UTILIZATION OF ACID WHEY ON FORMULATION OF POMEGRANATE RTS BEVERAGE AND ITS STORAGE QUALITY EVALUATIONS

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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 ***Utilization of Acid Whey on Formulation of Pomegranate RTS Beverage and its Storage Quality Evaluations***. presented by **Sujan Prasad Bolakhe** has been accepted as the partial fulfillment of the requirement for the **B. Tech. degree in Food Technology.**

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(Sujan Prasad Bolakhe)

Abstract

The present work was planned to prepare whey utilized pomegranate RTS beverage. Different variants of beverages were formulated using different levels of water replacement with whey i.e. 0%, 25%, 50%, 75% and 100% and named as sample A, B, C, D and E respectively. The TSS and acidity was maintained at 13 °Bx and 0.3 percent. The samples were subjected to sensory evaluation (color, taste, flavor and overall acceptance) by quality scoring method for consumer acceptability and the sensory data were analyzed by two-way ANOVA (no blocking) using GenStat and means were compared using LSD at 5% level of significance. Results revealed that on increasing whey incorporation, nutritional quality was enhanced, but poor in sensory properties. Beverage with 25% whey addition was found most acceptable with overall acceptability. The beverage was pasteurized at 80°C for 10 min and stored in 250 mL PET bottles at normal and refrigeration for 28 days. The effects of storage time and temperature on physicochemical (TSS, pH, acidity, reducing sugar) and microbial (TPC and coliform) properties were evaluated.

Total soluble solid (TSS), pH, moisture, protein, acidity, reducing sugar, and non-reducing sugar of pomegranate juice was found to be 13.6°Bx, 3.75, 85.64%, 0.22%, 6.76% and 5.32% and for whey these values were 5.8°Bx, 5.14, 92.62%, 0.46%, 0.28%, 4.7% and 8.99% respectively. Among the different proportion, the RTS prepared with 25% whey addition was found acceptable from sensory analysis. The TSS, pH, fat, moisture, protein, ash, calcium, acidity as citric acid, reducing sugar (as dextrose), non-reducing sugar (as dextrose) of RTS beverage was found to be 12.9°Bx, 3.21, 0.21%, 86.43%, 0.36%, 0.47%, 148mg/L, 0.3%, 7.27% and 6.23%. Total soluble solids, pH and reducing sugar increased while acidity content decreased with processing storage time in both storage condition. During storage analysis TSS, pH, acidity, and reducing sugar content ranged from 12.9-13.7°Bx, 3.21-3.12, 0.29-0.45% and 7.06-7.59% respectively under refrigeration condition and from 12.9-14.2°Bx, 3.21-3.05, 0.29-0.68% and 7.06-7.97% respectively under normal storage condition. From all the analysis, it can be observed that the RTS beverage can be kept unspoiled for 28 days.

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List of Abbreviation

Abbreviation	Full form
RTS	Ready to serve
COD	Chemical Oxygen Demand
TPC	Total Plate Count
PDA	Potato Dextrose Agar
TSS	Total soluble solid
ANOVA	Analysis of variance
LSD	Least Significant Difference
PCA	Plate Count Agar
CGMP	Casein glycol macro peptide
MFGM	Milk fat globule membrane
WPI	Whey protein Isolate
GSH	Glutathione
GIT	Gastro intestinal tract
GMP	Glycomacropeptide
TAG	Triacylglycerols

Part I

Introduction

1.1 General introduction

The soft beverage industry has seen significant advancements in production in recent years. Despite this progress, Nepal still offers a limited variety of flavors. Globally, many syrups, sherbets, and soft drinks with artificial fruit flavors are widely recognized (Tanwar *et al.*, 2022). Ready-to-Serve (RTS) beverage have gained significant popularity recently, catering to consumer demand for convenient, health-conscious option. These pre-packaged drinks are ready for immediate consumption without extra preparation. Creating RTS beverages with fruit juices, such as pomegranate , requires balancing taste, nutritional value , and stability (Maheswarlu *et al.*, 2010b).

Pomegranate is rich in valuable nutrients, including hydrolysable and condensed tannins, flavanols, anthocyanins, phenolic, and organic acids (Kandylis and Kokkinomagoulos, 2020b). It has a low pH, high acidity (up to 20 g of citric acid/L), and a sugar content of 70-180 g/L(mainly fructose and glucose). Its composition varies with factors like cultivar, soil, climate, ripening cultivation, processing, and storage. Pomegranate's bioactive compounds, such as polyphenols, provide antioxidant, anti- inflammatory, and antihypertensive benefits. Studies suggest it may help treat cancer, diabetes, and heart disease (Prakash and Prakash, 2011).

Whey is a valuable by-product obtained during manufacture of cheese, channa, casein paneer and shrikand as watery portion of milk after coagulation and removal of curd (Mohamed *et al.*, 2014). World whey production is 180 to 190 × 106 tones/year with an annual increment of 1-2% and only 50% of whey is utilized/processed. Whey contains 45-50% total milk solids, 70% milk sugar (lactose) 20% milk proteins and 70-90% milk minerals and almost all the water-soluble vitamins originally present in milk. So, whey disposal becomes a serious environmental pollutant being loaded with high amounts of organic matter (Ismail *et al.*, 2011). It has gained popularity not only as a byproduct of cheese production but also as a nutritional supplement due to its numerous health benefits.

This whey is thrown away into the environment which causes serious environmental pollution as it possesses high Biological Oxygen Demand (Melnik *et al.*) and Chemical

Oxygen Demand (COD). Whey protein possesses a very high biological value and is a complete protein with the presence of all essential and nonessential amino acids. The presence of lactose, minerals, protein and water-soluble vitamins make whey a highly nutritious product. The addition of whey into various fruit beverages is one of the most attractive avenues for its effective utilization. The main advantages associated with whey beverages is the healthful combination of the fruit-based vitamin containing components and the dairy-based calcium and whey proteins.(Shiby *et al.*, 2013).

