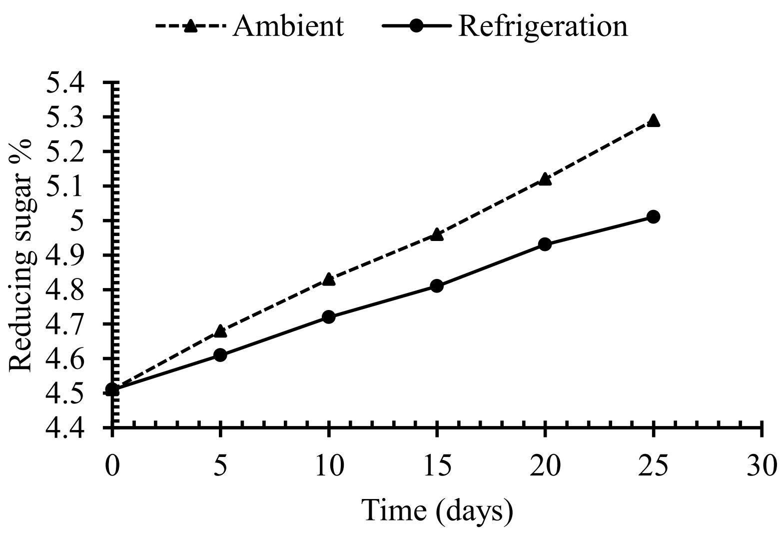


Fig.4.10 Effect of storage time and temperature on reducing sugar content of prepared beverage

4.4.2.5 Effect on ascorbic acid content

Data presented in the Fig 4.11 revealed that the ascorbic acid content in the prepared beverages decreased significantly during storage period which was more prominent in ambient storage conditions compared to refrigerated storage conditions. During 25 days of storage period, the initial ascorbic acid content of 4.50 mg/100g decreased to 1.04 and 2.98 mg/100g during ambient (25±5°C) and refrigerated (7±1°C) storage condition respectively.

Ascorbic acid (vitamin C) is an important nutrient, which has the capacity to antioxidize and defend against free radicals. It is also considered an important indicator of the nutritional quality of the beverage (Ahmed *et al.*, 2023). The declined ascorbic acid level could be explained by the fact that the ascorbic acid is sensitive to oxygen, heat and light and oxidizes rapidly in the presence of oxygen (Davey *et al.*, 2000). One prominent event linked to ascorbic acid degradation during storage is the breakdown of ascorbic acid in the beverage to carbolic acid, dehydroascorbic acid furfural etc. (Ilamaran and Amutha, 2007). A decrease in ascorbic acid content with the increase in storage intervals in storage stability of jack fruit RTS beverage was reported by Krishnaveni *et al.* (2001). LSD showed that there was significant difference on ascorbic acid at 5% level of significance.

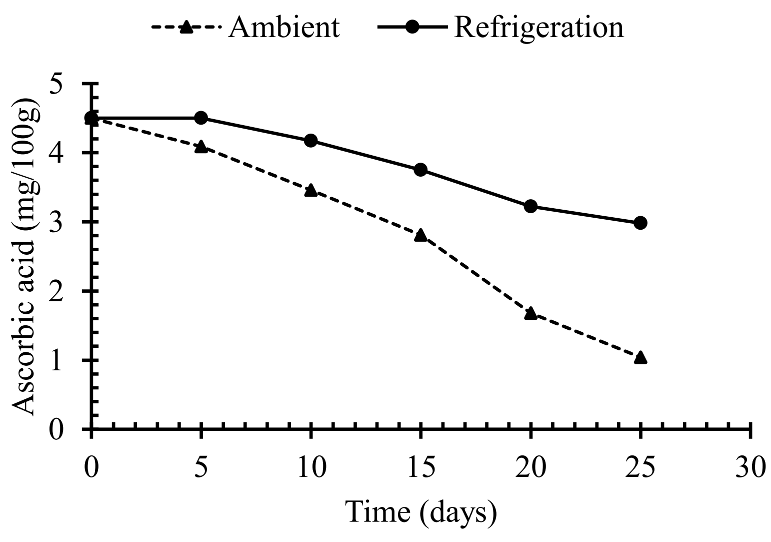


Fig 4.11 Effect of storage time and temperature on ascorbic acid content of prepared beverage

Part V

Conclusions and recommendations

5.1 Conclusions

From the obtained results and discussion, the conclusions drawn from the research work are:

1. From sensory analysis, whey-dragon fruit beverage with 62.5% whey (V/V), 37.5% dragon fruit juice (V/V) and 13.1°Bx TSS was found superior with respect to color, flavor, taste and overall acceptance.
2. During 25 days of storage TSS, acidity and reducing sugar increased while pH and ascorbic acid content decreased. These changes in refrigerated storage condition (7±1°C) were found to be minor with respect to normal storage condition (25±5°C).
3. Production cost (NRs 29.17 per 100 ml) of prepared beverage was reasonable, within the reach of general population and much lower than commercial fruit juices production cost.
4. The beverage can be stored at refrigeration temperature without adding any chemical preservatives with desirable acceptability up to 25 days.

5.2 Recommendations

Based on the present study, following recommendations can be made.

1. Formulation of such type of beverage can be done with varying proportion of different fruit juices and paneer whey.
2. Shelf life of beverage (storage stability) can be studied using different chemical preservatives, different storage conditions and with different packaging materials.
3. Changes in nutritional and phytochemical properties during storage can be studied.

Part VI

Summary

Whey is one of the highly nutritious by-product obtained from the dairy industry producing cheese, chhanna and paneer. It constitutes almost 45-50% of total milk solids, 70% of milk sugar mainly lactose, 20% of milk proteins, 70-90% of milk minerals and most of the water-soluble vitamins originally present in the milk which has indispensable value as human food. About 50% of the whey produced worldwide is dumped as by product. Utilization of whey in the preparation of whey-based dragon fruit beverage can be a good solution.

