PREPARATION OF WHEY BASED PINEAPPLE BEVERAGE AND ITS STORAGE QUALITY EVALUATION

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A dissertation submitted to the ***Food Technology Instruction Committee*** *in 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 of Whey Based Pineapple Beverage and its Storage Quality Evaluation*** *by* **Gajendra Bohora** has been accepted as a partial fulfillment of the requirements for the B. Tech degree in food technology

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(Gajendra Bohora)

Abstract

The utilization of pineapple (*Ananas comosus* (L.) *Merr.* Family: Bromeliaceae*)* in the development of whey based beverage showed a great benefit to the dairy industry. The aim was to develop whey based pineapple beverage utilizing design expert® (v 7.0) under mixed condition. Eight formulations of beverage were obtained with varying levels of whey and pineapple in the range of 50-100% and 0-50% respectively. Formulated samples were analyzed by sensory evaluation and the beverage sample with 50% whey and 50% pineapple juice were found to be superior. However, sensory parameters viz. appearance, color, flavor, taste and overall acceptability of different formulations were significantly affected ($p<0.05$) by variation of whey and pineapple beverage. The beverage was pasteurized at 82.5°C for 20 min and stored in 250 mL plastic bottles at normal (25±5◦C) and refrigeration (7±1°C) for 20 days. The effects of storage time and temperature on physicochemical (TSS, pH, acidity, reducing sugar, ascorbic acid) and microbial (TPC, yeast & mold count) properties were evaluated.

Total soluble solids, pH and reducing sugar increased while acidity and ascorbic acid content decreased with progressing storage time in both storage condition. During the storage analysis TSS, pH, acidity, reducing sugar and ascorbic acid content ranged from 12.8-13.43%, 4.43-4.37, 0.41-0.49%, 5.09-5.71% and 26-22.3 mg/100 g respectively under refrigerated condition and from 12.8-13.77%, 4.43-4.31, 0.41-0.60%, 5.09-5.44% and 26-17.36 mg/100 g respectively under normal storage condition. From all the analysis, it can be observed that the juice beverage can be kept unspoiled for 20 days without preservatives.

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List of abbreviation

Abbreviation	Full form
BU	Biological unit
CCT Lab	Central Campus of Technology, Laboratory
CW	Cheese whey
DDC	Dairy development corporation
HIV	Human immune virus
LC	Least count
LSD	Least significance difference
MT	Metric ton
NT	Normal temperature (25±5°C)
PET	Polyethylene tetracycline
RT	Refrigerated temperature (7±1°C)
RTS	Ready to serve
SNF	Solid not fat
TSS	Total soluble solid
WP	Whey protein
WPC	Whey protein concentrate
WPI	Whey protein isolate

PART I

Introduction

1.1 General introduction

Whey is the milk serum, yellowish liquid portion of milk left after the formation of curd / coagulated products that result from acid or proteolytic enzyme. It is the main by-product, left after the manufacture of *paneer*, channa, chakka, cheese, casein, etc. About 80-90% whey is obtained during the manufacturing of these products and only 10- 20% portion of milk is recovered as an end product. Whey contain 45-50% of total milk solid, 70% of milk sugar (lactose), 20% of milk protein and 70-90% of milk minerals and almost all the water soluble vitamins present in the milk (Wit, 1995).

Whey, the by-product of cheese or casein production, is of relative importance in the dairy industry due to the large volumes produced and the nutritional composition. Worldwide whey production is estimated at around 180 to 190×10^6 ton/year; of this amount only 50% is processed (Baldasso *et al.*, 2011). Approximately 50% of worldwide cheese-whey (CW) production is treated and transformed into various foods and feed products. About half of this amount is used directly in liquid form, 30% as powdered cheese-whey, 15% as lactose and its byproducts and the rest as cheese whey- protein concentrates (Lacta, 2012). There are two different types of whey: sweet whey and acid whey. Sweet whey is a by-product of ripened cheese production (pH 5.8–6.6) whereas acid whey is obtained from cottage cheeses (pH 3.6–5.1) (Park and Haenlein, 2013).

A tendency to use substitutes of ingredients in recipes of many products has been observed for several years in the food processing industry. It pertains to foods with reduced fat and sugar, or food products for vegetarians and people with lactose intolerance (Bolumar *et al.*, 2015; Serna *et al.*, 2014). Whey and its preparations may serve as substitutes having a positive impact not only on the consumers' health, but also on the finances of many companies by reducing the costs of raw materials, and thus lowering production costs (Bozanic *et al.*, 2014).

Modern industrial processing techniques such as ultrafiltration (UF), reverse osmosis (RO), new drying methods, hydrolysis, electro dialysis, ion-exchange, fermentation and protein fractionation, among others, have converted whey into a major source of ingredients with differing functional and nutritional properties, that could be used in food and dairy industry. The predominant driving force behind the development of whey utilization has been stringent regulations imposed by the environmental pollution agencies all over the world. Other aspect relates to economic return from whey, which contains almost half the solids of original milk. Presence of lactose, protein, minerals and water-soluble vitamins make the whey a highly nutritious product (Anon., 2015c).

Being a rich source of lactose, whey is a good fermentation media for a number of fermented products. The production of whey beverages, whey protein concentrates, lactose, and many others products from whey have prominence in advanced dairy countries. Whey cheeses like Gjetost, Mysost and Gudbrandsdulsost are produced in Norway, while Manouri, Anthotryos, Cryzittroa and Giza in Greece. Despite significant gains in the amount of whey being processed, a large amount of whey produced still is disposed as raw whey. In small plants, the choice remains some form of disposal, be it municipal treatment, spreading raw whey on local farm lands for its nutrient value or feeding to local livestock. Further, acid whey, because of high mineral content and low pH pose considerable difficulties in utilization and, therefore, mostly remain unutilized (Anon., 2015c).

1.2 Statement of the problems

The use of whey for the manufacture of whey-based beverage has been the most traditional approach to whey utilization for human nutrition. Whey based fruit beverages are manufactured by mixing of appropriate fruit pulp/ juice or juice concentrate and processed whey. A variety of whey beverages consisting of plain, carbonated, alcoholic, soy and fruit have been successfully developed and marketed all over the world, because they hold great potential for utilizing whey solids. Disposal of unutilized milk by-product (whey) leads to environmental pollution (Anon., 2014). In Nepal no. of large and small scale industries produce cheese and *paneer* products and whey so 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 liters (Anon., 2015a).

1.3 Objectives

1.3.1 General objectives

The general objectives of this dissertation is production of whey (*paneer* whey) based pineapple beverage and its storage quality evaluation.

1.3.2 Specific objectives

1. To carry out nutritive analysis of fresh whey, pineapple juice and prepared beverage.

2. To formulate the proportion of whey and pineapple for best sensory quality.

3. Self-life study with respect to microbial (TPC & yeast and mold) and chemical properties.

1.4 Significance of study

Growing environmental pollution and problems has pressurized the cheese and *paneer* manufacturers to stop dumping whey into the streams and sewage system. As a remedy solution, whey utilization in the production of beverage is common practices around the globe. But still the whey utilization in different dairy products and beverage production remains unexplored.

A whey drink can replace much of the lost organics and inorganics to the extracellular fluid. Whey, which is so rapidly assimilable, forms an ideal metabolic substrate. Utilization of whey as an excellent beverages base has been recognized as it is genuine thirst quencher. Whey drinks are light, refreshing, healthful and nutritious but less acidic than fruit juices, and offers good profit margins. Whey improves the flavors, texture, appearance and shelf life beverages. At present fruit juices are generally synthetic, flavored, bottled and sold in the global market. If this could be substituted with whey beverage a large part of dairy waste would be utilized that not only nutritionally and economically benefits the producer and consumer but also environmentally benefits the world.

1.5 Limitation of the study

1. Due to the time constraints only single variety of pineapple pulp was taken and analyzed.
2. Only the *paneer* whey was utilized.
3. For the microbial analysis TPC, yeast and mold count was done for pasteurized product only.
4. Shelf life of product was studied only for 20 days.
5. Detail nutritional analysis could not be done due to time and resource limitation.

PART II

Literature review

2.1 Milk

Milk is an important nutrition source for people around the world. Cow milk is comprised of approximately 3.3% protein, 4% fat, 87.1% water, 4.6% lactose, contains many essential vitamins (vitamin E and A), and is major source of calcium (Dissanayake, 2011). Traditionally milk proteins have been divided in three crude groups, whey proteins, milk fat globular membrane (MFGM) proteins, and casein. Milk proteins play an important role for growth factors, enzymes, immune system function, hormones, and antibodies (Clare and Swaisgood, 2000). A higher amount of milk protein may build-up muscles, prevent bone breakdown, improve muscle movement, raise satiety, control blood sugar, and decrease the risk of some cancers (Melnik *et al.*, 2013). Milk proteins consist of 80% casein and 20% whey proteins. Casein is responsible for transporting calcium and phosphate and aiding efficient stomach digestion. Other major proteins in milk are α-lactalbumin, β-lactoglubulin, bovine serum albumin and immunoglobulin (Haug *et al.*, 2007).

2.2 Whey

Milk whey is one of the highly nutritious by-products 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 almost all the water soluble vitamins originally present in milk (Horton, 1995; Shukla *et al.*, 2004). It resulted into unraveling the secrets of whey proteins and other components and established a sound basis for their nutritional and functional value (Smithers, 2008). Depending on the types of coagulant used whey is categorized as Acid whey or Sweet whey. Acid whey is the milk serum obtained after the production of rennet cheese (hard cheese); whereas Sweet whey is the milk serum results from the production of cottage cheese. Sour/Acid Whey has pH of 3.8 - 4.6 while Sweet Whey has pH of 5.4 - 6.7 (Bordenave *et al.*, 2005).

2.3 Whey background and production

Whey is the largest byproduct of dairy industry obtained during manufacture of casein, cheese, *paneer*, chhana etc. Whey had been considered as the milk by products for years and it has been mainly dumped into lands, sewage, waterways, and oceans while partly used as animal feed. Dumping of whey globally pushed the environmental pollution to further increment which leads searching the new possibility for the utilization of whey. Global production of liquid whey from cheese and casein amounted to 192 million MT in 2015 and annual average growth of ~3-4%. The European Union and United state produce ~70% of whey in the world (Affertsholt, 2015).

Lactose is the major product while whey proteins, water soluble vitamins, and minerals are the secondary products. Much of the whey is spray dried to whey powder, whey protein concentrates and isolates which in return is used in food fortification (Smithers *et al.*, 1996).

2.4 Nutritional and health effects of whey

Whey protein is a mixture of beta-lactoglubulin, alpha lactalbumin, bovine serum albumin, and immunoglobins. It also contains proteins, albumins and globulins, which have outstanding nutritional qualities. The glutathione in whey protein is an antioxidant reduces the risk of cancer in animals, suggesting an avenue for future medical research. Whey protein is considered a complete protein as it contains all 9 essential amino acids i.e. isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. It also contains lactose, minerals and biologically active components (Kamble, 2009).

Whey proteins have antimicrobial, antiviral and anti- oxidant properties can offer a kind of protection against cancer and heart diseases and assist in the enhancement of immune-defense (Nordqvist, 2015). Other benefits include:

1. Aiding weight loss with significantly loss of body fat and greater preservation of lean muscle.
2. Anti-cancer properties.
3. Lowering cholesterol.
4. Improve the immune response in children with asthma.

5. Reduced blood pressure in patients with hypertension and their risk of developing heart disease or stroke.

6. Reducing weight loss in people with HIV.

Nutritional value of sweet whey is presented in Table 2.1.

Table 2.1 Nutritional value per 100 g (3.5 oz) 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)

2.5 Whey components

2.5.1 Whey proteins

Whey protein are of two types major proteins and minor proteins, Major whey proteins are β-lactoglobulin-65%, α-lactalbumin-25%, serum albumin-8% and minor proteins/peptides are Glycomacropeptide (GMP), Bovine serum albumin, lactoferrin, Immunoglobulin, Phospho-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 and McSweeney, 1998).

2.5.1.1 β-lactoglobulin

β-lactoglubulin(β-Lg) is the most important protein in whey with ~ 18 kDa molecular weight. It represents 50% of whey protein and also 12% of total protein in milk. β-Lg is able to bind to fatty acids and retinol (vitamin A) and because of this, it has great foaming and gelation properties (Heino, 2010).

2.5.1.2 α-lactalbumin

α-lactalbumin (α–La) is the second most important protein in whey and milk with a molecular weight of ~ 14 kDa. It comprises 20 % of total whey protein and also 3.5 % of total protein in milk. It has dependency on calcium (Ca^{2+}) ions and it is known as a metallo-protein. It has a high tryptophan (Trp) content that is very useful for human brain function. It also has high level of the amino acids useful in muscle protein synthesis (Thompson *et al.*, 1988).