Fruit and dairy waste based products are attaining considerable attention due to delicious taste, increased energy value and high nutritional value and market for such food products has incredible potential. Whey based fruits beverages are more suitable for health as compared to other drinks. Production of nourishing pleasant whey based fruit RTS beverages is one of the most promising trends in the utilization of dairy waste, whey. The present study was planned to prepare pomegranate RTS by incorporating different level of whey and study its storage behavior (Nagar *et al.*, 2013).

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. 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 2079/2080 was estimated to be more than 4.8 million liters. By utilizing whey in the formulation, the study may explore sustainable practices within the dairy industry, promoting the use of a byproduct in creating value-added beverages. This aligns with the increasing consumer interest in environmentally friendly and sustainable food and beverage options. Pomegranate and whey protein individually offer various health benefits. The study aims to uncover potential synergies that may result in a beverage with enhanced nutritional and health-promoting properties, contributing to preventive health strategies. When considering whey-based beverages versus whey protein isolate (WPI) for athletes and bodybuilders, it's essential to evaluate both cost and nutritional efficiency. Whey-based beverages, while convenient, tend to be less economical compared to WPI. Despite the convenience of whey-based beverages, their higher cost per gram of protein makes them a less budget-friendly option compared to WPI. WPI, while requiring some preparation, offers a more cost-effective solution by providing a higher protein content per dollar spent. For athletes and bodybuilders who need to balance efficiency with budget considerations, whey protein isolate is often the more economical choice.

1.3 Objectives

The objectives of the study are as follows:

1.3.1 General objective

The general objectives of this dissertation is production of acid whey utilized pomegranate beverage and its storage quality evaluation.

1.3.2 Specific objective

1. To carry out nutritive analysis of fresh whey, pomegranate juice and prepared beverage.
2. To Formulate the proportion of whey replacement with water.
3. Self-life study with respect to microbial and chemical properties.

1.4 Significance of the study

The significance of a study on whey-utilized pomegranate Ready-to-Serve (RTS) beverages lies in the intersection of consumer demand for convenient, nutritious options and the potential health benefits offered by the combination of whey protein and pomegranate. Understanding and exploring this unique beverage category can provide valuable insights and contributions to both the academic and practical aspects of the food and beverage industry. the study deepens the scientific understanding of how different ingredients interact in a beverage matrix. This understanding can contribute to the broader field of food science, providing insights into the optimization of formulations for taste, texture, and nutritional content.

The study on the utilization of dairy industry waste whey in the formulation of pomegranate RTS (Ready-to-Serve) beverages holds significant importance for several reasons. Firstly, it addresses environmental concerns by offering a solution for managing whey, a by-product of the dairy industry that poses disposal challenges and potential pollution. Repurposing whey into beverage formulation promotes sustainability and contributes to a circular economy. Economically, this approach reduces production costs and adds value to dairy operations by creating new revenue streams. Nutritionally, whey is rich in proteins, vitamins, and minerals, enhancing the health profile of pomegranate beverages and aligning with the growing consumer demand for functional foods. This study also fosters innovation in the food and beverage sector, encouraging the development of novel products that combine the antioxidant benefits of pomegranate with the nutritional value of whey. Evaluating storage quality and consumer acceptability provides insights into market potential and product optimization.

1.5 Limitation of the study

1. Only acid whey was utilized.
2. Shelf life of product was studied only for 28 days.
3. Optimization is done only by sensory evaluation.

Part II

Literature review

2.1 Back ground

The global juice market is expanding and it is likely driven by the fitness conscious consumer and the demand for healthy food products. Nowadays juice manufacturers are customer centered and focus on introducing different juices varieties, flavors, and mix juices along with innovative packaging and detailed nutrition and health claims. The global juice market is predicted to witness strong growth at a compound annual growth rate of 3% during the period 2016-20 (Ceclu and Nistor, 2020). The market for RTS beverages is dynamic and continuously evolving, driven by consumer demand for convenience, variety, and nutritional value. Major players in the beverage industry are investing in research and development to create appealing and innovative products that align with current consumer trends (Nilugin and Mahendran, 2011).

2.2 Pomegranate

Pomegranate (*Punica granatum L.*) belongs to the Punicacea family (Harde *et al.*, 1970). It is one of the important and commercial horticultural fruits which is generally very well adapted to the Mediterranean climate. Different parts of its tree (leaves, fruits and bark skin) have been used traditionally for their medicinal properties and for other purposes such as in tanning (Jbir *et al.*, 2008). It is proved to have high antioxidant activity and good potency for cancer culture in Iran, fruit characteristics determination is of prevention (María I Gil *et al.*, 1995). The edible part of the fruit contains considerable amounts of acids, sugars, vitamins, polysaccharides, polyphenols and important minerals. Studies to determine weather great variability exists in the antioxidant activity and other physical and chemical properties among different pomegranate cultivars allow breeders to select and breed genotypes with higher levels of compounds. It also provides a way of increasing the dietary intake of antioxidant compounds (Maria I Gil *et al.*, 2000b).

Pomegranate (*Punica granatum*) is an old, beloved plant and fruit belong to the order Myrtales. Recent research finding corroborate traditional use of pomegranate as a medicinal remedy as all parts of this plant have several bioactive metabolites (Zarban *et al.*, 2007). It was cultivated in ancient Egypt and in early Greece and Italy. The fruit was very popular in Iraq. Over time, it spread into Asia, North Africa and Mediterranean, Europe and gets popular in various regions. The best soils for pomegranate cultivation are considered to be fertile, rich with humus, deep, medium density with good drainage and especially alluvial soils. The edible part of the pomegranate fruit (50%) consists of 40% arils and 10% seeds. Arils contain 85% water, 10% total sugars, mainly fructose and glucose, and 1.5% pectin, organic acid, such as ascorbic acid, citric acid, and malic acid, and bioactive compounds such as phenolics and flavonoids, principally anthocyanins (Sreekumar *et al.*, 2014).