For the study, dragon fruit and sugar were purchased from the local market of Dharan whereas paneer whey was collected from Kamdhenu Dairy Development Coop Ltd, Itahari. Whey was separated by coagulating with 2% citric acid during paneer making while dragon fruit juice was extracted by blending and filtered through muslin cloth, the separated juices were stored in refrigerated conditions for further analysis. Different formulated beverages were evaluated on a 9-point hedonic rating (1=dislike extremely, 9=like extremely) by nine semi-trained panelist. Prepared beverage (whey-dragon fruit) in 62.5:37.5 ratio and TSS 13.2°Bx was found to be best in overall sensory evaluation.

The superior beverage from the sensory evaluation was analyzed for chemical composition and storage stability under refrigerated (7±1°C) and normal (25±5°C) condition up to 25 days. Further study could not be done. The chemical composition of best sample was found to be T.S.S (13.1°Bx), moisture (86.68%), pH (5.47), acidity (0.32%), reducing sugar (4.51%), total sugar (10.93%), proteins (0.55%), ash (0.74%), fat (0.24%), ascorbic acid (4.5mg/100g), total phenolic content (24.84 mg GAE/100g), total flavonoids (2.45 mg QE/100g), antioxidant activity (31.82 %) and betacyanin content (8.23 mg/100g) . During 25 days storage, TSS, pH and reducing sugar increased while acidity and ascorbic acid content decreased under both storage conditions.

From the study it was concluded that by substituting fruit juice and large part of dairy whey, it would be eco-friendly and economically beneficial to consumer, dairy industry and fruit growers to solve whey disposal problem.

References

Abd Manan, E., Abd Gani, S. S., Zaidan, U. H., & Halmi, M. I. E. (2019). Characterization of antioxidant activities in red dragon fruit (Hylocereus polyrhizus) pulp water-based extract. *J. Adv Res. Fluid Mech Therm Sci.*, *61*(2), 170-180.

Ahmed, T., Sabuz, A. A., Mohaldar, A., Fardows, H. S., Inbaraj, B. S., Sharma, M., Rana, M. R., & Sridhar, K. (2023). Development of novel whey-mango based mixed beverage: effect of storage on physicochemical, microbiological, and sensory analysis. *Foods*, *12*(2), 237.

Alfaifi, M., & Stathopoulos, C. (2010). Effect of egg yolk substitution by sweet whey protein concentrate (WPC), on physical properties of Gelato ice cream. *Int. Food Res. J*, *17*(3), 787-793.

Anon. (2015). Annual bulletin of DDC [Report. In D. D. corporation. (Ed.). Nepal.

AOAC. (2005). *Official Methods of Analysis* (18th ed.). Association of Official Analytical Chemists Washington, DC.

Arivalagan, M., Karunakaran, G., Roy, T., Dinsha, M., Sindhu, B., Shilpashree, V., Satisha, G., & Shivashankara, K. (2021). Biochemical and nutritional characterization of dragon fruit (Hylocereus species). *Food Chem. 353*, 129426.

Arsić, S., Bulatović, M., Zarić, D., Kokeza, G., Subić, J., & Rakin, M. (2018). Functional fermented whey carrot beverage-qualitative, nutritive and techno-economic analysis. *Rom. Biotechnol. Lett.*, *23*(2), 13496-13504.

Aruna, K., Vimala, V., Giridhar, N., & Raio, D. (1997). Studies on preparation and storage of nectar prepared from papaya. *Beverage and Food World*, *24*(1), 29-32.

Atreya, P. N., Shrestha, C. M., Suvedi, B., & Pandey, S. (2020). Emerging fruits of Nepal: pomegranate, kiwifruit, avocado, dragon fruit and grape; opportunities, challenges and ways forward. Proceeding of 11th National Horticulture Seminar, Babar, R., Salunkhe, D., Chavan, K., & Thakare, V. (2008). Utilization of pomegranate juice for the preparation of chakka whey beverage. *J. Dairying, Foods Home Sci*. *27*(2), 87-93.

Barek, M. L., Hasmadi, M., Zaleha, A., & Fadzelly, A. M. (2015). Effect of different drying methods on phytochemicals and antioxidant properties of unfermented and fermented teas from Sabah Snake Grass (Clinacanthus nutans Lind.) leaves. *I. Food Res. J. 22*(2), 661.

Barone, G., O'Regan, J., Kelly, A. L., & O'Mahony, J. A. (2022). Interactions between whey proteins and calcium salts and implications for the formulation of dairy protein-based nutritional beverage products: A review. *Compr. Rev. Food Sci. Food Saf. 21*(2), 1254-1274.

Barthlott, W., & Hunt, D. R. (1993). Cactaceae. In *Flowering Plants· Dicotyledons: Magnoliid, Hamamelid and Caryophyllid Families* (pp. 161-197). Springer.

Begum, T., Islam, Z., Siddiki, M. S. R., Habib, R., & Rashid, H.-u. (2019). Preparation of fermented beverage from whey-based watermelon (Citrullus lanatus) juice. *Asian J. Dairy Food Res*. *38*(4), 301-306.

Beucler, J., Drake, M., & Foegeding, E. A. (2005). Design of a beverage from whey permeate. *J. Food Sci*. *70*(4), S277-S285.

Bhardwaj, R. L., & Pandey, S. (2011). Juice blends—a way of utilization of under-utilized fruits, vegetables, and spices: a review. *Crit. Rev. Food Sci Nutr*. *51*(6), 563-570.

Blancke, R. (2016). "Tropical fruits and other edible plants of the world" (1st ed.). Cornell University Press. London, England.

Bohara, G. (2018). Preparation of Whey Based Pineapple Beverage and its Storage Quality Evaluation*.* Tribhuvan Univ., Nepal.

Bordenave-Juchereau, S., Almeida, B., Piot, J.-M., & Sannier, F. (2005). Effect of protein concentration, pH, lactose content and pasteurization on thermal gelation of acid caprine whey protein concentrates. *J. Dairy Res*. *72*(1), 34-38.