2.5.1.3 Bovine serum albumin

Bovine serum albumin (BSA) with the molecular weight of 66 kDa is another cow milk proteins. BSA has significant biological effect on human health but its role in food and milk are not well known. BSA has only a slight effect on whey physiochemical properties due to its low concentration in milk (Heino, 2010).

2.5.1.4 Lactoferin

Lactoferin (LF) has a molecular weight of about 76.5 kDa and is a multi-functional protein from the transferrin family. It exists in different liquids like milk, nasal, saliva and others. LF has antibacterial activity in humans and interacts with nucleic acids (Yang *et al.*, 2013).

2.5.1.5 Glycomacropeptide

Glycomacropeptide (GMP) is the C-terminal portion of kappa casein and is sometimes called casein macro peptide (CMP). It has a molecular weight of 6-10 kDa. The amino acid composition of this protein is very unique. GMP has various chemical attributes like extensive emulsifying properties and is stable in a wide range of pH (Sharma *et al.*, 2013).

2.5.1.6 Immunoglobulin

Immunoglobulin (Ig) is the immunological part of the milk. Immunoglobulin's are antibodies that can protect people against a wide range of bacteria and viruses. Human milk has the highest amount of Ig but cow's milk has low level of Immunoglobulin. Immunoglobulin has a molecular weight of 150-1000 kDa. These proteins have immune-active peptides and therefore the presence of this protein is beneficial for a whey product (Tovar *et al.*, 2012).

2.5.2 Lactose

Lactose, the major component of whey, is probably the least valuable component and most difficult to utilize. Lactose comprises about 70% of the total solids of whey (Jelen, 2002).

2.5.3 Minerals

Whey is a good source of electrolytes including sodium and potassium, which are required during recovering from diarrhea. Minerals such as calcium, magnesium, and phosphorus are present in solution and also partly bound to proteins. Zinc is present in trace amounts. Lactose also promotes absorption of Mg and Zinc ions, which even in trace amount helps in better diarrheal management (Jelen, 2002).

2.5.4 Vitamins

During the manufacturing process, the water-soluble vitamins are transferred into whey in a varying extent: 40-70% of vitamin B12; 55-75% of vitamin B6 and pantothenic acid; 70-80% of riboflavin and biotin; 80-90% of thiamine, nicotinic acid, folic acid and ascorbic acid. In the case of vitamin B12, more of it was transferred into the whey when a rennet coagulation rather than acid coagulation was used (Jelen, 2002).

2.5.5 Composition of whey

The composition of whey is given in Table 2.2.

Table 2.2 Composition of whey

Parameter	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	o.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)

2.6 Utilization of whey

Worldwide only about 50% of the total whey production is "dried or further processed into whey protein concentrate, lactose and other products for use in formulating human food and animal feed products. The remainder of the whey, especially from small cheese manufacturing plants, is treated by private or municipal waste treatment, fed to livestock or spread on agricultural land as fertilizer. Processing of whey is limited due to less demand for whey powder and further processed whey products, plus the generally unfavorable economics of whey processing, especially for the smaller sized processing operation (Bozanic *et al.,* 2014) .

A substantial portion of whey powder, whey concentrate, reduced lactose/ reduced mineral whey, whey protein concentrate and lactose is 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).

2.7 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 - an iron-binding protein, Glycomacropeptide (GMP) which derives after cheese making using rennet and is naturally free of phenylalanine and alpha-lactalbumin which is a calcium binding protein. Due to presence of lactoferrin whey beverages can be used as functional food intended to improve iron absorption from food and/or help to keep pathogens from attaching to the intestinal walls. That is very important for nutrition of little children and babies. Furthermore, these beverages may improve absorption of calcium important for older population which is often suffering from osteoporosis (Miller, 2005).

Whey possesses potent antioxidant activity mainly contributed by cysteine-rich proteins that aid in the synthesis of glutathione (GSH), a potent intracellular antioxidant, also investigated as an anti-aging agent. Detoxifying property contributed by Glutathione peroxidase, which is derived from selenium and cysteine, that converts lipid peroxides into less harmful hydroxyl acids and α-lactalbumin, which chelates heavy metals and reduces oxidative stress because of its iron-chelating properties. Immunoglobulin and lysozyme in whey provides immunity enhancing benefits to infants and others. Antihypertensive peptides isolated from bovine β-lactoglubulin, reduces blood pressure (Miller, 2005).

Various minerals like, potassium, involved in the transmission of nerve impulses and muscular contractions; magnesium, depolarizes the nerve or muscle causing relaxation and help to lower blood pressure and supports an alkaline tissue pH; Calcium, used by the body to maintain an alkaline tissue pH, maintain bone density, cell wall integrity and nerve impulses. Lactoperoxidase inhibits the growth of iron dependent bacteria. Lactoferrin, inhibits the growth of bacteria (including pathogenic bacteria) and fungi, also regulates iron absorption and bio-availability. Whey also possesses the vitamins necessary for its utilization. It contains vitamins A, B_1, B_2, B_3, B_5, B_6, C, D, and E (Onwulata and Huth, 2008).

2.7.2 Whey proteins as a functional ingredient

Along with physiological benefits, WP possess inherent excellent functional properties and desirable sensory characteristics enabling them to be used in numerous food applications including sport beverages, liquid meat replacements, baked products and processed meats, pasta, salad dressings, spreads and dips, artificial coffee creams, soups, ice cream, confectionary infant foods and various other dairy products. The use of whey proteins as functional ingredient is given in Table 2.3. In order for them to be used in these applications, WP should be extracted from whey using different fractionation methods, mainly membrane processing (Fitzsimons *et al.*, 2007; Onwulata and Huth, 2008).

Table 2.3 Whey protein as 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 flavour.	Baked products, beverages, confectionery, snacks
Gelling and heat 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: Anon. (2012)

2.8 Processing of whey ingredients

The principle of industrial recovery processes of whey ingredients occurs before evaporation and/or spray drying. Membrane processes (18A) are used for the separation of ingredients with different molecular sizes. Microfiltration is used for the removal of bacteria and fat globules, ultrafiltration for the fractionation of proteins, nanofiltration for desalting, and reverse osmosis for the separation of water (DeWit, 2002; Varnam and Sutherland, 1994).

2.8.1 Demineralization of whey

The salts in whey have a significant effect on its taste, and may hamper the use of whey in food products. In delactosed whey (so-called mother liquor) the lactose content has been reduced to 50% and the protein content increased from 13 to 28%, but the mineral content has also been increased to about 20% on total solids. This makes the taste of mother liquor still more unfavorable for applications in human food which can be solved by desalting. Demineralization of whey or mother liquor may be realized by using ionic exchange. Electrodialysis is a cheaper and more current demineralization process for whey (DeWit, 2002).

2.8.2 Membrane filtration of whey

Well-known fractionation processes for the production of whey protein concentrates (WPC's) are based on membrane separation, which include microfiltration (MF), ultrafiltration (UF), Nano filtration (NF), and reverse osmosis (RO). Separation takes place through semi-permeable membranes, using a hydrostatic pressure gradient as the driving force. The separation mechanism is generally based on a sieving effect through thin filters of controlled pore size. Membranes may possess additional separation characteristics when they are charged, which is important for the separation of ions of a particular charge. Microfiltration is used for the removal of bacteria and fat (globules) from whey, by using membranes with fairly wide pores (> 0.1 µm). Ultrafiltration is used for the separation of whey proteins, and these membranes are usually characterized by separation capabilities on molecular weight (usually indicated as "cut-off"). Nanofiltration is used to fractionate mixtures of smaller molecules, e.g. partial demineralization of whey products. Nanofiltration can be applied as an alternative desalting process for Electrodialysis. Reverse osmosis is applied to remove water against an osmotic pressure, and requires much higher pressures than the other membrane techniques. Reverse osmosis is not a filtration process, but a phenomenon that opposes osmosis (DeWit, 2002).

2.8.3 Chromatographic separation of whey proteins

In the 1970‘s column chromatography was introduced as a separation technique for proteins on an industrial scale. There are several useful methods in this category for the isolation of Salts, proteins from whey according to specific protein properties. Well-known are gel filtration chromatography (based on differences in molecular sizes), ionic exchange chromatography (on the basis of charge differences), and affinity chromatography (as a technique using specificities in molecule-molecule interactions) (DeWit, 2002).

2.8.4 Recovery of lactose from whey

Lactose is the main component of whey and is recovered by crystallization from sweet and/or acid whey. There are two basic methods for the recovery of lactose, depending on the composition of the source:

1. Crystallization in concentrated whey
2. Crystallization in concentrated deproteinated whey (e.g. ultrafiltration permeate from whey) (DeWit, 2002).

2.8.5 Recovery of milk salts from whey

Table salt (NaCl) is one of the most used additives in food products either as preservative or as an enhancer of taste. Although Na^+ and Cl^- ions are essential constituents of all forms of human life; the average salt intake is estimated to be about 20 times the required daily need. One of the risk factors of a high sodium intake is hypertension (too high blood pressure). In response to current health concerns about the overconsumption of sodium, food processors are looking for salts with a reduced sodium content to replace table salt. One of the options is to fractionate milk salts from whey in their natural (physiological) composition, which reveal a high (soluble) calcium concentration as an additional nutritional benefit. Moreover it appears that fractionated whey salts provide the same salt-sensation as table salt does. One of the sources for milk salt production is delactosed whey; a by-product from the production of lactose (Varnam and Sutherland, 1994).

2.9 Applications of whey products

In the past whey has been regarded as a cure for many illnesses, and was used in thermal baths or as a medicine in cure centers. The unbalanced composition of whey solids limited the application of whey and whey powder in human food products. In particular the dominant presence of lactose (72%) and minerals (8%) were difficulties which had to be overcome for application of whey in food products. The increasing production of whey and whey powder stimulated their use as nutritional supplements for animal feed, particularly as a cheap replacer for skim milk powder. The introduction of fractionation and isolation techniques for whey components further increased the application possibilities in food products. Nowadays, potential uses for whey components either as functional or as nutritional supplements in food products are numerous (Anon., 2015c).

Applications in confectionery and bakery products are important outlets for whey and whey products in human foods. Lactose, the major component of whey, contributes to color and flavour in these products. Whey and whey-based products have been found to improve the flavour, aroma, color, texture and (in some cases) also the shelf life of bakery products. The use of demineralized whey is preferred, because of its blander taste. The high nutritional quality of whey proteins and the presence of specific growth factors make whey an important source for infant formula and elderly foods. Highly nutritional minor components may be isolated from whey by using column chromatography (Kamble, 2009).

2.9.1 Dairy products

2.9.1.1 Yoghurt

A growth in the consumption of fermented milk beverages has been observed in recent years; the most important of these is the consumption of yoghurt. For this reason, the quality characteristics of the finished product are very important. These characteristics can be successfully modified using whey preparations (Kozioł *et al.*, 2014; Liu *et al.*, 2016).

Whey products used in the production of yoghurt includes:

1. Sweet whey powder, which may replace skimmed milk powder at the level 2–5.2%.

2. WPCs, which are most often used by manufacturers of yoghurt. Addition of WPC34 at the level 0.7–2.0% or WPC80 at 0.5–0.8% is sufficient in the case of mixed yoghurt (a greater amount of the additive may adversely affect some quality characteristics). Replacing skimmed milk powder with WPCs causes, among other effects, an increased gel strength in solid yoghurt, increasing the

viscosity of mixed yoghurt, and reduces the risk of syneresis in both types of yoghurt.

3. WPI which, due to the low content of lactose and milk fat, is used in yoghurts with reduced lactose content.

4. Demineralized whey powder, with reduced mineral content, which accelerates the fermentation process. On the other hand, low mineral content weakens the structure of the gel, so it is necessary to add milk protein hydrolysates when this type of formulation is used (DeWit, 2002).

Furthermore, the addition of whey protein gives yoghurt a smooth and creamy texture; it also increases its nutritional value. The bioactive components present in whey and whey protein can stimulate the growth of probiotic bacterial cultures (both in the finished product, and in the human digestive tract) (Hugunin, 2009). The addition of *Bifidobacterium bifidum* to standard yoghurt cultures (*Lactobacillus delbrueckii ssp. bulgaricus*, *Streptococcus thermophiles*), increases the number of viable *B. bifidum* in the samples that contain the sweet whey or whey proteins (Kozioł *et al.*, 2014).

2.9.1.2 Ice-cream

Whey preparations used in the manufacture of ice-creams and sundaes include whey powder, demineralized whey powder, WPCs and WPI. Along with the most important nutritional, beneficial properties it also includes: water binding capacity, ability to form foam, and high nutritional value (Young, 2007). On the other hand, from the point of view of ice-cream manufacturers, it is important to reduce the costs of production. Rationalization of these costs can be achieved by using cheaper substitutes of certain ingredients of the recipes. The relatively expensive components include milk powder and egg yolk can be replaced with WPCs and with protein-fat preparations (Alfaifi and Stathopoulos, 2010; Jasińska *et al.*, 2012).