2.2.1 Historical background and current situation of Pomegranate

Pomegranates have a long and rich history dating back thousands of years. Pomegranates are grown in various parts of the world, including the Mediterranean region, Middle East, Central Asia, California, and parts of South America. Iran is the largest producer of pomegranates, followed by India and the United States (Chandra *et al.*, 2010). The demand for pomegranates and pomegranate products has been growing steadily due to increasing awareness of their health benefits and culinary versatility. This has led to expansion in cultivation and production in many regions.

Pomegranate cultivation in Nepal is primarily concentrated in the mid-hill and Terai regions, ranging from 200 to 1,500 meters above sea level. Several pomegranate varieties are suitable for cultivation in Nepal (Poudel *et al.*, 2017). Some popular varieties include Wonderful, Ganesh, Ruby, and Bhagwa. Further research and extension services are needed to disseminate best practices, develop locally adapted varieties, and address specific challenges faced by pomegranate growers in Nepal (Atreya *et al.*, 2020).

2.2.2 Functional component of Pomegranate

Pomegranates contain numerous functional components that contribute to their health-promoting properties. Some of the key functional components of pomegranates include:

2.2.2.1 Antioxidant

Pomegranate is well known for its antioxidant properties. Studies in chemical analysis have shown pomegranate juice to be a rich source of antioxidants (Lazeeza, 2021). It is rich in antioxidants, including polyphenols such as flavonoids, tannins, and anthocyanins. These compounds help neutralize harmful free radicals in the body, thereby reducing oxidative stress and lowering the risk of chronic diseases like heart disease, cancer, and diabetes (Maria I Gil *et al.*, 2000a).

2.2.2.2 Vitamins and Minerals

Pomegranates are a good source of vitamins, including vitamin C, vitamin K, and several B vitamins, as well as minerals such as potassium and copper. These nutrients play essential roles in various physiological processes and contribute to overall health and well-being (Kandylis and Kokkinomagoulos, 2020a).

2.2.2.3 Dietary Fiber

Pomegranates are rich in dietary fiber, both soluble and insoluble. Fiber aids digestion, promotes gut health, and helps regulate blood sugar levels and cholesterol levels. It also contributes to a feeling of fullness, which can support weight management (Kandylis and Kokkinomagoulos, 2020b).

2.2.3 Nutritional Profile of pomegranate

Pomegranate is a well-known source of valuable nutritional substances. It contains hydrolysable tannins, condensed tannins, flavanols, anthocyanins, and phenolic and organic acids. The antioxidant capacity of pomegranate juice was shown to be three times higher than that of red wine and green tea, based on the evaluation of the free-radical scavenging and iron reducing capacity of the juices (Al-Maiman and Ahmad, 2002). Pomegranates are particularly known for their high antioxidant content, including punicalagins and anthocyanins, which may contribute to their potential health benefits. Antioxidants help combat oxidative stress and inflammation in the body. It's worth noting that the nutritional content can vary slightly based on the size of the fruit and its ripeness. Additionally, pomegranate juice, while still nutritious, may have a higher concentration of sugars and calories compared to the arils alone (Kulkarni *et al.*, 2004).

2.2.4 Health Benefits of pomegranate

Fruits, in general, are essential for maintaining a balanced diet. They supply a wealth of macro-and micronutrients, along with bioactive compounds that promote health. In recent decades, numerous studies have highlighted the importance of fruit consumption in reducing health risks, and there have been campaigns to encourage the inclusion of fruit in children's diets (Faria and Calhau, 2010).

A substantial amount of research has focused on the potential health benefits and nutritional value of pomegranate and its components. Positive findings have spurred increased interest in particular fruit over the past few years. Pomegranate, abundant in bioactive compound like polyphenols, has demonstrated various health-related properties such as antioxidant, anti-inflammatory, and antihypertensive effects in both in vivo and in vitro studies. These health-promoting properties are primarily attributed to the presence of punicalagin, with other metabolites like flavonols and anthocyanins also contributing to its benefits (Syed *et al.*, 2007).

Table 2.1 Characteristics of pomegranate juice

Parameter	Values
Moisture (%)	85.4
Total Sugar(g/100mL)	10.6
Reducing Sugar(g/mL)	10.5
Non-reducing sugar(g/100mL)	0.1
Pectin(g/mL)	1.4
Total acidity as citric acid (g/100mL)	0.1
Ascorbic acid(mg/100ml)	0.7
Free amino acid(mg/100ml)	19.6
Ash(g/100ml)	0.05
Potassium (ppm)	49.2
Magnesium (ppm)	2.4
Sodium (ppm)	3.0

Source: Kandylis and Kokkinomagoulos (2020)

2.2.5 Use of pomegranate for RTS making

The use of pomegranate in Ready-to-Serve (RTS) beverage manufacturing offers a myriad of possibilities to create a refreshing and nutritious drink. Pomegranates are valued not only for their distinctive flavor but also for their potential health benefits, making them an attractive ingredient for innovative and health-conscious beverage formulations (Vardin and Fenercioğlu, 2003). Blending pomegranate juice with other fruit juices to create a more complex and well-rounded flavor profile. This can enhance the overall taste of the RTS beverage and contribute to its uniqueness. Pomegranate juice is known for its vibrant color. To preserve this appealing visual aspect, consider incorporating antioxidants like ascorbic acid (vitamin C) or natural color stabilizers to prevent oxidation and color changes during storage. the natural sweetness of the pomegranate juice and adjust it to meet the desired taste profile. Additionally, consider the acidity of the juice and make necessary adjustments to achieve a balanced flavor (Vegara *et al.*, 2013).

2.2.6 Innovation in pomegranate Beverage Production

Innovation in pomegranate beverage production has seen significant advancements across various fronts. Recent developments focus on enhancing both the flavor profiles and nutritional benefits of pomegranate drinks. Advanced processing techniques have streamlined the extraction of pomegranate juice, ensuring higher yields and improved quality (Fahmy *et al.*, 2020). Novel formulations cater to evolving consumer preferences, emphasizing natural ingredients and functional additives that enhance health benefits. Innovative packaging solutions have been introduced to prolong shelf life and maintain the sensory attributes of pomegranate beverages, addressing both convenience and sustainability concerns.