Borkar, M., Zinjarde, R., Chore, N., & Prajapati, A. (2020). Preparation of chakka whey beverage blended with watermelon (Citrullus lanatus) Juice. *J. Pharmcogn. Phytochem*. *9*(6), 1280-1283.

Britton, N. L., & Rose, J. N. (1963). "The Cactaceae: descriptions and illustrations of plants of the cactus family" (Vol. 3). Dover publications. New York, USA.

Budhathoki, R., Bhutia, K. D., Das, T., Chettri, S., Sharma, L., & Upadhyay, S. (2023). Physico-Chemical Characteristics of Dragon Fruit Hylocereus polyrhizus (Weber) Britton and Rose from Different Locations of Nepal. *Environ. Ecol*. *41*(4), 2853-2858.

Chatterjee, G., De Neve, J., Dutta, A., & Das, S. (2015). Formulation and statistical evaluation of a ready-to-drink whey based orange beverage and its storage stability. *Revista mexicana de ingeniería química*, *14*(2), 253-264.

Chavan, R., Shraddha, R., Kumar, A., & Nalawade, T. (2015). Whey based beverage: its functionality, formulations, health benefits and applications. J. Food process Technol.

Chen, N. J., & Paull, R. E. (2019). Overall Dragon Fruit Production and Global Marketing Overall Dragon Fruit Production and Global Marketing. *FFTC Agricultural Policy Platform*, *9*(2), 229-239.

Choo, W. S., & Yong, W. K. (2011). Antioxidant properties of two species of Hylocereus fruits. *Adv. Appl. Sci. Res. 2*(3), 418-425.

Clare, D., & Swaisgood, H. (2000). Bioactive milk peptides: a prospectus. *J. Dairy Sci. 83*(6), 1187-1195.

Darade, R., & Ghodake, S. (2012). An overview of whey beverages. Res. J. Anim. Husbandry Dairy Sci.

Davey, M. W., Montagu, M. V., Inze, D., Sanmartin, M., Kanellis, A., Smirnoff, N., Benzie, I. J. J., Strain, J. J., Favell, D., & Fletcher, J. (2000). Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. *J. Sci. Food Agric. 80*(7), 825-860.

Devi, L., Singh, D., & Chandra, R. (2017). Development, chemical analysis and sensory evaluation of whey based pineapple juice beverages. *Int. J. Food Sci. Nutr*, *2*(5), 102-105.

Devi, L. S., Singh, D., & Chandra, R. (2017). Study on sensory quality evaluation of whey based fruit juice beverages. *Pharm. Innov. J. 6*(9), 310-314.

Dhamsaniya, N., & Varshney, A. (2013). Development and evaluation of whey based RTS beverage from ripe banana juice. *J. Food Process Technol.*

Dissanayake, M. (2011). Modulation of functional properties of whey proteins by microparticulation. Published Ph.D. Thesis.Victoria Univ., Nepal.

Divyasree, G., Swarajya Lakshmi, K., Rama Krishna, M., & Arunodhayam, K. (2018). Studies on Physico-Chemical, Sensory Quality of Sweet Orange Based RTS Blends under Refrigerated Storage. *Int. J. Current Microbiol. Appl. Sci. 7*(9), 1403-1413.

Djurić, M., Carić, M., Milanović, S., Tekić, M., & Panić, M. (2004). Development of whey-based beverages. *Eur. Food Res.Technol. 219*, 321-328.

Elmarzugi, N., Cheah, L., Eid, A., Aziz, A., & Ariffin, F. (2016). Phytochemical properties and health benefits of Hylocereus undatus. *Nanomed Nanotechnol*, *1*(1), 1-10.

Foegeding, E. A., Davis, J. P., Doucet, D., & McGuffey, M. K. (2002). Advances in modifying and understanding whey protein functionality. *Trends Food Sci. Technol. 13*(5), 151-159.

Fox, P. F. (2008). Milk: an overview. *Milk proteins*, 1-54.

Fox, P. F., Mcsweeney, P. L., & Paul, L. (1998). Dairy chemistry and biochemistry.

Frid, A. H., Nilsson, M., Holst, J. J., & Björck, I. M. (2005). Effect of whey on blood glucose and insulin responses to composite breakfast and lunch meals in type 2 diabetic subjects. *Amer. J. Clin. Nutr. 82*(1), 69-75.

Guan, J., & Brunner, J. (1987). Koumiss produced from a skim milk-sweet whey blend. *Cult. Dairy Prod. J.*

Gunasena, H., Pushpakumara, D., & Kariyawasam, M. (2006). Dragon Fruit: Hylocereus undatus (Haw) Britton and Rose-A fruit for the future. *SriLanka Council For Agricultural Policy*, *114*.

Harshal, W., Sheshrao, C., Rameshwar, J., Nilesh, K., & Mahesh, K. (2023). Effect of Storage Temperature and Interval on Sensory, Physico-chemical and Microbiological Properties of Orange-based Paneer Whey Beverage. *J. Food Chem. Nanotechnol*. *9*(S1), S308-S315.

Haug, A., Høstmark, A. T., & Harstad, O. M. (2007). Bovine milk in human nutrition–a review. *Lipids in health and disease*, *6*, 1-16.

Heino, A. (2010). Microfiltration in cheese and whey processing. Published Academic. Thesis. Univ. of Helsinki., Finland.

Hossain, F. M., Numan, S. M. N., & Akhtar, S. (2021). Cultivation, nutritional value, and health benefits of Dragon Fruit (Hylocereus spp.): A Review. *Intl. J. Horti. Sci. Technol. 8*(3), 259-269.

Huffman, L. M. (1996). Processing whey protein for use as a food ingredient. *J. Food. Technol.* 2, 49-52.

Hugunin, A. (1999). Whey products in yogurt and fermented dairy products. *US Dairy Export Council*, 1-8.

Ilamaran, M., & Amutha, S. (2007). Effect of total soluble solids and CO2 pressure on physico-chemical and sensory qualities of carbonated banana and sapota beverages. *J. Food Sci. Technol.* 44(2), 178-182.