2.9.1.3 Cheese, processed cheese, and their analogues

Whey preparations, such as sweet whey powder, powdered whey with reduced lactose content, WPCs and WPI can be successfully used in the production of processed cheese and processed cheese analogues. Liquid, sweet whey left after cheese making from cow and sheep milk, can be used in manufacturing whey cheeses. For the production of processed cheese analogues, cheese is replaced by milk proteins (casein, whey proteins) or vegetable proteins. The addition of whey protein preparations to processed cheese analogues, as well as replacing casein with these proteins, increases the hardness of the final product which may be important for the preparation of products for slicing. In turn, the analogues supplemented with whey products exhibited lower meltability compared to cheese analogues prepared solely on the basis of acid casein in a pH range of 5.0–7.0 (Philippopoulos and Papadakis, 2001; Salvatore *et al.*, 2014).

Control of lactose content during the production process to prevent its crystallization and browning of the product (therefore, it seems reasonable to use products with a low content of milk sugar – WPCs, WPI). The recommended addition of whey preparations to cheese mixtures is as follows: sweet whey powder: 4–8%, whey with reduced content of powder lactose: 5–8%, WPC34 and WPC80: 1–5%, WPI90: 0.5–1% (Young, 2007). The most popular whey cheeses include Myzithra, Ricotta, Mysost, Gjetost, Manouri, Anthotyros and Giza (Wendorff, 2008).

2.9.2 Bakery and confectionery Products

2.9.2.1 Bakery products

Whey may be widely used in the baking, confectionery, and pastry industries for the production of breads, cakes, cookies, biscuits, crackers, muffins, and icing. Eggs contribute to the development of cakes structure and taste. Replacing eggs with whey proteins is also an effective means of reducing production costs (the obtained product crumbles less during cutting and packaging – which means a lack of additional costs). Furthermore, as a result of the presence of cholesterol in egg yolk, due to dietary reasons, there is a growing interest in the replacement of this component with the addition of WPCs (Stoliar, 2009).

WPC34 is suitable for products such as spice cookies or chocolate chip cookies as a partial replacement for both egg and fat. On the other hand, WPC80 is a good substitute for eggs in products such as bread, cakes and biscuits (both dry and soft) and muffins. In baking, confectionery and pastry, lactose (from whey) is often used as a substitute for sucrose as it enhances the Maillard reaction, improves emulsification and crumb structure, and enhances the flavor. In addition, whey proteins contain a high level of essential amino acids; they are also considered a source of high quality protein. In addition, they are characterized by a high content of calcium and other minerals, such as potassium and zinc which makes whey protein a valuable additive to bakery products (Stoliar, 2009).

2.9.2.2 Confectionary products

Whey products, including, demineralized whey powders, low-lactose whey powders, WPCs and isolates, and lactose have been used in the following confectionery: chocolates and chocolate chips, candies, jellies and chewing gums. Lactose – milk sugar – can serve as a bulking agent. It is slightly sweet, less soluble than sucrose, and has a low hygroscopicity level; however, it influences the color, the taste, and the texture of the finished product and takes part in the Maillard reaction. For these reasons, use of lactose can be more or less reduced, which depends on many characteristics of the confectionery. Another use of derivatives of WPCs is the production of the so-called aerated confectionery and chocolate. The foaming properties of concentrates are used in this case. In addition, WPI and concentrates of high protein content (WPC80) can be successfully used in the production of protein bars for athletes (Pernot, 2008; Stoliar, 2009).

2.9.3 Meat and meat products

Whey processing products used in meat industries especially in the production of comminuted products, such as: frankfurters, sausages, mortadella's, luncheon meat, or surimi. Whey protein partially replaces meat protein, as well as partially or completely substitutes soy protein and other binding agents, fillers, modified starch and hydrocolloids (Prabhu, 2006).

Whey proteins with improved flavour and increased functionality are obtained with new technologies. While choosing a particular whey product, it is essential to match its function to the characteristics we want to achieve. For example, high protein concentrates or isolates are used to modify fat content. A slight increase in sweetness occurs, especially with the addition of sweet whey (which enables reduced addition of sweeteners) (Youssef and Barbut, 2011).

Different properties of whey proteins used in the processing of meat products, poultry, and fish are water binding capacity that prevents the depletion of mass during thermal processing and storage of the product, increases juiciness of the final product and facilitates cutting cold meat products into slices; viscosity which improves the consumers' palatable impressions during consumption of the product (biting and chewing); formation of stable emulsions, which is particularly important in the production of finely comminuted meat products (Youssef and Barbut, 2011).

Furthermore, the addition of whey proteins affects the taste and improves the gelation, used in the production of edible sausage casings. They are also used in the finishing of semi-products, as their addition has a positive effect on the adhesion of batter to portions of meat, poultry, or fish. They also exhibit antioxidant activity (this refers to the oxidation of fat in pork meat, in salmon meat, or in products rich in lipids) (Prabhu, 2006; Youssef and Barbut, 2011).

2.9.4 Beverage products

Beverages based on whey or partially based on whey are numerous from the technological point of view, whey drinks can be made from natural whey or whey permeate or refined whey or ultra-filtered whey or whey concentrate. These may be classified as under the following heading (Kamble, 2009):

2.9.4.1 Whey beverages based on fruit juices

Mixtures of fruit juices and unprocessed or deproteinated whey or UF permeates are the most common types of whey drinks to be manufactured. These products usually fulfill a role similar to typical fruit juices, including breakfast-type beverage, healthful fruit juice, snack-type drink, or drinks with a healthful image as a source of vitamins. The main two basic ingredients are typically liquid whey and liquid fruit juice or, more likely, fruit juice concentrate. The flavors used in these beverages most often include citrus fruits (mainly orange, followed by lemon, rarely grapefruit), as well as mango, passion fruit, pear, apple, strawberry, raspberry or fruit juice combinations with exotic descriptive terms (‘tropic mix’, ‘multifruit’, ‘fruit nectar’, etc.) since they have proved to be very efficient in covering up the undesirable odor of cooked milk and salty-sour flavor of fresh whey. The additions of berries which are known as a good source of iron and antioxidants have proved to be very useful (Duric *et al.*, 2004).

whey based mango herbal beverage (water 48% and panner whey 32%) with 12 per cent mango pulp, 8% sugar and lemon grass 1.5% obtained highest sensory score (Sahu *et al.*, 2005). Whey based mango RTS beverage with 70% whey and 30% mango juice, scored at maximum for all sensory attributes viz., color, flavour, taste and overall acceptability (Sakhale *et al*., 2007). Custard apple whey beverage with combination of whey: pulp 90:10, 85:15, and 80:20. Out of these combinations, 85:15 (whey: pulp) with 10% sugar was the best combination and obtained maximum scored (Ingale *et al.*, 2009). Organoleptic evaluation showed that whey based Pineapple fruit juice beverage prepared by using whey & water (70:30) and 20 % fruit juice was found to be more acceptable as compared to sample prepared by using different combination of whey, water and fruit juice concentration, as it gave good Flavor & Taste and Overall acceptability (Devi *et al.*, 2017).

2.9.4.2 Dairy-type whey beverages

There are two basic types of dairy beverages:

1. Unfermented milk and milk derivatives, milk shakes, flavoured milk and similar products (based on skim, partially skimmed, fullfat or even fat-enriched products);
2. Fermented products such as sour milk, buttermilk, kefir and other similar cultured dairy beverages.

The main difference is the pH; which is to the neutral range (pH 6.2–6.5) in the former, while most fermented dairy products and acid whey are quite acidic, with the pH of 4.8–4.5 (Athanasiadis *et al.*, 2004).

Production of whey beverages with low alcohol content includes deproteinizing whey, whey concentration, fermentation of lactose (usually by yeast strains *Kluyveromyces fragilis* and *Saccharomyces lactis*) or addition of sucrose until reaching the desired alcohol content (0.5–1%), flavoring, sweetening and bottling. Some of noted beverages belonging to this category are 'Milone' obtained by fermentation with kefir culture and whey sparkling wine 'Serwovit' produced in Poland. One of the most famous whey beverages obtained by fermentation with *Lactobacillus rhamnosus* is 'Gefilus' which is being produced in Finland. In some recent studies whey was fermented by using following strains *Lactobaciluus acidophilus, Lactobacillus delbrueckiis* sp. *bulgaricus, Streptococcus thermophillus, Lactobacillus rhamnosus* and *Bifidobacterium animalis* sp. *lactis* (Almeida *et al.*, 2008; Pescuma *et al.*, 2008).

Big attention has been paid to development of probiotic whey beverages, since beneficiary effects of probiotic strains on human health like lowering cholesterol level in blood, improving lactose metabolism, lowering blood pressure, anticancerogenic properties and immune system stimulation are known for a long period of time. Fermentations with probiotic strains *Lactobacillus reuteri* and *Bifidobacterium* managed to produce an acceptable probiotic whey beverage with addition of sugar and Pectins (Mendoza *et al.*, 2007; Shah, 2007).

2.9.4.3 Whey based thirst-quenching carbonated beverages

Now-a-days the addition of CO_2 combined with fruit added in it to overcome the undesirable flavor and odor of cooked milk. The most typical product representing this type of whey beverage is the Swiss ‘Rivella’, a sparkling, crystal clear infusion of alpine herbs prepared by fermenting deproteinized whey with lactic acid bacteria, filtering, condensing to a 7:1 concentrate, adding sugar and flavoring, refiltering, diluting and carbonating after which the product was bottled and pasteurized (Philippopoulos and Papadakis, 2001).Other beverage Bodrost, (an alcoholic beer-like beverage), Tai (a soft drink fortified with whey protein concentrate to contain 1.5% proteins), Big MR, Frusighurt, and Taksi (Chavan *et al.*, 2015).

2.9.4.4 Alcoholic whey beverages

Since lactose is the main constituent (70%) of whey dry matter, whey is a very good material for production of alcoholic beverages (Jelicic *et al.*, 2008). Alcoholic whey beverages are divided into:

1. Low alcohol beverage (≤ 1.5%)
2. Whey beer
3. Whey wine

2.9.4.4.1 Low alcohol beverage

Production of whey beverages with low alcohol content includes deproteinizing whey, whey concentration, fermentation of lactose (usually by yeast strains *Kluyveromyces fragilis* and *Saccharomyces lactis*) or addition of sucrose until reaching the desired alcohol content (0.5 - 1%), flavoring, sweetening and bottling. Thereby, a certain amount of lactose is being transformed to lactic acid which gives a refreshing sour taste to the end product, while the rest ferments to alcohol (Jelicic *et al.*, 2008).

2.9.4.4.2 Whey beer

Whey beer can be produced with or without addition of malt; it can be fortified with minerals or can contain starch hydrolysates and vitamins. Some of problems that can occur here are presence of milk fat since which can cause loss of beer foam, undesirable odor and taste due to low solubility of whey proteins and inability of beer yeasts to ferment lactose (Jelicic *et al.*, 2008).

2.9.4.4.3 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 (Jelicic *et al.*, 2008).

2.9.5 Other application of whey

2.9.5.1 Infants formula

Whey preparations, being a source of high quality protein and of active peptides, are widely used by manufacturers of baby foods. It also increases amount of amino acids in infant formulas especially lysine, methionine and threonine (Murphy *et al.*, 2015).

2.9.5.2 Media

Whey preparations are also used as media in the microencapsulation of sensitive food ingredients which are fragrances, dyes, or various types of probiotic bacteria (e.g. *Bifidobacterium*-BB-12). The use of whey protein supports protection of active ingredients and prevents the loss of their properties in the long-term. Additionally, after the microencapsulation process, a product is obtained in the form of powder or granules, which allows for controlled release of the component and new uses for food additives (Pinto *et al.*, 2015).

2.9.5.3 Edible coating

Another protective use of whey proteins is as an edible coating for food. Coating based on whey preparations is characterized by good mechanical properties, a good barrier against lipids, aromatics and, especially, oxygen. Fruit and vegetables can be coated successfully with whey protein (Pintado *et al.*, 2009).

2.10 Need for development of whey based beverage

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

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

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

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

1. Whey is having a broad range of solubility i.e. from pH 3-8.
2. Whey is having a bland flavor and on higher temperature, casein they can act as carrier for the aroma compounds.
3. Buffering capacity of whey can be explored for survival of probiotic bacteria in the gastro intestinal tract.
4. Addition of whey improves the ‘mouthfeel’ of the drink by increasing the viscosity of the beverage.

5. Whey can be also used to solve the problems associated with cloudiness of tropical fruit juices and produce a cloud stable juice.