Technological innovations play a crucial role by preserving the bioactive compounds and antioxidants inherent in pomegranates, thereby enhancing their nutritional value. The integration of new ingredients and additives aims to further enrich the functional properties of these beverages, appealing to health-conscious consumers (Vilas-Boas *et al.*, 2022). Consumer-driven innovations in packaging and marketing strategies are also shaping market trends, reflecting a demand for transparency and health-focused branding. Overall, ongoing research and development continue to explore novel applications and health-promoting potentials of pomegranate beverages, driving the evolution of this dynamic sector.

2.3 Milk

Milk is the "lacteal secretion, practically free from colostrum, obtained by the complete milking of one or more healthy cows"(Johnson, 2012). 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). 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).

2.3.1 Milk proteins

Milk contains a large variety of proteins that typically can be broken into two classes, the caseins and the whey proteins. From a processing perspective, the caseins are those proteins that aggregate into the curds during cheese production, and whey proteins are those dissolved in the aqueous portion and not retained within the curd. Whereas this is a useful industrial simplification, even with respect to nutritional properties, there are important differences among the types of whey proteins that depend on the method used to produce cheese. For example, acid-precipitated caseins contain all of the casein proteins. Conversely, when the curd is formed in response to the enzyme chymosin, which attacks specifically the κ-casein on the casein micelle surface,(Farrell *et al.*, 1999) the effective stabilizing constituent caseinoglycomacro peptide (CGMP), a polypeptide of 64 amino acid residues derived from the C-terminal part of bovine κ-casein, is released from the surface of the casein micelle. The remaining caseins aggregate to form a curd and are processed to cheese. The CGMP becomes part of the whey. Because CGMP has significant biological properties (Yvon *et al.*, 1994) its presence in sweet whey is an example of a difference in the net nutritional properties of whey processed via different methods. Approximately 80% of the proteins in cow's milk are caseins. A goal of modern cheese-making is to entrap as much of the components of milk as possible within the casein curd. Nevertheless, a large fraction of the soluble constituents remaining in the milk that are not entrapped in the clotted casein network emerge as co-products of cheese manufacture or whey. This whey portion contains various proteins, peptides, amino acids, lactose, minerals, vitamins, and varying quantities of lipids. Commercial processes produce whey protein concentrates that contain 34 to 90% protein, whey protein isolates that contain more than 90% protein, and various lactose fractions. The major whey proteins are β-lactoglobulin and αlactalbumin. Whey is also abundant in other proteins, including bovine serum albumin, immunoglobulins, lactoperoxidase, and lactoferrin, as well as a variety of milk fat globule membrane proteins whose abundance varies with the method used to produce whey. Whey also contains peptides formed by the hydrolysis of other milk proteins, including the caseins. The most notable of these casein peptides is CGMP; however, a variety of other peptides whose biological activities have not been well characterized are also produced (Meisel and Bockelmann, 1999).

2.4 Whey

Whey is a valuable by-product liquid obtained from protein precipitation in milk from the cheese industry (Silva e Alves *et al.*, 2018). Moreover, over the years, several methods have been explored to convert huge volumes of whey into food-grade products. The reason behind this is that only about 60% of whey generated globally is used to manufacture products. The remainder of the material is disposed of as waste (León-López *et al.*, 2020). Traditionally, whey has been discarded in surface water or fed to cattle. Whey dumping on soil causes serious environmental pollutants by changing the physico-chemical properties of soil, which leads to lower crop yields. Furthermore, whey waste can also decrease dissolved oxygen and inhibit biodegradability when it is released into bodies of water, and causes a serious threat to aquatic life and human health (Begum *et al.*, 2019). whey contains various nutrients, such as calcium, magnesium, phosphorus, vitamins (riboflavin and thiamine), and protein (α-lactoalbumin, β-lactoglobulin, immunoglobulins, serum albumin), which accounts for around 85–95% of the volume of milk, and it retains up to 55% of its contents after processing (Chatterjee *et al.*, 2015).

Table 2.2 General Proximate Composition of Whey

Constituent%	Sweet Whey	Acid Whey
Water	93-94	93-94
Dry matter	6-6.5	5-6
Lactose	4.5-5.0	3.8-4.3
Protein	0.6-0.65	0.6-0.65
Lactic Acid	Traces	Up to 0.8
PH	6.2-6.4	4.6-5.0

Source: Omole *et al.* (2012)

2.5 Overview of whey production

Whey had long been considered to be nothing more than a waste product. Whey used to be sent back to the farms for land disposal, as a component of animal feed, or it was dumped into drains, waterways, and oceans. The annual global production of whey is approximately 80 billion liters (Smithers *et al.*, 1996). Increased environmental concern in the early 1990s pushed for a more cost-effectively and environmentally friendly method of disposal.

Numerous efforts have been undertaken to incorporate whey into different dairy product formulations, however, there remains ample opportunity to explore its potential applications in beverages, especially within the health-focused energy drink sector (A. K. Singh *et al.*, 2009). The use of whey in beverage production is more cost effective and adds value than other methods of treatments and disposal. The majority of the whey produced is spray dried into whey powder, whey protein concentrates, and whey protein isolates. These whey powders are utilized as ingredients in a variety of food products, with protein enriched goods being one of the most commercially valuable product lines (Huffman, 1996).

2.6 Nutritional health benefits of whey

Whey proteins are the group of milk proteins that remain soluble in milk serum or whey after precipitation of casein at pH 4.6 and 20°C. This group includes β-Lactoglobulin, Lactalbumin, serum albumin, immunoglobulins, lactoferrin and proteose-peptone fractions (Farrell Jr *et al.*, 2004). Broader classifications assign lactoferrins, lactoperoxidase, β-microglobulin, lysozyme, insulin-like growth factor, -globulins and several other small proteins to minor whey proteins (Yalcin, 2006).