Ingale, M., Ranveer, R., & Nagargoje, K. (2009). Development of whey based custard apple beverage. *J. Beverage and Food World*, *36*(5), 143-144.

Jasińska, M., Trzciński, J., Dmytrów, I., & Mituniewicz-Małek, A. (2012). Whey protein concentrates and whey-fat preparations as powdered milk substitutes in soft serve ice cream. *Acta Agrophysica*, *19*(1), 37-50.

Jelen, P. (1992). Whey cheeses and beverages. In *Whey and lactose processing* (pp. 157-193). Springer.

Jeličić, I., Božanić, R., & Tratnik, L. (2008). Whey based beverages-new generation of dairy products. *Mljekarstvo: časopis za unaprjeđenje proizvodnje i prerade mlijeka*, *58*(3), 257-274.

Jenssen, H., & Hancock, R. E. (2009). Antimicrobial properties of lactoferrin. *Biochimie*, *91*(1), 19-29.

Jeronimo, M. C., Orsine, J. V. C., & Novaes, M. R. C. G. (2017). Nutritional pharmacological and toxicological characteristics of pitaya (Hylocereus undatus): A review of the literature. *Afr. J. Pharm. Pharmacol. 11*(27), 300-304.

Jiang, H., Zhang, W., Li, X., Shu, C., Jiang, W., & Cao, J. (2021). Nutrition, phytochemical profile, bioactivities and applications in food industry of pitaya (Hylocereus spp.) peels: A comprehensive review. *Tren. Food Sci.Technol. 116*, 199-217.

Kakade, V., Jinger, D., Dayal, V., Chavan, S., Nangare, D., & Wakchaure, G. (2020). Dragon Fruit: Wholesome and remunerative fruit crop for India. *Food and Scientific Reports*, *1*(12), 44-48.

Kakade, V., Morade, A., & Kadam, D. (2022). Dragon fruit (Hylocereus undatus). *Tropical Fruit Crops: Theory to Practical; Ghosh, SN, Sharma, RR, Eds*, 240-257.

Kalra, S., Tandon, D., Garg, N., & Singh, B. (1991). Quality evaluation of some market fruit drinks. *Indian Food Packer*, *45*(3), 48-53.

Keri Marshall, N. (2004). Therapeutic applications of whey protein. *Alternative medicine review*, *9*(2), 136-156.

Khamrui, K. (1998). Making profits from whey. *Indian Dairyman*, *50*, 13-18.

Kharel, G. P. (1999). "Elementry Food Analysis". Bharabi Polytechnic Institute. Nepal.

Kohli, D., Kumar, A., Kumar, S., & Upadhyay, S. (2019). Waste utilization of amla pomace and germinated finger millets for value addition of biscuits. *Current Research in Nutrition and Food Science Journal*, *7*(1), 272-279.

Koziol, J., Gustaw, W., Wasko, A., Skrzypczak, K., Slawinska, A., & Solowiej, B. (2014). Wpływ wybranych preparatów białek mleka na wzrost i przeżywalność

Lactobacillus acidophilus oraz właściwości reologiczne mlecznych napojów fermentowanych. *Żywność Nauka Technologia Jakość*, *21*(3).

Krishnamurthy, G., Giridhar, N., Satyanarayana, A., & Venkateswata Rao, M. (1990). Studies of commercial utilization of Rangpur limes. *Indian Food Packer*, *44*(6), 37-41.

Krishnaveni, A., Manimegalai, G., & Saravanakumar, R. (2001). Storage stability of jack fruit (Artocarpus heterophyllus) RTS beverage. *J. FoodSci.Technol.* 38(6), 601-602

Kumar, K., Singh, J., & Chandra, S. (2017). Formulation of whey based pineapple herbal beverages and its storage conditions. *Chem Sci Rev Lett*, *6*(21), 198-203.

Larsen, L. B., Wedholm-Pallas, A., Lindmark-Månsson, H., & Andrén, A. (2010). Different proteomic profiles of sweet whey and rennet casein obtained after preparation from raw versus heat-treated skimmed milk. *Dairy Sci. Technol. 90*(6), 641-656.

Liaotrakoon, W. (2013). Characterization of dragon fruit (Hylocereus spp.) components with valorization potential. Unpublished Ph.D. Thesis. Ghent Univ., Belgium.

Lim, S., Yusof, Y., Chin, N., Talib, R., Endan, J., & Aziz, M. (2011). Effect of extraction parameters on the yield of betacyanins from pitaya fruit (Hylocereus polyrhizus) pulps. *J. Food Agri. Environ.* 9(2), 158-162.

Liu, H., Chen, W.-L., & Mao, S. (2007). Antioxidant nature of bovine milk β-lactoglobulin. *J. Dairy Sci. 90*(2), 547-555.

Liu, K., Tian, Y., Stieger, M., van der Linden, E., & van de Velde, F. (2016). Evidence for ball-bearing mechanism of microparticulated whey protein as fat replacer in liquid and semi-solid multi-component model foods. *Food Hydrocolloids*, *52*, 403-414.

Luu, T.-T.-H., Le, T.-L., Huynh, N., & Quintela-Alonso, P. (2021). Dragon fruit: A review of health benefits and nutrients and its sustainable development under climate changes in Vietnam. *Czech J. Food Sci. 39*(2), 71-94.

Maberley, D. (1993). "The Plant-book: A portable dictionary of the higher plants". Cambridge University Press. Cambridge, Great Britain.

Macwan, S. R., Dabhi, B. K., Parmar, S., & Aparnathi, K. (2016). Whey and its utilization. *Int.J. Current Microbiol. Appl. Sci. 5*(8), 134-155.

Majumdar, T., Wadikar, D., Vasudish, C., Premavalli, K., & Bawa, A. (2011). Effect of storage on physico-chemical, microbiological and sensory quality of bottlegourd-basil leaves juice. *Am. J. Food Technol*. 6(3), 226-234.