2.11 Use of fruits in preparation of whey beverage

Singh *et al*. (2014) prepared guava blended beverage by using whey and guava pulp in the proportion 67.5:20 and was found to be more acceptable and of good quality in terms of protein and vitamin C content.

A delicious and nutritious RTS beverage from the ripe banana juice and milk whey using *Mentha arvensis (*mint*)* extract as a natural flavoring agent was prepared by (Dhamsaniya and Varshney, 2013). The proportion of banana juice, *M. arvensis* extract and milk whey was varied from 5-15 ml, 1-5 ml and 72-86 ml per 100 ml of the prepared beverage, respectively. The screening of beverage samples was done on the basis of their physicochemical and sensory characteristics. Whey-banana-RTS beverage prepared having 77 ml milk whey+15 ml banana juices+3 ml M. *arvensis* extract+8 g sugar powder per 100 ml of the prepared beverage was found to be acceptable and recommended for large scale production. The other researches in whey beverages is presented in Table 2.4.

Table 2.4 Others examples of fruits use in whey beverage

No.	Types of whey	Products	References
1.	Channa whey	Mango RTS beverage	(Sikdar and Ghatak, 2003)
2.	*Paneer* whey	Kokum juice	(Rupnar, 2006)
		Pineapple juice	(Kesarkar, 2002)
		Guava whey beverage	(Singh and Kapoor, 1999)
3.	Cheese whey	Kinnow juice beverage	(Khamrui, 1998)
		Chocolate drink	(Singh, 1991)
4.	Chakka whey	Fruit jelly	(Joshi and Waghmare, 1985)
		Mango whey beverage	(Shende, 2006)

2.12 Use of pineapple in beverage

2.12.1 Introduction

Pineapple (*Ananas comosus* (L.) *Merr.* Family: Bromeliaceae) is one of the most important commercial fruit crops in the world. It is known as the queen of fruits due to its excellent flavour and taste (Baruwa, 2013). Pineapple is the third most important tropical fruit in the world after Banana and Citrus. Pineapples are consumed or served fresh, cooked, juiced and can be preserved. This fruit is highly perishable and seasonal. Mature fruit contains 14% of sugar; a protein digesting enzyme, bromelin, and good amount of citric acid, malic acid, vitamin A and B . Thailand, Philippines, Brazil and China are the main pineapple producers in the world supplying nearly 50% of the total output (Sabahelkhier *et al.*, 2010). Various food items like squash, syrup, jelly are produced from pineapple. Vinegar, alcohol, citric acid, calcium citrate etc. are also produced from pineapple. Bromelain as a proteolytic digestive enzyme found in pineapple When taken with meals, bromelin aids in the digestion of proteins, working to break proteins down into amino acids (Kader *et al.*, 2010).

2.12.2 Nutritional value

Pineapple is a wonderful tropical fruit having exceptional juiciness, vibrant tropical flavor and immense health benefits. Pineapple contains considerable amount of calcium, potassium, vitamin C, carbohydrates, crude fiber, water and different minerals that is good for the digestive system and helps in maintaining ideal weight and balanced nutrition. It contains 10-25 mg of vitamin. Pineapple juice contains ascorbic acid (Fights against bacterial and viral infections) which is an effective antioxidant and helps the body absorb iron. The chemical composition of pineapple pulp juice is presented in Table 2.5. Half a cup of pineapple juice provides 50% of an adult's daily recommended amount of vitamin C (Sabahelkhier *et al.*, 2010).

Table 2.5 Chemical composition of pineapple pulp juice

Parameters	Pineapple pulp
Moisture (%)	87.3
Ash content (mg/100 g)	1.8
Total soluble solids (%)	13.3
Crude fiber (g/100 g)	0.41
Total sugars (%)	18.6
Reducing sugars (%)	11.2
Non reducing sugars (%)	7.4
Titratable acidity (%)	2.03
Ascorbic acid (mg/100 g)	21.5

Source: Farid *et al.* (2015)

2.12.3 Uses as Food

Pineapple fruits exhibit high moisture, high sugars, soluble solid content ascorbic acid and low crude fiber. Thus pineapple can be used as supplementary nutritional fruit for good personal health. The pineapple fruits are normally consumed fresh or as fresh pineapple juice. Field ripe fruits are best for eating fresh, and it is only necessary to remove the crown, rind, eyes and core. Pineapple may be consumed fresh, canned, juiced, and are found in a wide array of food stuffs - dessert, fruit salad, jam, yogurt, ice cream, candy, and as a complement to meat dishes. The pineapple does not lend itself well to freezing, as it tends to develop off flavors. Canned pineapple is consumed throughout the world (Hemalatha and Anbuselvi, 2013).

2.12.4 Medicinal value

Pineapple fruits are an excellent source of vitamins and minerals. One healthy ripe pineapple fruit can supply about 16.2% of daily requirement for vitamin C. Vitamin C is the body's primary water soluble antioxidant, against free radicals that attack and damage normal cells. A powerful antioxidant, vitamin C supports the formation of collagen in bones, blood vessels, cartilage and muscle, as well as the absorption of iron. Vitamin C also retards the development of urinary tract infections during pregnancy and reduces the risk of certain cancers. Malic acid boosts immunity; promotes smooth, firm skin; helps maintain oral health; and reduces the risk of toxic metal poisoning. Pineapple is a digestive aid and a natural anti-inflammatory fruit. Fresh pineapples are rich in bromelain used for tenderizing meat. Bromelain supplements are particularly popular among athletes for treating all sorts of physical aches and injuries (Anon., 2015c).

Pineapples are rich in manganese, a trace mineral that is needed for body to build bone and connective tissues. One cup of pineapple provides 73% of the daily recommended amount of manganese. The benefits of pineapple can affect the growth of bones in young people and the strengthening of bones in older people. Pineapple juice's high manganese content means it is a good choice for boosting fertility through sperm quality. Bromelain has demonstrated significant anti-inflammatory effects, reducing swelling in inflammatory conditions such as acute sinusitis, sore throat, arthritis and gout and speeding recovery from injuries and surgery. Pineapple enzymes have been used with success to treat rheumatoid arthritis and to speed tissue repair as a result of injuries, diabetic ulcers and general surgery. Pineapple reduces blood clotting and helps remove plaque from arterial walls. Pineapple enzymes may improve circulation in those with narrowed arteries, such as angina sufferers. Pineapples are used to help cure bronquitis and throat infections. Pineapple is an excellent cerebral toner; it combats loss of memory, sadness and melancholy. Pineapple is effective in getting rid of intestinal worms and also keeps the intestines and kidneys clean. It helps prevent gum disease and also prevents the formation of plaque, thus keeping the teeth healthy (Farid *et al.*, 2015).

2.13 Microbiology of milk and milk products

Milk is virtually sterile when it is synthesized in a healthy cow's udder (mammary gland). Cows are natural reservoirs of bacteria. Many of these bacteria are not harmful to humans, but some may be harmful to humans even though the cows are not affected and appear healthy. Milk may become contaminated with bacteria during or after milking. The mammary glands of cows can become inflamed due to a bacterial infection called mastitis. During a mastitis infection, very high numbers of bacteria present can be in the udder and in the milk. Some disease causing organisms (pathogens) can be shed through cow feces and may contaminate the outside of the udder and teats, the farm environment and the milking equipment. Although optimal growth conditions for bacteria are different for different organisms, milk contains important nutritional components for mammal growth, and, therefore, it is also an ideal medium for the growth of many different bacteria. Temperature plays an important role in bacterial growth. Many bacteria prefer to grow at body temperature (86-98°F, 30-37°C), but will grow at lower temperatures (such as refrigerator temperature) at slower rates (Anon., 2015b).

The area of dairy microbiology is large and diverse. The bacteria present in dairy products may cause disease or spoilage. Human illness from milk borne pathogens is usually associated with consumption of raw milk or products made from raw milk such as fresh cheeses. In the past 20 years, foodborne illnesses from dairy product consumption have been predominantly associated with *Salmonella enterica, Listeria monocytogenes, Campylobacter jejuni,* and *Escherichia coli*. Because there is a risk of pathogen contamination in milk produced from healthy cows under sanitary milk conditions, pasteurization of milk prior to consumption will destroy pathogens and provide protection for illness associated with consumption of dangerous microbes. Occasionally, human illness has been linked to pasteurized milk products but these cases usually have been a result of contamination of the product after pasteurization or improper pasteurization (Anon., 2015b).

Part III

Materials and methods

3.1 Materials

3.1.1 Milk

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

3.1.2 Sugar

Crystal sugar was obtained from local store of Dharan.

3.1.3 Pineapple

Pineapple was obtained from the Amarhat fruit store of Dharan. It was washed thoroughly in the water to remove foreign materials and pulping was done in pulper. Later detailed study was done.

3.1.4 Bottle

PET bottles of 250 ml capacity purchased from the local market of Dharan.

3.1.5 Chemicals

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 SeO_2, 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
4. Phenolphthalein
5. Conc. Sulphuric Acid (H_2SO_4)

6. Conc. nitric Acid (HNO_3)
7. Sodium hydroxide (NaOH)
8. Oxalic acid
9. Dextrose
10. Meta-phosphoric acid
11. Indophenol
12. Sodium carbonate
13. Carraz-I and carraz-II

3.1.6 Glassware and apparatus

The glassware and 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
2. Muffle furnace
3. Desiccator
4. Kjeldahl digestion apparatus
5. pH meter
6. Thermometer
7. Weighing balance (LC: 0.001g)
8. Refractometer

3.2 Methodologies

3.2.1 Preparation of whey

Standardized milk was used for the preparation of good quality whey. Milk was heated to 80°C and when temperature decreased to 40-45°C, 2% citric acid solution (40-50 ml per liter of milk) gradually added followed by continuous stirring which resulted in complete coagulation of milk protein (casein). The liquid (whey) was filtered using muslin cloth and stored for further use. The process flowchart for the preparation of whey is presented in Fig. 3.1

Standard milk

↓

Heated to 80°C
(Pasteurization temperature)

↓

Cooled to (40-45°C)

↓

Coagulated with 2% citric acid

↓

Filtered in muslin cloth

↓

Whey

↓

Stored at refrigerator at (7±1°C)

Fig. 3.1 Flowchart for preparation of whey

Source: Awsi and Dorcus (2012)

3.2.2 Preparation of pineapple juice

Ripe pineapples were selected, washed with water peeled, the crown and stem were removed and the peeled fruits were sliced using slicer. The prepared slices were crushed and juice extracted by juice extractor. The juice was strained through the muslin cloth. The juice was kept at a refrigeration temperature (7±1°C) until used. The extraction procedure of clear pineapple juice is given in Fig. 3.2.

Pineapple
(Fresh and ripened)

↓

Peeled and eyes removed

↓

Stem, crown removed and washed with clean water

↓

Sliced into pieces

↓

Juice extraction
(Mechanical juice extractor)

↓

Filtered in muslin cloth

↓

Pineapple juice

↓

Stored at refrigerator at (7±1°C)

Fig. 3.2 Flowchart for preparation of pineapple juice

Source: Revathi and Singh (2014)

3.2.3 Preparation of whey pineapple beverage

Different formulation of whey beverage was prepared by heating whey at 45°C with addition of sugar, pineapple pulp juice, as shown in flow chart. After proper mixing it was filtered through clean muslin cloth to obtain whey-pineapple beverage, filled into PET bottle of 250 ml capacity and it was crown corked. After crown corking it was heated at 85-90°C for 15min and immediately cooled and subjected to sensory evaluation to find out best formulation which was stored in refrigeration and ambient temperature for further study. The complete process flowchart for the preparation of beverage is presented in Fig. 3.3.

Clarified filtered Whey
(100%, 94%, 88%, 75%, 69%, 63%, 57%, 50%)

↓

Heated to 45°C

↓

Pineapple pulp juice → Mixing ← Grinded sugar
(13% TSS maintained)

↓

Filtration through clean muslin cloth

↓

Whey pineapple beverage collection

↓

Bottling (in 250 ml PET bottle)

↓

In bottle pasteurization (88℃ for 15 min)

↓

Cooling to room temperature

↓

Sensory evaluation

↓

Selection of best formulation

↓

Storage at refrigeration temperature (7±1°C)

↓

Analysis of beverage at every 5 days interval up to 20 days
(TPC, yeast and mold count, pH, acidity, TSS, reducing sugar, ascorbic acid)

Fig. 3.3 Flowchart for preparation of whey beverage

Source: Revathi and Singh (2014)

3.3 Analytical procedure

3.3.1 Physical and chemical examination

Different parameter like total solid, total soluble solid, fat content, acidity, pH, acidity, ascorbic acid and microbiological analysis was done for raw whey, pineapple pulp and prepared beverage.

3.3.1.1 Total soluble solid (TSS)

Total soluble solids were determined with hand refractometer (0-30) and values were expressed as °Brix according to Ranganna (2000).