Whey is a by-product of the dairy industry, which for years was thought to be insignificant and was either used as an animal feed or it was disposed of as waste. Over the last years several studies were carried out concerning the importance of whey is nutritional value and the properties of its ingredients. It is now accepted that main content, whey proteins, have antimicrobial, antiviral and anti-oxidant properties (Macwan *et al.*, 2016).

a) With growing age, muscle loss and its negative health implications is a growing concern, both in terms of volume and medical costs. Good nutrition and adequate amounts of high quality whey protein may help in maintaining strong muscles during aging, especially when combined with an exercise and resistance training program (Marcelo and Rizvi, 2008).
b) Another benefit of whey protein for seniors is the ability to help prevent bone loss.
c) Whey protein is a naturally complete protein, meaning that it contains all of the essential amino acids required in the daily diet. It has the ideal combination of amino acids to help improve body composition and enhance athletic performance.
d) It is estimated that a nursing infant ingests about 3 g lactoferrin daily during the first week of life, whereas a calf drinking two liters of milk a day ingests about 2 g lactoferrin daily. Whey protein contains many of the same components found in human breast milk and for this reason, is a key ingredient in a wide variety of infant formulas, including those for premature infants (Keri Marshall, 2004).
e) Branched-chain amino acids (BCAAs): These are metabolized in the muscle to manufacture glutamine, a precursor to GSH and another important component of the immune system (Kadam *et al.*, 2018).

f) Lactoferrin: It has been shown to exhibit immune-modulating activity through both antimicrobial and antitoxin activity. It may also provide protection against viruses such as hepatitis, cytomegalovirus and influenza (Harper, 2004).

g) Cysteine An amino acid found in high levels in whey proteins, is involved in the intracellular production of GSH (Glutathione) (Kadam *et al.*, 2018).

h) Immunoglobulins: They may confer disease protection to infants through passive immunity and to adults by promoting the activity level of the immune system (Harper, 2004).

i) Research has shown that whey proteins may work against hypertension. Human clinical trials and animal studies have shown that hydrolyzed whey protein isolate assists in reducing the blood pressure of borderline hypertensive individuals (Frid *et al.*, 2005b).

j) It has been observed that whey proteins may reduce the cholesterol level in individuals (Frid *et al.*, 2005a).

k) Whey protein concentrates have been researched extensively in the prevention and treatment of cancer. In a review of whey protein concentrates in the treatment of cancer, (Bounous, 2000) discusses the antitumor and anticarcinogenic potential.

2.7 Whey components

2.7.1 Whey proteins

2.7.1.1 β-Lactoglobulin

β-Lactoglobulin is the major whey protein in ruminants and pigs; however, it is not found in abundance in the milk of many other species. Although isolated over 60 years ago, the function of this protein is still unknown, but it does bind calcium and zinc and a variety of hydrophobic small molecules. Bovine milk β-lactoglobulin and serum retinol-binding protein both enhance the uptake of retinol in the intestine of suckling rats (Said *et al.*, 1989). β-Lactoglobulin, retinol-binding protein, apolipoprotein D, α-2-urinary globulin, and bilin-binding protein all belong to a family of proteins that function to bind and/or transport small hydrophobic molecules (Ali and Clark, 1988). Although this protein is similar in sequence to retinol-binding proteins, their functions may not be the same. β-Lactoglobulin may also function as a fatty acid or lipid-binding protein (Pérez and Calvo, 1995). X-ray crystallographic studies have shown that fatty acids and retinoids bind independently and simultaneously to β-lactoglobulin, and that palmitic acid binds in the central cavity of the molecule in a manner similar to the binding of retinol to the related lipocalin, serum retinol-binding protein (Wu *et al.*, 1999).

2.7.1.2 α-Lactalbumin

α-Lactalbumin comprises 2 to 5% of skim milk total protein. This protein is a calcium-binding protein and has a high affinity for other metal ions, including Zn+2, Mn+2, Cd+2, Cu+2, and Al+3. α-Lactalbumin is the "B" protein of the lactose synthase enzyme complex that catalyzes the last step in the biosynthesis of lactose. For this reason, it is of major interest in terms of the control of milk secretion. Purified α-lactalbumin is used commercially in infant formula because it is structurally and compositionally similar to the major protein in human breast milk. Purified α-lactalbumin is also used as a sports food protein because it is a good source of branched-chain amino acids (Tawa Jr *et al.*, 1992). Branched-chain amino acids are utilized by muscle for energy as well as protein synthesis and several reports have associated improved performance with the consumption of diets enriched with these amino acids (Blomstrand and Newsholme, 1992).

2.7.1.3 Blood Proteins

Two minor proteins found in whey are blood proteins. Serum albumin is derived from the maternal bloodstream, and it is not synthesized by the mammary gland. Serum albumin is presumed to enter the milk via nonspecific transcytotic mechanisms, that is, "leakage," although there may be a more specific mechanism of transport. The concentration of serum albumin in milk whey increases during mastitis and during mammary involution. The function of this protein in milk is unknown, although it does bind fatty acids and other small molecules. Albumin may be neutral from an evolutionary perspective, its presence creating no negative selective pressure. The blood proteins in whey are actually a family of proteins. The immunoglobulins include IgG1, IgG2, IgA, and IgM. The concentration of immunoglobulins is very high in colostrum, and it is lower in milk. Immunoglobulin content constitutes part of the passive immunity conferred to the neonate via colostrum (Gabriel Michael *et al.*, 1971). Colostral immunoglobulins currently are being developed as food-grade antimicrobials.

2.7.1.4 Lactoferrin

Lactoferrin comprises only a small portion, 0.2 to 0.8%, of skim milk protein but is a higher proportion of whey, and it is relatively easily enriched by taking advantage of its unusual cationic binding properties. Lactoferrin has antibacterial and antioxidant properties, it is the major nonspecific disease resistance factor in the mammary gland and it probably mediates protection against microbial infection of the mammary gland (Seyfert *et al.*, 1994).

2.7.2 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 and Technology, 1992).

2.7.3 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 and Technology, 1992).

2.7.4 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 and Technology, 1992).