Matthews, D. E. (2005). Observations of branched-chain amino acid administration in humans. *The Journal of nutrition*, *135*(6), 1580S-1584S.

Mehta, B. M. (2014). Chemical Composition of Milk and Milk Products. In P. C. K. Cheung & B. M. Mehta (Eds.), *Handbook of Food Chemistry* (pp. 1-34). Springer Berlin Heidelberg.

Melnik, B. C., Schmitz, G., John, S. M., Carrera-Bastos, P., Lindeberg, S., & Cordain, L. (2013). Metabolic effects of milk protein intake strongly depend on pre-existing metabolic and exercise status. *Nutrition & metabolism*, *10*, 1-6.

Merten, S. (2003). A review of Hylocereus production in the United States. *J. PACD*, *5*, 98-105.

Miller, G. (2005). Healthy growth ahead for wellness drinks. *Food technology (Chicago)*, *59*(10), 20-26.

Mishra, L. (2024). Preparation and study on storage stability of mixed RTS beverage of dragon fruit and pineapple incorporated with mint. Unpublished B. Tech thesis. Tribhuvan Univ., Nepal.

MR, A. (2010). Antioxidant study of pulps and peels of dragon fruits: a comparative study. *Int. Food Res. J.*, *17*, 367-375.

Mukherjee, P., Raj, N., & Sivaprakasam, S. (2023). Harnessing valorization potential of whey permeate for D-lactic acid production using lactic acid bacteria. *Biomass Conversion and Biorefinery*, *13*(17), 15639-15658.

Nagadevi, V., & Puraikalan, Y. (2015). Formulation and quality evaluation of wheybased fruit juice. *Int. J. Sci. Res*, *4*(4), 859-861.

Naik, Y., Khare, A., Choudhary, P., Goel, B., & Shrivastava, A. (2009). Studies on physico-chemical and sensory characteristics of whey based watermelon beverage. *Asi. J. Res. Chem.*, *2*(1), 57-59.

Neelima, Sharma, R., Rajput, Y. S., & Mann, B. (2013). Chemical and functional properties of glycomacropeptide (GMP) and its role in the detection of cheese whey adulteration in milk: a review. *Dairy Science & Technology*, *93*, 21-43.

Nerd, A., Sitrit, Y., Kaushik, R. A., & Mizrahi, Y. (2002). High summer temperatures inhibit flowering in vine pitaya crops (Hylocereus spp.). *Scientia Horticulturae*, *96*(1-4), 343-350.

Nobel, P. S. (2002). "Cacti: biology and uses"(1st ed.) . University of California Press. Canifornia, USA.

Nurmahani, M., Osman, A., Hamid, A. A., Ghazali, F. M., & Dek, M. (2012). Antibacterial property of Hylocereus polyrhizus and Hylocereus undatus peel extracts. *Int. Food Res. J. 19*(1).

Nurul, S., & Asmah, R. (2014). Variability in nutritional composition and phytochemical properties of red pitaya (Hylocereus polyrhizus) from Malaysia and Australia. *Int. Food Res. J. 21*(4).

Onwulata, C., & Huth, P. (2009). "Whey processing, functionality and health benefits" (Vol. 82). John Wiley & Sons Ltd., Publication. State Avenue, USA.

Pandey, S., & Ojha, P. (2020). Preparation and quality evaluation of mango based whey beverage. *J. Food Sci. Technol. Nepal*, *12*(12), 59-61.

Panico, A., Garufi, F., Nitto, S., Di Mauro, R., Longhitano, R., Magrì, G., Catalfo, A., Serrentino, M., & De Guidi, G. (2009). Antioxidant activity and phenolic content of strawberry genotypes from Fragaria x ananassa. *Pharmaceutical Biology*, *47*(3), 203-208.

Patel, K. (2012). Development of selfcarbonated probiotic whey beverage. M. Tech Thesis. Anand Agricultural Univ., India.

Patel, M. T., Kilara, A., Huffman, L. M., Hewitt, S. A., & Houlihan, A. V. (1990). Studies on whey protein concentrates. 1. Compositional and thermal properties. *J. Dairy Sci. 73*(6), 1439-1449.

Patel, S., & Ishnava, K. (2019). In-vitro antioxidant and antimicrobial activity of fruit pulp and peel of Hylocereu sundatus (Haworth) Britton and Rose. *Asi. J. Ethnopharmacol. Med. Foods*, *5*(2), 30-34.

Pernot-Barry, A. (2008). Importance of whey ingredients in confectionery products. 5th International Whey Conference, Paris, France.

Perween, T., Mandal, K., & Hasan, M. (2018). Dragon fruit: An exotic super future fruit of India. *J. Pharmacog. Phytochem. 7*(2), 1022-1026.

Philippopoulos, C. D., & Papadakis, M. T. (2001). Current trends in whey processing and utilization in Greece. *Int. J. Dairy Technol. 54*(1), 14-19.

Prazeres, A. R., Carvalho, F., & Rivas, J. (2012). Cheese whey management: A review. *J. Env. Mgmt.*, *110*, 48-68.

Raj, S. P., Nirmala, C., Deccaraman, M., & Sagadevan, E. (2014). Production of Single Cell Protein using Kluveromyces marxianus isolated from paneer whey. *Int. J. Biomed. Adv. Res*.

Ranganna, S. (1986). "Handbook of Analysis and Quality Control for Fruit and Vegetable Products" (2 ed.). Tata McGraw-Hill Education. New York, USA.

Rao, C., & Sasanka, V. (2015). Dragon fruit 'The Wondrous Fruit'for the 21st century. *Glo. J Res. Anal*, *4*(10), 261-262.

Rapin, J.-D., Marison, I. W., von Stockar, U., & Reilly, P. J. (1994). Glycerol production by yeast fermentation of whey permeate. *Enzyme and Microbial Technology*, *16*(2), 143-150.

Revathi, R., & Singh, A. (2014). Chemical analysis of whey based pineapple. *Int. J. of home Sci. extension and Commun. Manage*, *1*(1), 32-34.