3.3.1.2 Fat content

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

3.3.1.3 Total and reducing sugar content

It was determined by Lane and Enyon’s method according to Ranganna (2000).

3.3.1.4 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 (2000).

3.3.1.5 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.

3.3.1.6 Ascorbic acid

Ascorbic acid content in the sample was determined by the visual titration method using the dye 2, 6- dichlorophenolindophenol according to Ranganna (2000).

3.3.1.7 Protein content

Protein content in the sample was determined by kjeldahl method (by estimating nitrogen content) according to Ranganna (2000).

3.3.1.8 Ash content

Total ash content of the samples was determined by using dry ashing according to Ranganna (2000).

3.3.1.9 Total solids

It was determined by subtracting the moisture from the 100 according to Ranganna (2000)

3.3.1.10 Moisture content

It was determined according to Ranganna (2000).

3.3.2 Experimental design

Different recipe formulation was formulated using Design expert.

Table 3.1 Different recipe samples

S.N.	Code	Whey (ml)	Pineapple juice (ml)
1.	A (Control)	100	0
2.	B	93.7	6.3
3.	C	87.4	12.6
4.	D	75	25
5.	E	68.9	31.1
6.	F	62.6	37.4
7.	G	56.3	43.7
8.	H	50	50

3.3.3 Sensory evaluation

The coded sample of the beverage were sensorically evaluated for appearance, flavor, texture, juiciness, taste and overall palatability on 9 point hedonic scale. The panelists were given instruction to give 9 points to extremely liked and 1 points to the extremely disliked point sample. The coded samples were randomly presented. For the above hedonic rating test, semi trained panelist of B.Tech 4th year and teachers of Central Campus of Technology were taken. The specimen card for sensory evaluation is shown in Appendix A. The panelists were untrained. Differences in the quality were determined by statistical analysis according to Ranganna (2000).

3.3.4 Statistical analysis

The analyses were carried out in triplicate. Statistical calculations were performed in Microsoft office Excel 2013. All the data obtained in this experiment were analyzed for significance by Analysis of Variance (ANOVA) using the statistical program known as Genstat Release 12.1 (2009). From this, means were compared using Fisher‘s protected LSD (Least Significance Difference) at 5% level of significance (Payne, 2007).

3.3.5 Microbiological analysis

Total Plate Count (TPC) was determined by pour plate technique on Plate Count Agar (PCA) medium (incubated at 30℃/48 h). Yeasts and molds count was determined by pour plate technique on Potato Dextrose Agar (PDA) medium (incubated at 25-27℃/48-72 h) (AOAC, 2005).

3.3.6 Storage studies

Whey-pineapple beverage were aseptically filled in the PET bottles. The bottles containing beverages were stored at refrigerated condition (7±1°C) and normal condition (20-25°C) for 20 days. Samples were drawn at intervals of 5 days and evaluated for physico-chemical properties (TSS, acidity, pH, reducing sugar and ascorbic acid) and microbiological qualities (TPC and yeast and mold count).

Part IV

Results and discussion

Whey pineapple beverage was prepared at the laboratory of Central Campus of Technology for the present study. The pineapple juice and *paneer* whey were the major ingredient. Pineapple juice was extracted using juice extractor while the *paneer* whey was obtained by 2% citric acid coagulation. The extracted raw materials were filtered and different formulated sample was prepared and pasteurized at 88°C for about 15 min. The samples were analyzed through sensory evaluation and best scored sample was stored for 20 days at refrigerated (7±1°C) and normal (25±1°C) for study of storage stability.

4.1 Analysis of raw material

In the preparation of whey based pineapple beverage, whey and pineapple pulp were the major ingredient which were analyzed for their chemical composition presented as in Table 4.1.

Table 4.1 Chemical composition of Raw materials

S.N.	Parameter	Whey	Pineapple
1.	T.S.S (°Bx)	5.50 (0.10)	11.9 (0.1)
2.	Moisture (%)	92.92 (0.02)	88.22 (0.015275)
3.	pH	5.18 (0.01)	3.76 (0.01)
4.	Acidity (%)	0.21 (0.011547)	0.67 (0.01)
5.	Reducing sugar (%)	4.67 (0.011547)	7.79 (0.017320)
6.	Non-reducing sugar (%)	4.32 (0.02)	7.43 (0.03)
7.	Total sugar (%)	8.99 (0.030550)	15.22 (0.017320)
8.	Proteins (%)	0.48 (0.005774)	0.47 (0.01)
9.	Ash (%)	0.57 (0.005774)	0.18 (0.01)
10.	Fat (%)	0.46 (0.005774)	-
11.	Ascorbic acid (mg/100 g)	-	41.7 (0.4)
12.	Total solids (%)	7.08 (0.005773)	11.80 (0.017320)

Figures are the means of triplicate. Values in the parentheses are standard deviation.

This chemical composition data for the fresh whey and pineapple juice was found to be a bit different than the data obtained by (Rupnar, 2006) and (Awsi and Dorcus, 2012) for the *paneer* whey and pineapple respectively. This may be due to the variation of species, variety of the pineapple produced. Also variation on the whey composition may be due to the variation in the milk constitute obtained and produced in the different parts of the world.

4.2 Effect of formulations on sensory characteristics

Eight different samples of varying proportion of whey and pineapple juice was taken and coded as A, B, C, D, E, F, G and H respectively. The TSS of the samples was kept constant *viz.* 13°Bx. Then the formulations having different proportion of whey and pineapple juice was subjected to sensory evaluation.

4.2.1 Appearance

The mean sensory score for the appearance of the beverage of eight samples A, B, C, D, E, F, G and H were determined to be 5.4, 5.0, 5.6, 5.6, 6.7, 7.4, 7.0 and 7.1 respectively (Fig. 4.1). Statistical analysis showed that there was significant effect of proportion variation of whey and beverage on the appearance of beverage at 5% level of significance (Appendix C). LSD shows that products A&B&C&D and G&H were not significantly different while the others product samples were found to be significantly different from each other. The appearance of sample F was found to be superior to other with highest mean score 7.4.

Devi *et al.* (2017) reported increase in the sensory score for the appearance of beverage while increasing the pineapple juice content in the preparation of whey based pineapple juice. Nagadevi and Puraikalan (2015) and Zaman *et al.* (2016) also reported similar result.

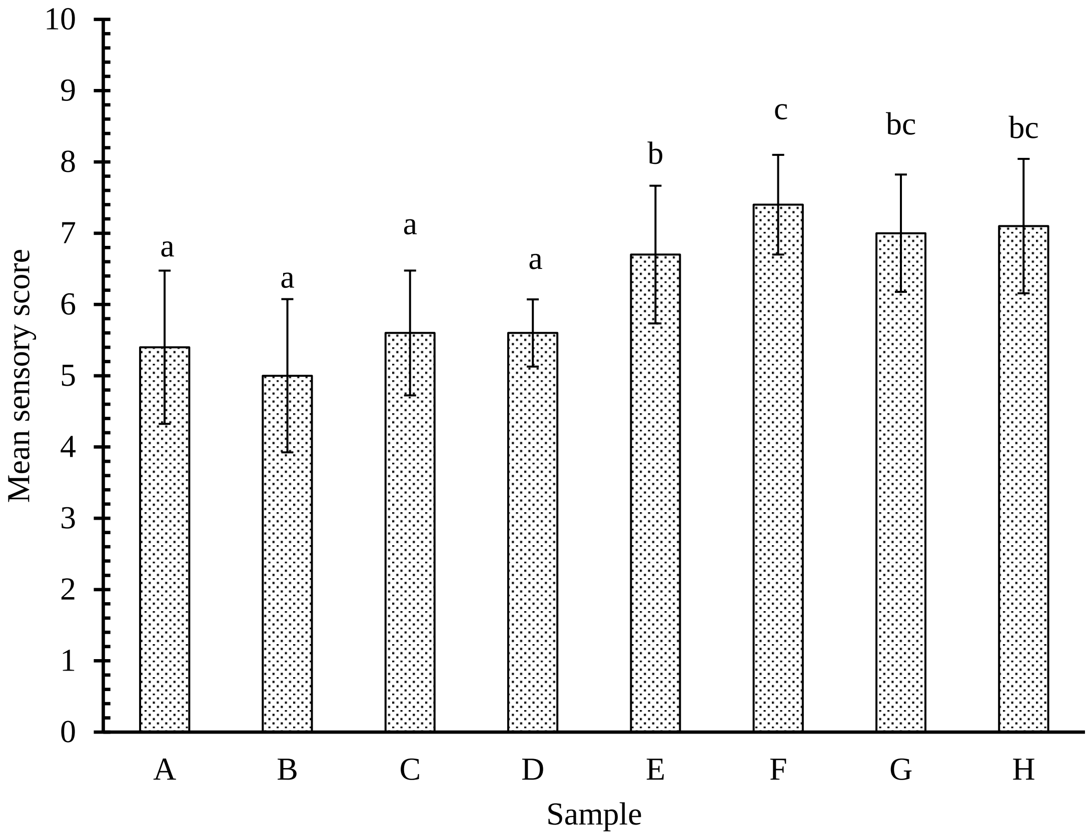

Fig. 4.1 Mean sensory score for appearance

Fig 4.1 represents the mean sensory scores for appearance of whey-pineapple 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.

4.2.2 Color

From the sensory evaluation for the color of the eight different samples A, B, C, D, E, F, G and H the mean sensory score were found to be 5.4, 5.1, 5.4, 5.8, 7.5, 6.9 and 7.1 respectively (Fig. 4.2) . LSD showed that products A&B&C&D and E&F&G&H were not significantly different while the others samples were found significantly different at 5% level of significance **(**Appendix C).

Devi *et al.* (2017) reported increase in the sensory score for the color of beverage while increasing the pineapple juice content in the preparation of whey based pineapple juice. Zaman *et al.* (2016) and Nagadevi and Puraikalan (2015) also reported similar result of increasing score for the color.

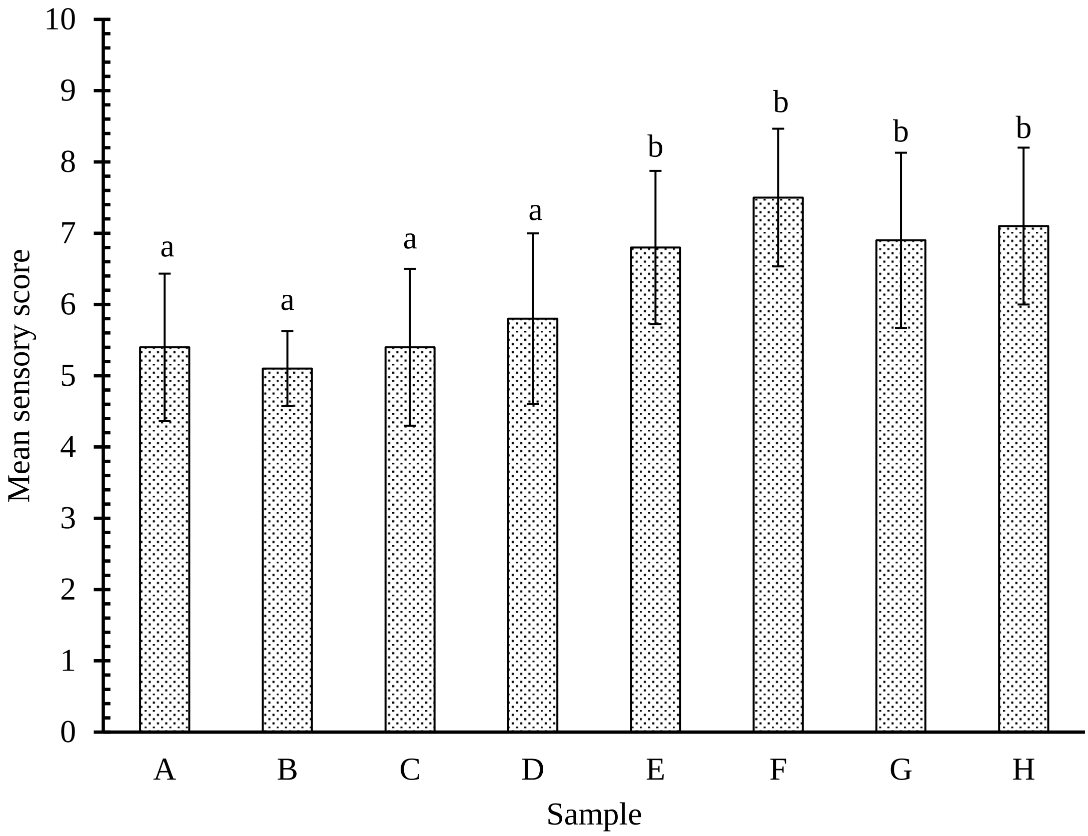

Fig. 4.2 Mean sensory score for color

Fig 4.2 represents the mean sensory scores for color of whey-pineapple 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 Flavor

Eight samples A, B, C, D, E, F, G and H of different proportion of juice was subjected to sensory evaluation and the mean sensory score were observed to be 5.6, 5.3, 5.5, 5.9, 6.5, 7.0, 6.7 and 7.3 respectively (Fig. 4.3). Samples A&B&C and F&G were not significantly different but other samples were found to be significantly different at 5% level of significance (Appendix C). The sample H (50% whey + 50% pineapple juice) was found to be superior in flavor with mean sensory score of 7.3.