2.8 Therapeutic value of whey

Due its health benefits it targets all kinds of age groups from old to young people it also helps in treating some illness, like digestive tract diseases, etc. It has a great history bound to it for showing best benefits in diarrhea and other skin related diseases, scales in urinary tract, and helps in intoxication. As the beverage is having high amount of amino acids it is very much useful for the athletes. Mainly known for the branched chain amino acids like isoleucine, leucine, and valine. These are the first amino acids used during the resistance training which are directly metabolized into muscle tissues. They include GSH, GMP, lactoferrin with help of these component especially the beverage can be used to check the absorption capacity for the iron content and may be also used to improve the iron content absorption in the GIT (more useful in infants and neonates) (Anirudh *et al.*, 2022). It also has improve calcium. Absorption in the older people who are suffering from osteoporosis it also poses antioxidant properties, antiaging agent (GSH), Glutathione peroxidase which is from cysteine and selenium they convert the lipid peroxides into hydroxyl acids. Reduction of the oxidative stress is possible with the chelation done by the alpha lactalbumin bovine beta lactoglobulin lowers the blood pressure as they are isolated peptides. Minerals like potassium, involves in nerve transmission, muscular contractions, calcium used to maintain body alkaline pH and bone density with the effect of depolarization through magnesium. Iron beneficial bacteria inhibited by Lactoperoxidase, fungi growth is inhibited by lactoferrin. Whey contains many essential vitamins some are D,B2,A,B3,C,B5,B1 E and B6 (Keri Marshall, 2004).

2.9 Whey protein as a functional ingredient

Along with physiological benefits**,** whey protein offers outstanding functional attributes and appealing sensory qualities that make it suitable for a wide range of food uses. These include sports beverages, liquid alternatives to meat, baked goods, processed meats, pasta, salad dressings, spreads and dips, coffee creamers, soups, ice cream, confectionery, infant foods, and various dairy products. The uses of whey protein as functional ingredient id given in Table 2. In order for them to be used in these application, whey protein should be extracted from whey using different fractionation methods, mainly membrane processing (Fitzsimons *et al.*, 2007; Onwulata and Huth, 2009). Its rapid digestion and absorption make it ideal for post-workout recovery, enhancing muscle protein synthesis. Beyond its nutritional benefits, whey protein contributes to food formulation through its emulsifying, foaming, and gelling properties, which are advantageous in products such as protein shakes, baked goods, and dairy items (McIntosh *et al.*, 1998).

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 flavor	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: Bohora (2018)

2.10 Biological application of whey

2.10.1 Muscle Building

Branched chain amino acids are high in whey which stimulates the protein synthesis, muscle building, and retension.it also protects the degradation of the muscle, it is the building block of the amino acids that can further help in building muscle in low tone athletes or people. It is absorbed very quickly in the blood as it is termed as fast acting protein. After practice session when athletes take this whey beverage it readily helps in the speedy recovery of the muscles by providing the BCCA to the athletes (Gangurde *et al.*, 2011). During competitive events low level of BCCA should be replaced within 1 hour or less the depending upon the game which the athlete is playing. Bodybuilders highly prefer whey because of its wonderful nutritional characters which helps in building muscle in lean athletes. It is very good in the powder or beverage form which gives rapid energy for overcoming the fatigue, over dosage of anything is quite harmful to the body or the athletes out is suggested not take more than 30gms of protein in one sitting as more then that will create high load on liver this beverage if taken in consistent form and will result in wonderful efficiency (Gangurde *et al.*, 2011).

2.10.2 Cardiovascular disease

Increased consumption of the high fat rich diet creates many health problems mainly cardiovascular disease as it is not only related one factors many other cofactors are also related to this scenario as they are old age, obesity, sedentary lifestyle high alcohol intake, fat diet. Milk is one of the best composition of 12 types of fats includes oleic acids, free sterols, cholesterol, sphingolipids these milk products lowers the blood pressure and minimises hypertension (Keri Marshall, 2004) . In a study of 20 people where fermented milk with whey was given for the subjects to check whether there is a change in the lipid and blood pressure levels it was conducted for 8 weeks and control group was also present this showed a variable results as the test regime showed high density lipoproteins and low TAG and systolic blood pressure but in the control the result was vice versa (Keri Marshall, 2004).

2.10.3 Human Immunodeficiency Virus

Use of whey for these patients is done for the increase in the levels of glutathione and cysteine proteins in HIV positive individuals. In a study, 30 subjects with HIV gets a daily dose of 45 g whey protein from few sources Immunocal, Protectamin The two products have different amino acid profiles and Protectamin mob showed increased glutathione levels but Immunocal group had no significant elevation (Keri Marshall, 2004).

2.10.4 Obesity

Obesity in people can be reduced by high carbohydrate and less fat diet. Whey proteins have 95% protein, and it has a remarkable commercial significance in weight reduction program.

BMI is increased with amino acids. For reducing obesity calcium breaks the lipids with no ill effects on muscle in the animals (Mehmood *et al.*, 2019).

2.11 Utilization of whey

The dominant presence of lactose (72%) and minerals (8%) has to overcome. The possibilities of new techniques have created more application in bakery industries. Lactose, huge part of whey. They enhance the flavor, color, smell, texture, and durability in the bakery industry. The use of demineralized whey is preferred, because as it is having blander taste. With help of column chromatography we can extract the components of whey (Keri Marshall, 2004).

2.11.1 Dairy Products

2.11.1.1 Beverage products

whey is far better in technological way, drinks can be manufactured from permeate or refined whey or ultra-filtered whey concentrate .they are as follows (Pernot-Barry, 2008).

2.11.1.2 Whey beverages based on fruit juices

These products usually fulfill a part of the meal like breakfast type beverage, snack type beverage here the main components are healthy fruit juice composition along with the flavors which are mainly mango pulp, citrus, Apple, pear, strawberry, raspberry or fruit juice combination with various terms. As it proved to cover the undesirable taste or odor addition of these frits have proven to very essential for the commercialization of the product along with great health benefits for especially to athletes. Whey based mango beverage is basically a blend of 12% pulp and 8% sugar ,but in RTS we have 70% whey and 30% pulp which passed all the sensory tests, sample prepared with different combinations of whey and fruit juice concentrations as it gives good taste ,floor man best acceptability (Villarreal, 2017).