Rohit, I., Chavan, K., More, R., & Parmeshwar, W. (2019). Studies on healthy fruit based chhana whey beverage by using kiwi (Actinidia deliciosa) fruit extract. *J. Pharmacog. Phytochem.*, *8*(5), 1650-1653.

Sadasivam, S. (1996). "Biochemical Methods" (2nd ed.). New Age International. Coimbatore, India.

Sakhale, B., Pawar, V., & Ranveer, R. (2012). Studies on the development and storage of whey based RTS beverage from mango cv. Kesar. *J. Food Process. Technol*. 3(3) 148.

Salvatore, E., Pes, M., Falchi, G., Pagnozzi, D., Furesi, S., Fiori, M., Roggio, T., Addis, M., & Pirisi, A. (2014). Effect of whey concentration on protein recovery in fresh ovine ricotta cheese. *J. Dairy Sci. 97*(8), 4686-4694.

Satpute, D., Padghan, P., Patil, Y., & Suryawanshi, D. (2018). Effect of menthol (Mentha arvensis) and beet root extract on physico-chemical properties of paneer whey based beverage. *Int. J. Food Sci. Nutr. 3*(1), 99-105.

Sharma, R., Choudhary, R., Thakur, N., Bishist, R., & Thakur, A. (2020). Optimization of Fructooligosaccharide fortified low calorie apple-whey based RTS beverage and its quality evaluation during storage. *Curr. J. Appl. Sci. Technol.*, *39*(10), 17-28.

Sharma, S. C., Mittal, R., Sharma, A., & Verma, V. (2021). Dragon fruit: A promising crop with a growing food market that can provide profitable returns to farmers. *Int. J. Agric. Sci. Res*, *11*, 1-14.

Shrestha, K., & Dahal, A. (2021). Preparation of whey-based banana beverage and its quality evaluation. *Himalayan J. Sci. Technol. 5*(01), 60-66.

Shukla, F., Sharma, A., & Bakshi, A. (2000). Studies on the preparation offruit juice beverages using milk by-products. International Conference on Processed Foods for 21st Century. Calcutta, India. January,

Silva, G. d. S., da Costa Castro, C. D. P., Borges, G. d. S. C., De Freitas, S. T., Aidar, S. d. T., Biasoto, A. C. T., Rybka, A. C. P., & Cardarelli, H. R. (2023). Physicochemical and functional properties of new sports drink with ricotta cheese whey and a brazilian passion fruit variety. *J. Food Sci. Technol. 60*(2), 538-548.

Singh, A. K., Sinha, S., & Singh, K. (2009). Study on?-Galactosidase Isolation, Purification and Optimization of Lactose Hydrolysis in Whey for Production of Instant Energy Drink. *Int. J. Food Eng. 5*(2).

Singh, D., Singh, R., & Bhatt, F. (2014). Development, quality evaluation and shelf life studies of whey guava beverage. *Int. J. Curr. Eng. Technol. 4*(3), 2171-2175.

Singh, V., Hsu, C., Chen, D., & Tzeng, C. (1983). Fermentation processes for dilute food and dairy wastes. *J. Process Biochem.* 18(2), 16-17.

Siso, M. G. (1996). The biotechnological utilization of cheese whey: a review. *Bioresource Technol.*, *57*(1), 1-11.

Smithers, G. W. (2008). Whey and whey proteins—From 'gutter-to-gold'. *Int. Dairy J.*, *18*(7), 695-704.

Sonawane, M. S. (2017). Nutritive and medicinal value of dragon fruit. *Asi. J. Horticul.* 12(2), 267-271.

Srivastava, S., Hossain, S. A., Bharti, B. K., & Dixit, N. K. (2018). Sensory evaluation of beverages by incorporation of aonla and beet root juice in paneer whey. *J. Pharmacog. Phytochem. 7*(5), 2173-2178.

Stoliar, M. (2009). US whey ingredients in bakery products. *Applications monograph bakery. Published by US dairy export council. USA*, 1-8.

Szilagyi, A., & Ishayek, N. (2018). Lactose intolerance, dairy avoidance, and treatment options. *Nutrients*, *10*(12), 1994.

Thompson, M. P., Groves, M. L., Brower, D. P., Farrell Jr, H. M., Jenness, R., & Kotts, C. E. (1988). The calcium-dependent electrophoretic shift of α-lactalbumin, the modifier protein of galactosyl transferase. *Biochem. Biophy. Res. Commun.*, *157*(3), 944-948.

Tovar Jiménez, X., Arana Cuenca, A., Téllez Jurado, A., Abreu Corona, A., & Muro Urista, C. R. (2012). Traditional methods for whey protein isolation and concentration: effects on nutritional properties and biological activity. *J. Mex. Chem. Soc.*, *56*(4), 369-377.

Wakchaure, G., Minhas, P., Kumar, S., Mane, P., Kumar, P. S., Rane, J., & Pathak, H. (2023). Long-term response of dragon fruit (Hylocereus undatus) to transformed rooting zone of a shallow soil improving yield, storage quality and profitability in a drought prone semi-arid agro-ecosystem. *Saudi J. Bio. Sci.*, *30*(1), 103497.

Wit, J. N. (2001). "Lecturer's Handbook on Whey and Whey Products" (1st edi.). European whey product Association. Brussels, Belgium.

Yadav, R. B., Yadav, B. S., & Kalia, N. (2010). Development and storage studies on whey-based banana herbal (Mentha arvensis) beverage. *Am J Food Technol*, *5*(2), 121-129.

Young, S. (2007). Whey products in ice cream and frozen dairy desserts. *Applications Monograph Ice cream. Published by US dairy export council. USA.*

Zadow, J. (1994). Utilisation of milk components: Whey. In *Robinson: Modern Dairy Technology: Volume 1 Advances in Milk Processing* (pp. 313-373). Springer.

Zadow, J. (2012). *Whey and lactose processing*. Springer Science & Business Media.

Zadow, J. G. (1992). Lactose hydrolysis. In *Whey and lactose processing* (pp. 361-408). Springer.