Devi *et al.* (2017) reported increase in the sensory score for the flavor of beverage while increasing the pineapple juice in the preparation of whey based pineapple juice. Nagadevi and Puraikalan (2015) and Zaman *et al.* (2016) found flavor score increased on increasing the fruit juice content.

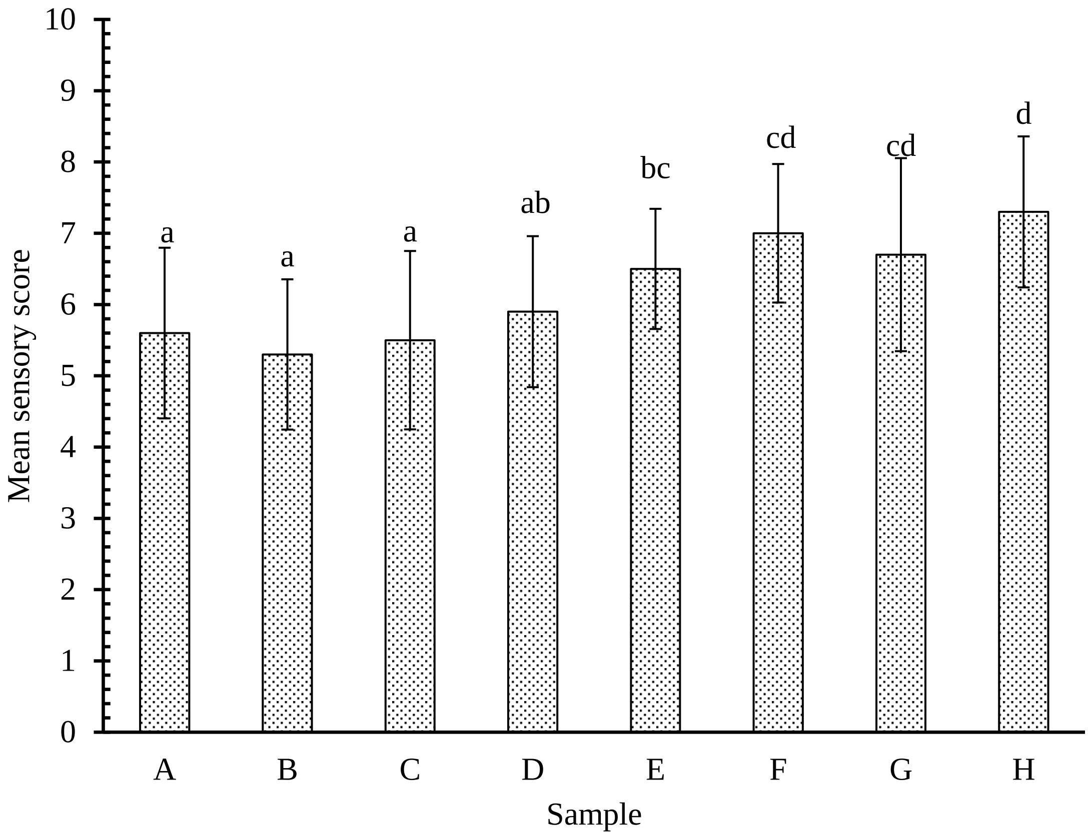

Fig. 4.3 Mean sensory score for flavor

Fig 4.3 represents the mean sensory scores for flavor of whey-pineapple 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.

4.2.4 Taste

Like other parameters, juice variation has significant effect on the taste at 5% level of significance. The mean sensory score, from sensory evaluation, given to the different samples A, B, C, D, E, F, G and H were found to be 5.4, 5.5, 5.7, 6.3, 6.4, 7.2, 6.6 and 7.2 respectively (Fig.4.4). The sample F&H were not significantly different while others samples were found to be significantly different from the statistical analysis (Appendix C).

Devi *et al.* (2017) reported increase in the sensory score for the taste of beverage while increasing the pineapple juice content in the preparation of whey based pineapple juice.

Zaman *et al.* (2016) and Nagadevi and Puraikalan (2015)stated that the taste was widely accepted for the increased juice content.

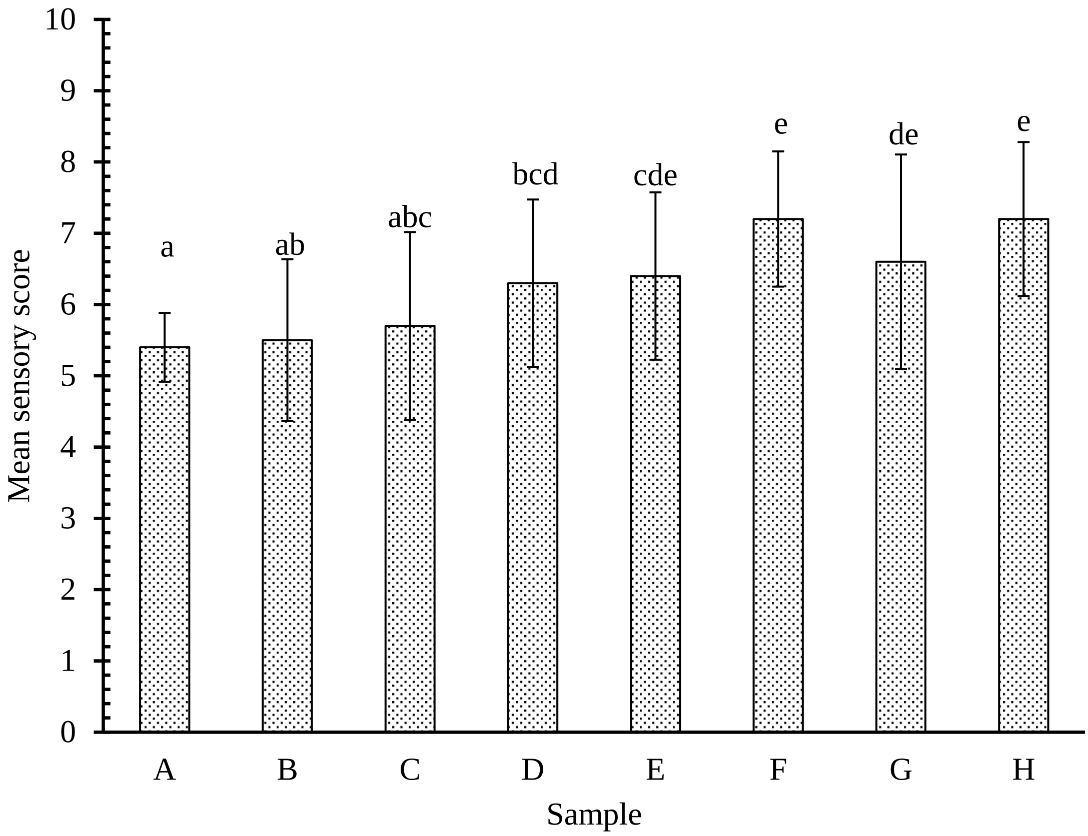

Fig. 4.4 Mean sensory score for taste

Fig 4.4 represents the mean sensory scores for taste of whey-pineapple 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 acceptability

Overall acceptance is the main factor for accepting the sample. The mean sensory score from the sensory evaluation of the eight different samples A, B, C, D, E, F, G and H were found to be 5.4, 5.3, 5.4, 6.2, 6.5, 6.8, 6.7 and 7.8 respectively (Fig.4.5). Samples A&B&C and D&E&F&G were not significantly different while others samples were found to be significantly different at 5% level of significance (Appendix C). The sample H in sensory evaluation (overall acceptance) was scored the highest i.e. 7.8.

Devi *et al.* (2017) reported increase in the sensory score for the overall acceptability of beverage while increasing the pineapple juice content in the preparation of whey based pineapple juice. Nagadevi and Puraikalan (2015) and Zaman *et al.* (2016) concluded the similar result stating that the overall acceptability of the product with increased fruit juice content scored best.

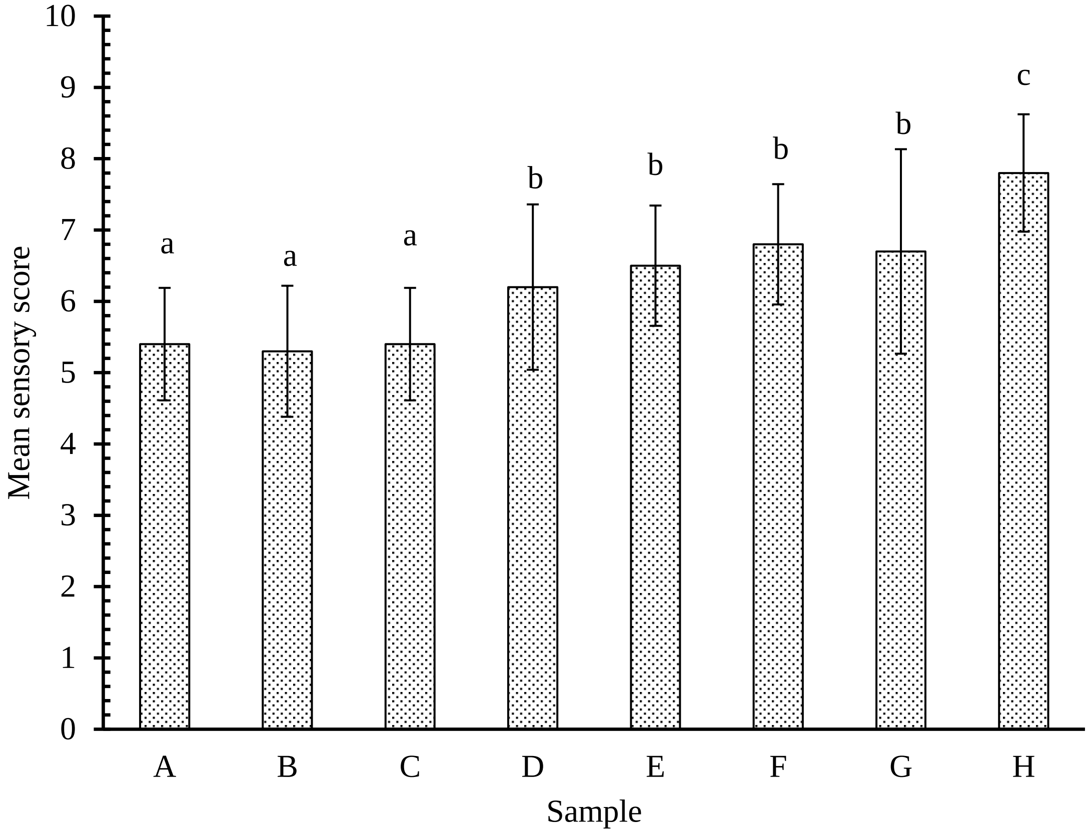

Fig. 4.5 Mean sensory score for overall acceptance

Fig 4.5 represents the mean sensory scores for overall acceptance of whey-pineapple 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 composition of final product (sample code-H)

The sample H with whey 50% (V/V) and pineapple juice 50% (V/V) is rated superior in the sensory evaluation. Pineapple bland flavor and pineapple taste on the beverage was accepted and rated superior. The chemical composition of the final product (sensorily scored superior) by the sensory panelist is presented in Table 4.3.

Table 4.3 Chemical composition of final product

S.N.	Parameter	Final product
1.	T.S.S (°Bx)	12.83 (0.06)
2.	Moisture (%)	86.67 (0.02)
3.	pH	4.20 (0.02)
4.	Acidity (%)	0.46 (0.01)
5.	Reducing sugar (%)	5.17 (0.01)
6.	Non-reducing sugar (%)	10.60 (0.1)
7.	Total sugar (%)	15.77 (0.11)
8.	Proteins (%)	0.42 (0.01)
9.	Ash (%)	0.32 (0.01)
10.	Fat (%)	0.22 (0.02)
11.	Ascorbic acid (mg/100g)	26.70 (0.7)
12.	Total solids (%)	13.33 (0.01)

Figures are the means of triplicate. Values in the parentheses are standard deviation.

4.4 Storage study

Prepared whey pineapple beverage was stored for 20 days' time period at refrigerated (R) condition (7±1°C) and ambient (A) 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 was done. There was no any colony observed during initial storage period. During preparation of beverage heat treatment at 88°C for 15 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. The microbial analysis is tabulated in Table 4.5.