2.11.1.2 Whey beer

This can be manufactured with Malt or without malt further fortified with mineral ,vitamins and hydrolysates of starch .this creates a foamy texture with bad odour with terrible taste with low solubility of proteins alone inhibition of the beer yeasts (Jeličić *et al.*, 2008a).

2.11.1.3 Whey wine

This product of the whey contains low alcohol amount around 11% giving fruit smell which starts with Deproteinization by beta galactosidase ,transferring it into smaller proportions, cooling yeast addition, fermentation, transferring it into smaller fractions, storing, purifying, and bottling (Jeličić *et al.*, 2008b).

2.12 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.*, 2015b).

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.*, 2015a):

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.

2.13 Use of fruits in preparation of whey based beverage

(Panghal *et al.*, 2017a) prepare papaya based beverage with whey incorporation (0, 25, 50 and 100%), which is a major environmental pollutant from dairy industry. RTS was evaluated for its nutritional composition, physicochemical attributes like titratable acidity, total soluble solids, total and reducing sugars and sensory quality. Microbial quality was also observed for 60 days. Results revealed that on increasing whey incorporation, nutritional quality was enhanced, but poor sensory and microbial quality was observed. Beverage with 25% addition was found most acceptable.

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 varies from 5-15ml, 1-5 ml and 72-86 ml per 100 ml of the prepared beverage, respectively. The screening of beverage sample 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 researchers in whey beverages is presented in Table 2.4.

Table 2.4 Others example of fruits use in whey beverage

No.	Types of whey	Products	References
1.	Channa whey	Mango RTS beverage	(Khedkar *et al.*, 2014)
2.	Paneer whey	Guava whey beverage	(W. Singh *et al.*, 1999)
3.	Cheese whey	Kinnow juice beverage	(Khamrui and Rajorhia, 1998)
		Chocolate drink	(Joshi *et al.*, 1985)
4.	Chakka whey	Mango whey beverage	(Hapase, 2004)

2.14 RTS

Ready-to-serve drinks refers to a category of beverages that have not been diluted before consumption. Typically, it is made from juice, pulp, or both, and is then colored and flavored with optional ingredients. Sweeteners like sugar and acidifiers like citric acid are also mixed in. Carbonation is present in ready-to-drink beverages. Depending on the product, different levels of carbonation are used. RTS are required to meet certain criteria, like having fruit components that are at least 10%, 10% TSS, and 0.3% acidity etc. as given in Table.

Table 2.5 Specifications for ready-to- serve drinks (RTS)

Parameters	Value
Fruit Content	Not less than 10%
Total soluble solid (TSS)	Not less than 10%
Acidity	0.2-0.3%
Sulphur Dioxide	Not more than 70ppm

Source: DFTQC (2018)

2.15 Sensory analysis methods

Sensory analysis of Ready-to-Serve (RTS) beverages is essential for evaluating consumer acceptability and quality based on attributes like color, flavor, taste, and texture. Common methods include the 9-point Hedonic Scale, where panelists rate their preference (Meilgaard *et al.*, 1999), and descriptive analysis (QDA), which quantifies sensory attributes (Lawless and Heymann, 2010) Discrimination tests, such as the triangle test, help detect formulation differences (Stone and Sidel, 2004). Statistical tools like ANOVA are used to analyze sensory data (Næs *et al.*, 2011) . These methods ensure reliable sensory evaluation of RTS beverages, guiding product optimization and consumer satisfaction.

Part III

Material and method

3.1 Materials

3.1.1 Milk

Standardized milk contained 5% fat and 8% SNF was purchased from local market of Dharan, Nepal. It was stored under refrigerated condition for further use.

3.1.2 Pomegranate

Pomegranate (*Punica granatum*) was purchased from local fruit store of Dharan, Nepal. It was washed thoroughly in the water to remove foreign materials and pulping was done in pulper. Later detail study was done.

3.1.3 Sugar

The table sugar was purchased from the local market of Dharan, Nepal. It was grinded for further use.

3.1.4 Water

Potable water from the campus drinking water source was used for the dilution and volume make of the ready to serve juice.

3.1.5 Bottle

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

3.2 Methodology

3.2.1 Preparation of whey

Standardized milk contained 5% fat and 8% SNF was used to prepare high-quality whey. The milk was heated to 90°C for 5 minutes, then cooled to 70°C. A 2% citric acid solution was gradually added while continuously stirring, resulting in the complete coagulation of milk protein (casein). The liquid whey was then 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 90°C for 5 minutes

(Pasteurization temperature)

↓

Cooling to 70 °C

↓

Coagulated with 2% citric acid

↓

Filtered in muslin cloth

↓

Whey

↓

Stored at refrigerator

Fig.3.1 Flowchart for preparation of whey

Source: Jan *et al.* (2012)

3.2.2 Preparation Pomegranate juice

Ripe pomegranates were carefully selected to ensure the highest quality and optimal ripeness for juice extraction. These selected fruits were thoroughly washed with clean water to remove any surface impurities or contaminants. Following the washing process, the arils (the seed sacs inside the pomegranate) were manually separated from the fruit. This manual separation was done with great care to avoid damaging the arils, which could affect the quality and yield of the juice.

Once the arils were separated, the juice extraction process began. The arils were first crushed to release the juice, followed by pressing the crushed arils through a muslin cloth consisting of two layers. This method was chosen to ensure that the juice was filtered effectively, removing any solid particles while retaining the rich color and flavor of the pomegranate juice.

After extraction, the freshly pressed pomegranate juice was immediately transferred to a storage container and kept at refrigeration temperature. This temperature was maintained to preserve the juice's freshness, prevent microbial growth, and retain its nutritional and sensory qualities until it was ready for further use or analysis. The detailed extraction procedure of pomegranate juice is illustrated in Fig. 3.2.