Zaman, Q. U., Sahar, A., Sameen, A., Ahmad, M. H., Muhammad, N., Huma, N., Afzal, M. F., Khalid, W., & Al-Farga, A. (2023). Development and storage stability of Whey sugarcane based functional beverage. *Int. J. Food Prop. 26*(1), 752-763.

Zandona, E., Blažić, M., & Režek Jambrak, A. (2021). Whey utilization: Sustainable uses and environmental approach. *Food. Technol. Biotechnol.*, *59*(2), 147-161. https://doi.org/[doi: 10.17113/ftb.59.02.21.6968]

Appendices

Appendix-A

Specimen card for sensory evaluation

Hedonic rating test

Name: …………………………   Date: ……………

Name of the product: Whey based dragon fruit beverage

Observe the product by testing. Use appropriate scale to show your attitude by checking at the point best described your feeling of products. An honest expression of your personnel feeling is warmly welcomed. Give point as follows;

Quality description:

9. Like extremely
8. Like very much
7. Like moderately
6. Like slightly
5. Neither like nor dislike
4. Dislike slightly
3. Dislike moderately
2. Dislike very much
1. Dislike extremely

S.N.	Samples	Color	Flavor	Taste	Overall acceptance
1.	A				
2.	B				
3.	C				
4.	D				
5.	E				
6.	F				
7.	G				

Comments if any:…………………………………………………………………………..

Signature: ……………..

Appendix B

ANOVA results of sensory analysis of whey-based dragon fruit beverage

Table B.1.1 Two-way ANOVA (no blocking) for color

Source of variation	d.f.	s.s	m.s.	v.r.	F pr.
Sample	6	141.3333	23.8889	67.64	<.001
Panelist	8	1.2698	0.1587	0.45	0.885
Residual	48	16.9524	0.3532		
Total	62	161.5556			

Table B.1.2 LSD for color

Table	Sample	Panelist
rep.	9	7
d.f.	48	48
l.s.d.	0.5633	0.6387

Table B.1.3 Two-way ANOVA (no blocking) for flavor

Source of variation	d.f.	s.s	m.s.	v.r.	F pr.
Sample	6	149.9683	24.9947	113.15	<.001
Panelist	8	2.2857	0.2857	1.29	0.269
Residual	48	10.6032	0.2209		
Total	62	162.8571			

Table B.1.4 LSD for flavor

Table	Sample	Panelist
rep.	9	7
d.f.	48	48
l.s.d.	0.4455	0.5051

Table B.1.5 Two-way ANOVA (no blocking) for taste

Source of variation	d.f.	s.s	m.s.	v.r.	F pr.
Sample	6	97.2063	16.2011	62.17	<.001
Panelist	8	10.6032	1.3254	5.09	<.001
Residual	48	12.5079	0.2606		
Total	62	120.3175			

Table B.1.6 LSD for taste

Table	Sample	Panelist
rep.	9	7
d.f.	48	48
l.s.d.	0.4838	0.5486

Table B.1.7 Two-way ANOVA (no blocking) for overall acceptance

Source of variation	d.f.	s.s	m.s.	v.r.	F pr.
Sample	6	127.9365	21.3228	107.83	<.001
Panelist	8	4.2857	0.5357	2.71	0.015
Residual	48	9.4921	0.1978		
Total	62	141.7143			

Table B.1.8 LSD for overall acceptance

Table	Sample	Panelist
rep.	9	7
d.f.	48	48
l.s.d.	0.4215	0.4779

Appendix C

ANOVA of chemical constituents of whey-based dragon fruit beverage

Table C.1.1 Two-way ANOVA for TSS as a variate

Sourceof variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	5	1.995556	0.399111	41.01	<.001
Condition	1	0.321111	0.321111	33.00	<.001
Residual	29	0.282222	0.009732		
Total	35	2.598889			

Table C.1.2 LSD for TSS

Table	Days	Condition
rep.	6	18
d.f.	29	29
l.s.d.	0.1165	0.0673

Table C.1.3 Two-way ANOVA for acidity as a variate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	5	0.211056	0.042211	12.74	<.001
Condition	1	0.098178	0.098178	29.62	<.001
Residual	29	0.096122	0.003315		
Total	35	0.405356			

Table C.1.4 LSD for acidity

Table	Days	Condition
rep.	6	18
d.f.	29	29
l.s.d.	0.06798	0.03925

Table C.1.5 Two-way ANOVA for pH as a variate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	5	0.1540472	0.0308094	32.98	<.001
Condition	1	0.0210250	0.0210250	22.51	<.001
Residual	29	0.0270917	0.0009342		
Total	35	0.2021639			

Table C.1.6 LSD for pH

Table	Days	Condition
rep.	6	18
d.f.	29	29
l.s.d.	0.03609	0.02084

Table C.1.7 Two-way ANOVA for reducing sugar as a variate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	5	1.695922	0.339184	89.16	<.001
Condition	1	0.162678	0.162678	42.76	<.001
Residual	29	0.110322	0.003804		
Total	35	1.968922			

Table C.1.8 LSD for reducing sugar

Table	Days	Condition
rep.	6	18
d.f.	29	29
l.s.d.	0.0728	0.0420

Table C.1.9 Two-way ANOVA for ascorbic acid as a variate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	5	29.5901	5.9180	43.50	<.001
Condition	1	7.7377	7.7377	56.88	<.001
Residual	29	3.9452	0.1360		
Total	35	41.2730			

Table C.1.1 LSD for ascorbic acid

Table	Days	Condition
rep.	6	18
d.f.	29	29
l.s.d.	0.4355	0.2515

Appendix D

Table D.1 List of equipment used

The apparatus required during this work were provided by CCT lab. The list of glassware and apparatus required during this work were:

1. Hot air oven	6. Thermometer
2. Muffle furnace	7. Weighing balance
3. Desiccator	8. Refractometer
4. Kjeldhal digestion apparatus	9. Spectrophotometer
5. pH meter	10. Glassware