Table 4.5 Microbiological analysis of product

Days/ parameter	Day-0	Day-5		Day-10		Day-15		Day-20	
		A	R	A	R	A	R	A	R
TPC (cfu/ml)	0	0	0	0	0	$4{\times}10^3$	$3{\times}10^3$	$13{\times}10^3$	$6{\times}10^3$
Yeast and mold count (cfu/ml)	0	0	0	0	0	$2{\times}10^3$	$1{\times}10^3$	$9.5{\times}10^3$	$4.5{\times}10^3$

4.4.2 Chemical analysis

Effects of storage period on chemical composition (TSS, pH, titrable acidity, reducing sugar and ascorbic acid content) of whey pineapple beverage were analyzed. The changes in physicochemical properties during storage is presented in Table 4.4.

Table 4.4 Physicochemical properties during storage

Days/ parameter	Day-0	Day-5		Day-10		Day-15		Day-20	
	A/R	A	R	A	R	A	R	A	R
TSS(°Bx)	12.8 (0.10)	12.87 (0.06)	12.83 (0.06)	13.13 (0.06)	12.97 (0.06)	13.43 (0.06)	13.16 (0.06)	13.77 (0.06)	13.33 (0.06)
pH	4.43 (0.01)	4.41 (0.01)	4.42 (0.01)	4.38 (0.01)	4.40 (0.01)	4.35 (0.01)	4.38 (0.01)	4.31 (0.01)	4.36 (0.06)
Acidity (%)	0.41 (0.01)	0.43 (0.01)	0.42 (0.01)	0.45 (0.06)	0.43 (0.01)	0.52 (0.01)	0.46 (0.01)	0.60 (0.01)	0.49 (0.01)
Reducing Sugar (%)	5.09 (0.01)	5.17 (0.01)	5.22 (0.01)	5.31 (0.06)	5.38 (0.01)	5.39 (0.01)	5.61 (0.01)	5.44 (0.01)	5.71 (0.01)
Ascorbic Acid (mg/100gm)	26 (0.23)	24.8 (0.10)	26 (0.10)	22.83 (0.06)	25.67 (0.01)	21.20 (0.17)	24.80 (0.06)	17.36 (0.01)	22.30 (0.06)

Figures are the means of triplicate. Values in the parentheses are standard deviation.

4.4.2.1 Effect on TSS

A slight increase in the TSS of the whey-pineapple beverage during 20 days of storage at both storage temperatures was observed (Fig 4.6). TSS for the beverage ranged from 12.8 to 13.77°Bx (25±3°C) and 12.8–13.43°Bx (7±1°C) during the 20 days of storage (Table 4.4). Retention or minimum increase in TSS content of juice during storage is desirable for preservation of good juice quality (Bhardwaj and Pandey, 2011). Similar results were observed in the preparation of mixed fruit RTS (Bull *et al.*, 2004; Deka and Sethi, 2001). LSD showed that there was significant difference on TSS at 5% level of significance (Appendix D).

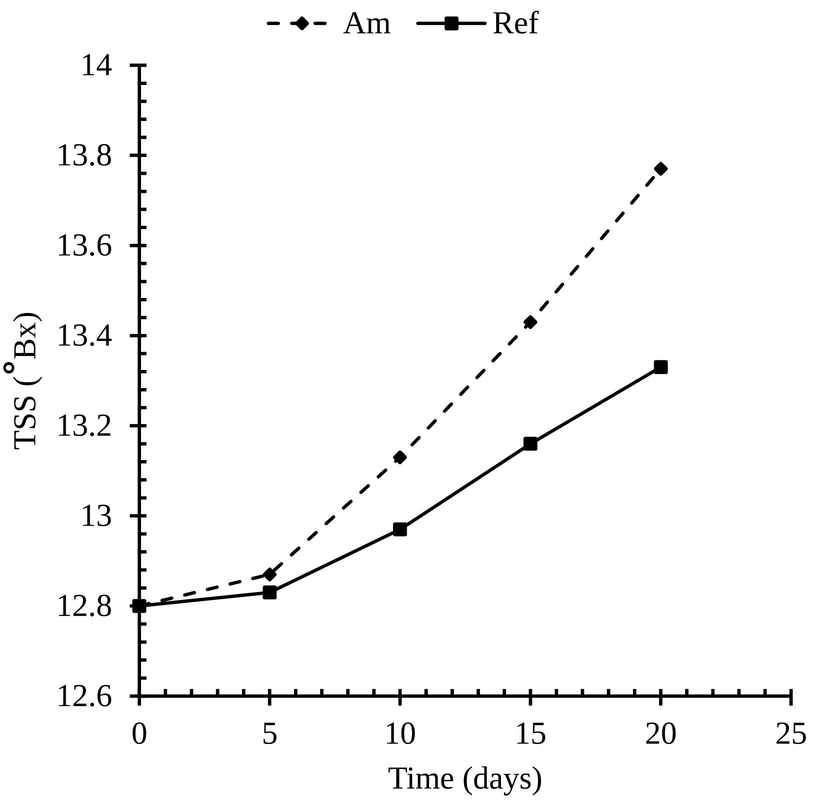

Fig. 4.6 Effect of storage time and temperature on TSS of prepared beverage

4.4.2.2 Effect on pH

There was a significant decrease in pH during storage (Table 4.4). pH is one of the important quality characteristics that describes the stability of bioactive compounds in fruit juice (Bhardwaj and Pandey, 2011). There was a slight decrease in pH during 20 days of storage (Fig 4.7) which ranged from 4.43 to 4.37 (7±1°C) and 4.43 to 4.31 (25±5◦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. Similar results were reported for a juice blend of bottle guard and basil leaves juice by Majumdar *et al.* (2011) and for pineapple juice blend with carrot and orange by Awsi and Dorcus (2012). LSD showed that there was significant difference on pH at 5% level of significance (Appendix D).

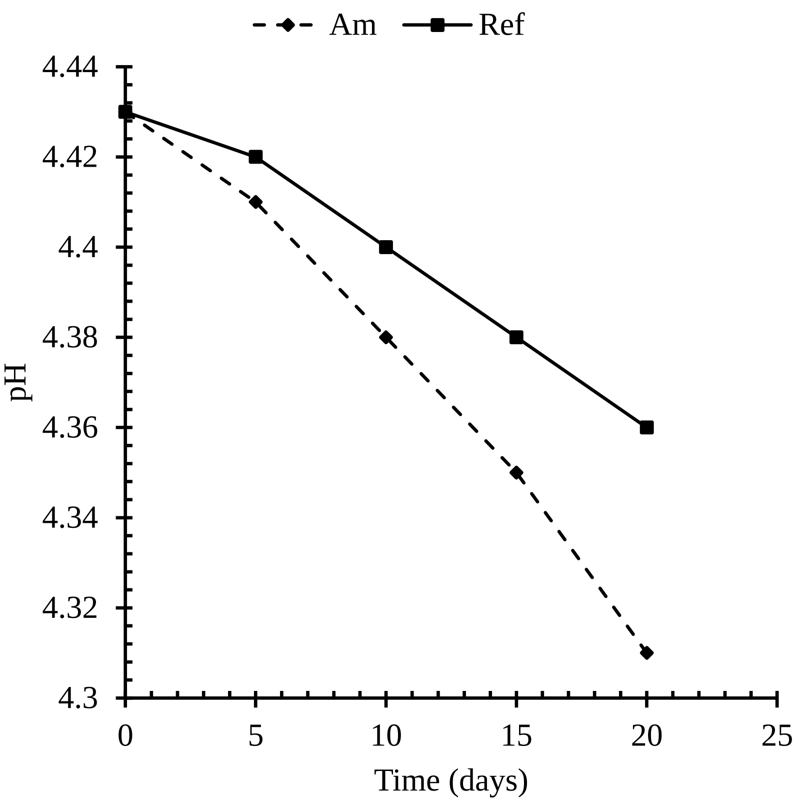

Fig. 4.7 Effect of storage time and temperature on pH of prepared beverage

4.4.2.3 Effect on titrable acidity

The effect of storage on the acidity of beverage is shown in (Fig 4.8). During 20 days 0f storage the acidity was found to have gradually increased from 0.41 to 0.60 (25±5 °C) and 0.41 to 0.49 (Table 4.4). The increase in acidity may be due to the production of organic acids and amino acids by the action of ascorbic acid on sugar and protein content of the beverages. Lactose and proteins are converted into lactic acid and amino acids leading to increase in acidity and decrease in pH of beverages (Kalra *et al.*, 1991). The results are in agreement with the findings reported in whey based banana herbal beverage (Yadav *et al.*, 2010) and also in whey based RTS from mango (Sakhal, 2012). LSD showed that there was significant difference on acidity at 5% level of significance (Appendix D).

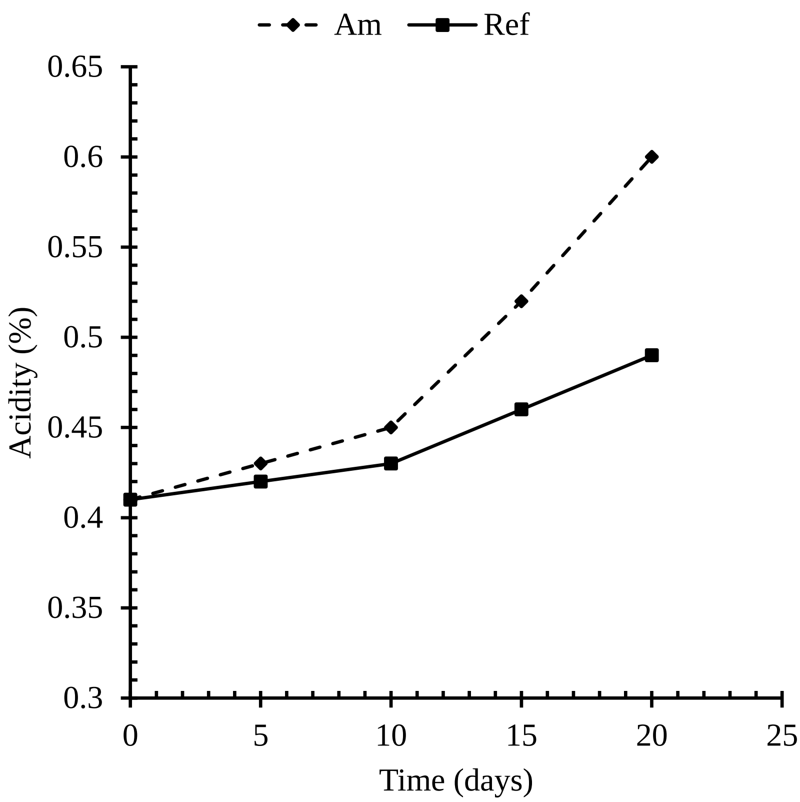

Fig. 4.8 Effect of storage time and temperature on acidity of prepared beverage

4.4.2.4 Effect on reducing sugar (RS)

Sugars are one of the most important constituents of fruit products, essential for and also act as a natural food preservative (Bhardwaj and Pandey 2011). The RS value for beverage ranged from 5.09 to 5.44 (25±5°C) and 5.09 to 5.71 (7±1 °C) during the 20 days of storage (Table 4.4). The results showed gradual increase in reducing sugar with increasing storage period (Fig 4.9). The sugar content of fruit juices usually increased with increased storage period. The increase was probably due to the hydrolysis of polysaccharides like cellulose, pectin, etc. and conversion into simple sugars (glucose, fructose) (Bhardwaj and Pandey, 2011). Increased reducing sugar content with increased storage time of a cucumber–melon functional drink (Kausar *et al.*, 2012) and bottled gourd–basil leave juice (Majumdar *et al.*, 2011). LSD showed that there was significant difference on reducing sugar at 5% level of significance (Appendix D).