Table D.2 List of chemicals used

The chemicals required during this work were provided by CCT lab. The lists of chemicals required during this work are:

1. Catalyst Mixture (Mixture of 2.5 g of powdered SeO2, 100 g K_2SO_4 and 20 g $CuSO_4.5H_2O$)
2. Mixed Indicator Solution (Mixture of 10 ml of 0.1% bromocresol green and 2 ml of 0.1% methyl red solution which is prepared separately in 95% ethanol)

3. Boric acid	11. Indophenol
4. Phenolphthalein	12. Sodium carbonate
5. Conc. sulphuric acid (H_2SO_4)	13. Carraz-I and carraz-II
6. Conc. nitric acid (HNO_3)	14. Gallic acid
7. Sodium hydroxide (NaOH)	15. DPPH (2,2-diphenyl-1-picrylhybrazyl)
8. Oxalic acid	16. Folin/Ciocalteau reagent
9. Dextrose	17. Sodium nitrate
10. Meta-phosphoric acid	18. Aluminium chloride

Appendix E

Table E.1 Cost evaluation (for every 250 ml bottle)

S.N.	Particulars	Quantity	Rate (Rs)	Amount (Rs)
1.	Whey	62.5 ml	10/L	0.625
2.	Dragon fruit juice	37.5 ml	480/L	18
3.	Sugar	5.55 g	100/kg	0.55
4.	Bottle (250 ml)	1	10/piece	10
	Total			29.17

The price of 100 ml of drink is NRs 29.17 and NRs 72.92 for 250 ml of drink.

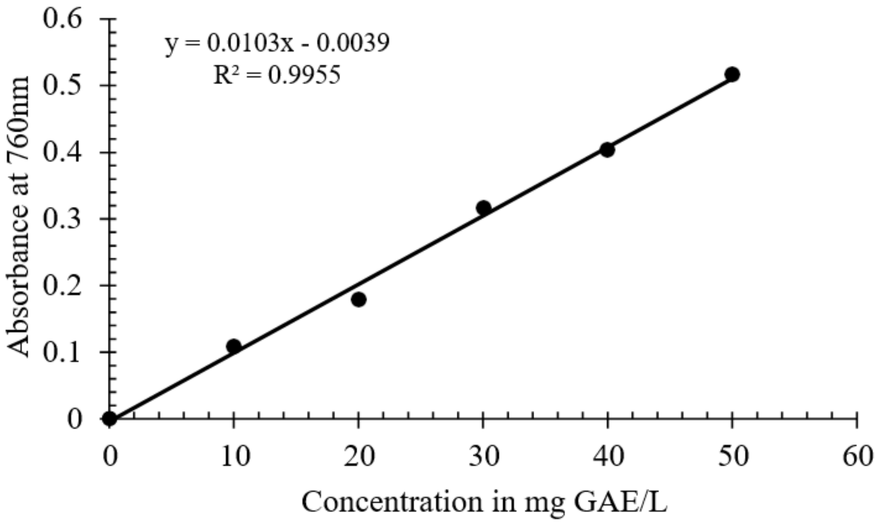
Appendix F

Summary of ANOVA of sensory evaluation of whey-based dragon fruit beverage.

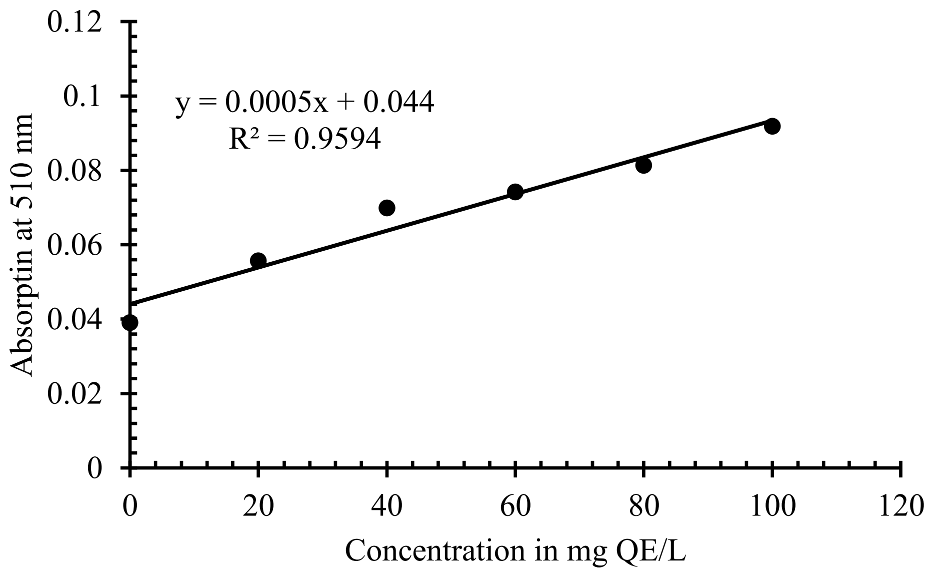
Sample	Color	Flavor	Taste	Overall acceptance
A	4.0±0.66a	4.4±0.49a	5.0±0.67a	4.3±0.47a
B	5.2±0.41b	4.5±0.57b	5.3±0.47a	5.1±0.31b
C	5.6±0.47bc	5.2±0.41b	6.1±0.73b	5.5±0.49b
D	6.3±0.47c	6.3±0.47c	7.1±0.73c	6.6±47c
E	7.2±0.78d	6.6±0.48c	7.5±0.49cd	7.2±0.62c
F	8.5±0.49e	8.6±0.47d	8.5±0.49e	8.5±0.49d
G	8.1±0.31e	8.1±0.31e	7.8±0.56de	7.8±0.34e

Appendix G

1. Standard curve for total phenolic content determination.



2. Standard curve for flavonoids determination



Color Plates

Plate 1 Seven different samples

Plate 2 Sensory evaluation

Plate 3 Protein estimation

Plate 4 Microbiological analysis

Plate 5 Analysis for reducing sugar