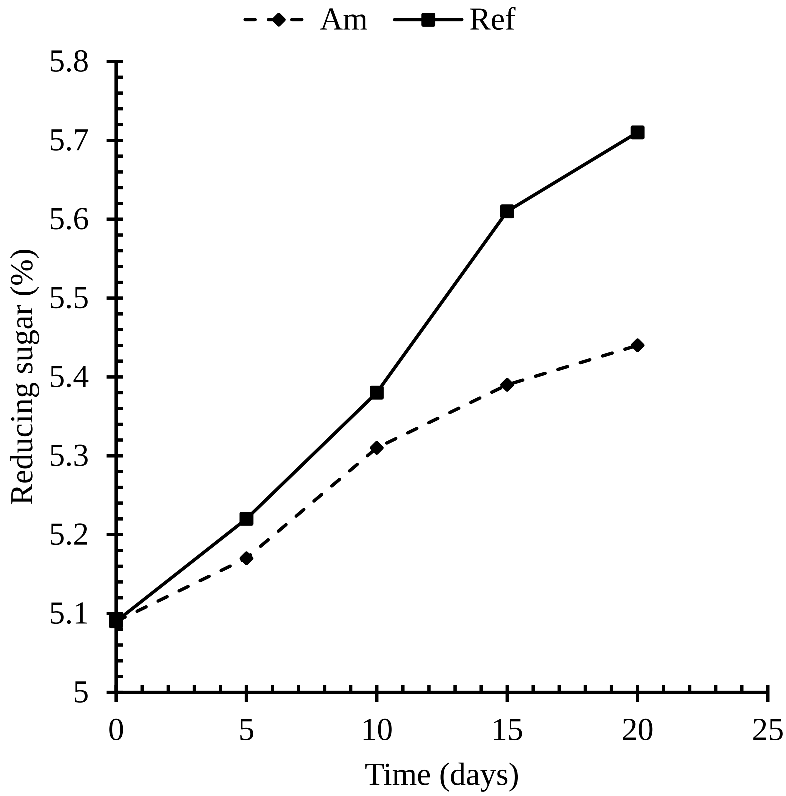

Fig. 4.9 Effect of storage time and temperature on reducing sugar content of prepared beverage

4.4.2.5 Effect on ascorbic acid

Ascorbic acid (Vitamin C) is an important nutrient that possesses antioxidant ability and provides the protection against free radicals (Esteve *et al.*, 2005). It is also considered an indicator of the nutritional quality of juices. Storage temperature, type of processing and packaging materials affect the rate of ascorbic acid degradation during storage (Bull *et al.*, 2004). The ascorbic acid content of the juice decreased significantly (Fig 4.10) from 26-22.3 mg/100 g (7±1°C) and 26-17.36 mg/100 g (25±5°C) during storage with the advancement of storage period (Table 4.4). This decrease was probably due to the fact that ascorbic acid being sensitive to oxygen, light and heat was easily oxidized in presence of oxygen by both enzymatic and non-enzymatic catalyst (Davey *et al.*, 2000). A decrease in ascorbic acid content with the increase in storage intervals in storage stability of jack fruit RTS beverage (Krishnaveni *et al.*, 2001) was reported. LSD showed that there was significant difference on acidity at 5% level of significance (Appendix D).

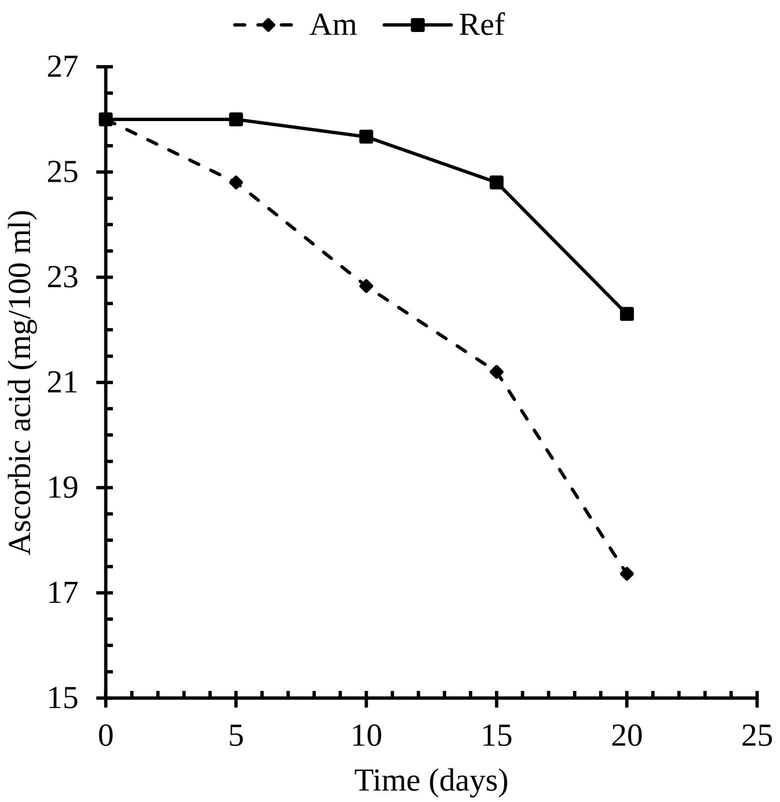

Fig. 4.10 Effect of storage time and temperature on ascorbic acid content of prepared beverage

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-pineapple beverage with 50% whey (V/V), 50% pineapple juice (V/V) and 13°Bx TSS was found superior with respect to appearance, color, flavor, taste and overall acceptance.

2. During 20 days of storage TSS, acidity and reducing sugar contents 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. The beverage can be stored at refrigeration temperature without adding any chemical preservative with desirable acceptability up to 20 days.

4. Production cost (NRs. 16.89) of prepared beverage was reasonable, within the reach of general population and much lower than commercial fruit juices production cost for the year 2018.

5. The product proved as a nutritionally as well as organoleptically desirable beverage with agreeable taste, flavor.

5.2 Recommendations

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

1. Formulation of such type of beverage can be done with varying proportion of juices and with incorporation of variable herbal type can be done.

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 as well as phytochemical properties can be done.

4. Pineapple of different varieties found in different places can be utilized to produce a nutritious, cheaper, thirst quenching drink of high vitamin C, carbonated drinks, fermented beverage etc.

Part VI

Summary

Milk whey is highly nutritious by-products obtained from the dairy industry producing cheese, chhanna and *paneer* constituting almost 45-50% of total milk solids, 70% of milk lactose, 20% of milk proteins, 70-90% of milk minerals and almost all the water soluble vitamins originally present in milk. About 50% of whey produced worldwide is dumped as by products. So the present work was connected to study the consumer acceptance of whey beverage, its chemical and storage quality.

For the study the milk, pineapple, and sugar were purchased from the local market. Whey was separated from the *paneer* by casein precipitation with 2% citric acid while the pineapple pulp juice was extracted using juice extractor and the separated juices were stored for the further analysis. Whey-pineapple beverage samples formulation was designed by experimental design software under mixed condition. Prepared beverage (whey-pineapple) in 1:1 ratio and TSS 13°Bx was found to be best in overall sensory evaluation analysis.

The superior beverage from the sensory analysis was analyzed for chemical composition and storage stability under refrigerated (7±1°C) and normal (25±5°C) storage condition. The analyzed chemical composition of best sample was found to be T.S.S (12.83 °Bx), Moisture (86.67%), pH (4.20), Acidity (0.46%), Reducing sugar (5.17%), Non-reducing sugar (10.60%), Total sugar (15.77%), Proteins (0.42%), Ash (0.32%), Fat (0.22%), Ascorbic acid (26.70 mg/100 g), and Total solids (13.33%). During storage study TSS, pH and reducing sugar increased while acidity and ascorbic acid content decreased under both storage conditions.

It was concluded that the present study of formulating whey-pineapple beverage was found nutritionally as well as organoleptically desirable. At present most of the fruit beverage in the market are generally synthetic, flavored, bottled and sold in market. If this could be substituted with whey beverage it would be eco-friendly and economically beneficial to consumer, dairy industry to solve whey disposal problem.

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Appendixes

Appendix A

Specimen card for sensory evaluation

Hedonic rating test

Name: **Date:**

Product: whey-pineapple 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.

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

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

Any comments…………………………………………………………………...

……………………………………......

Signature

Appendix B

Table B.1 Cost evaluation (for every 100 ml bottle)

S.N.	Particulars	Quantity	Rate(Rs)	Amount (NRs)
1.	Whey	50 ml	10/L	0.50
2.	Pineapple pulp juice	50 ml	180/L	9.00
3.	Sugar	5.22 gm	75/kg	0.39
4.	Bottle (250ml)	1	7/piece	7.00
	Total			16.89

The price of 100 ml whey drink cost NRs. 16.89. Thus, the price of 250 ml of drink is Rs. 31.725.

Appendix C

ANOVA for Sensory analysis of product

Table C.1.1 Two way ANOVA (no blocking) for appearance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	7	59.3500	8.4786	14.09	<.001
Panelist	9	18.7000	2.0778	3.45	0.002
Residual	63	37.9000	0.6016		
Total	79	115.9500			

Since F pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

Table C.1.2 Two way ANOVA (no blocking) for color

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	7	59.8000	8.5429	10.94	<.001
Panelist	9	30.0000	3.3333	4.27	<.001
Residual	63	49.2000	0.7810		
Total	79	139.0000	0.002		

Since F pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

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

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	7	39.3500	5.6214	7.63	<.001
Panelist	9	42.2000	4.6889	6.37	<.001
Residual	63	46.4000	0.7365		
Total	79	127.9500			

Since F pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

Table C.1.4 Two way ANOVA (no blocking) for taste

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	7	35.2875	5.0411	5.98	<.001
Panelist	9	40.0125	4.4458	5.28	<.001
Residual	63	53.0875	0.8427		
Total	79	128.3875			

Since F pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

Table C.1.5 Two way ANOVA (no blocking) for overall acceptance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	7	53.1875	7.5982	12.29	<.001
Panelist	9	29.3625	3.2625	5.28	<.001
Residual	63	38.9375	0.6181		
Total	79	121.4875			

Since F pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

Appendix D

ANOVA of chemical constituents of whey-pineapple beverage

Table D.1.1 Two way ANOVA for TSS as variate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Condition	1	0.147000	0.147000	17.82	<.001
Days	4	2.562000	0.640500	77.64	<.001
Residual	24	0.198000	0.008250		
Total	29	2.907000			

Since F pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

Table D.1.2 Two way ANOVA for pH as variate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Condition	1	0.0034133	0.0034133	23.05	<.001
Days	4	0.0342200	0.0085550	57.78	<.001
Residual	24	0.0035533	0.0001481		
Total	29	0.0411867			

Since F pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

Table D.1.3 Two way ANOVA for acidity as variate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Condition	1	0.0124033	0.0124033	22.53	<.001
Days	4	0.0766000	0.0191500	34.78	<.001
Residual	24	0.0132133	0.0005506		
Total	29	0.1022167			

Since F pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

Table D.1.4 Two way ANOVA for reducing sugar as variate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Condition	1	0.115320	0.115320	32.93	<.001
Days	4	0.995713	0.248928	71.08	<.001
Residual	24	0.084047	0.003502		
Total	29	1.195080			

Since F pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

Table D.1.5 Two way ANOVA for ascorbic acid as variate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Condition	1	47.1253	47.1253	49.57	<.001
Days	4	143.1353	35.7838	37.64	<.001
Residual	24	22.8180	0.9507		
Total	29	213.0787			

Since F pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

Appendix E

Summary of ANOVA of sensory evaluation of whey-pineapple beverage

Sample	Appearance	Color	Flavor	Taste	Overall Acceptance
A	$5.4^{a} \pm 1.07$	$5.4^{a} \pm 1.03$	$5.6^{a} \pm 1.19$	$5.4^{a} \pm 0.48$	$5.4^{a} \pm 0.78$
B	$5.0^{a} \pm 1.07$	$5.1^{a} \pm 0.52$	$5.3^{a} \pm 1.05$	$5.5^{ab} \pm 1.13$	$5.3^{a} \pm 0.91$
C	$5.6^{a} \pm 0.87$	$5.4^{a} \pm 1.10$	$5.5^{a} \pm 1.25$	$5.7^{abc} \pm 1.31$	$5.4^{a} \pm 0.78$
D	$5.6^{a} \pm 0.47$	$5.8^{a} \pm 1.19$	$5.9^{ab} \pm 1.05$	$6.3^{bcd} \pm 1.17$	$6.2^{b} \pm 1.15$
E	$6.7^{b} \pm 0.96$	$6.8^{b} \pm 1.07$	$6.5^{bc} \pm 0.84$	$6.4^{cde} \pm 1.17$	$6.5^{b} \pm 0.84$
F	$7.4^{c} \pm 0.70$	$7.5^{b} \pm 0.96$	$7.0^{cd} \pm 0.97$	$7.2^{e} \pm 0.95$	$6.8^{b} \pm 0.84$
G	$7.0^{bc} \pm 0.82$	$6.9^{b} \pm 1.22$	$6.7^{cd} \pm 1.35$	$6.6^{de} \pm 1.50$	$6.7^{b} \pm 1.43$
H	$7.1^{bc} \pm 0.94$	$7.1^{b} \pm 1.10$	$7.3^{d} \pm 1.06$	$7.2^{e} \pm 1.08$	$7.8^{c} \pm 0.82$
LSD (5%)	0.6932	0.7898	0.7670	0.820	0.7026

Photo Gallery

Plate 1 Samples

Plate 2 Sensory evaluation

Plate 3 Analysis of samples

Plate 4 Samples for analysis

Plate 5 Samples stored in refrigerated condition