Pomegranate

↓

Sorting and Washing

↓

Juice extraction (Mixer)

↓

Filtration (Muslin Cloth)

↓

Bottle Filling

↓

Storage (Refrigeration)

Fig.3.2 Flowchart for preparation of pomegranate juice

Source; Ayoub *et al.* (2017)

3.2.3 Preparation of whey pomegranate RTS beverage

The RTS beverage was formulated using a blend of whey, water, pomegranate pulp, sugar, and citric acid. To explore the impact of varying whey content on the beverage's characteristics, five different variants were prepared by adjusting the proportion of whey. The levels of whey incorporated were 0%, 25%, 50%, 75%, and 100%, with the remaining portion being replaced by water. Once the formulations were finalized, the prepared RTS beverages were carefully packed into sterilized bottles with a capacity of 250 ml. During the filling process, a ½-inch headspace was left. To ensure the microbiological safety and stability of the beverage, an in-bottle pasteurization step was employed.

The detailed steps involved in the preparation of this whey-based pomegranate RTS beverage are visually summarized in Figure 3.3. The flowchart outlines the key stages from ingredient mixing to final storage, providing a clear overview of the entire process.

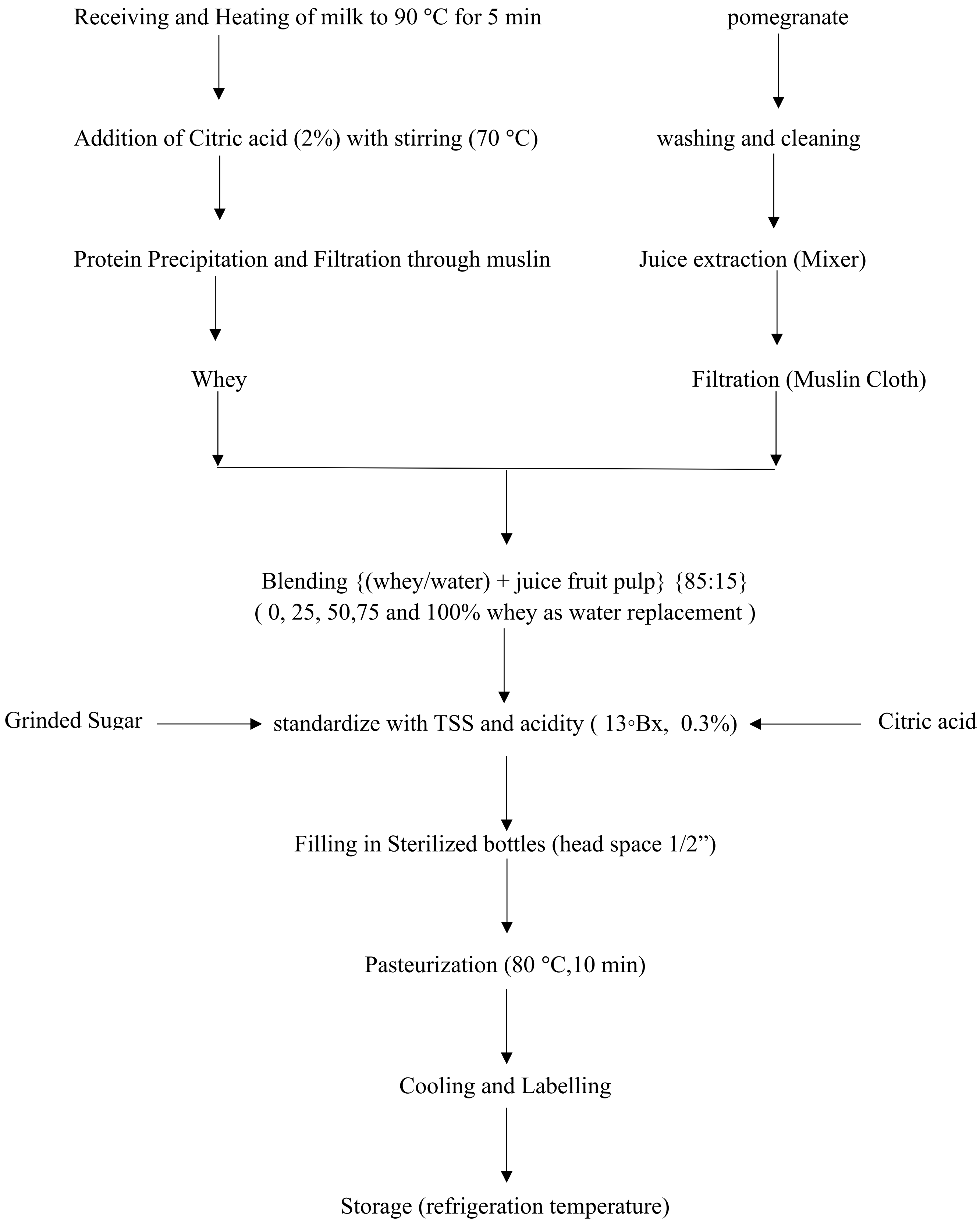

Fig:3.3 Flowchart for preparation of whey beverage Source: Panghal *et al.* (2017a)

3.3 Analytical procedure

3.3.1 Physical and chemical examination

Different parameter like total soluble solid, fat content, Protein content, acidity, pH, total sugar, total solids, calcium content, reducing sugar and microbiological analysis was done for raw whey, pomegranate juice and prepared RTS. The final product was then kept at room temperature and refrigeration temperature for 28 days and analysis was conducted on every 7 days which included determination of TSS, acidity and microbial analysis including TPC.

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 (1986).

3.3.1.2 Fat content

Fat content was determined by Gerber method according to Ranganna (1986).

3.3.1.3 Ph

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

3.3.1.4 Protein content

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

3.3.1.5 Titratable acidity

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

3.3.1.6 Total and reducing sugar content

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

3.3.1.7 Ash content

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

3.3.1.8 Calcium content

The calcium content of the sample was determined by flame photometer as method given by (Rai and Kc, 2007).

3.3.1.9 Alkalinity

Total alkalinity of water was determined by titration with HCl using methyl orange to pink end point as given by (Rai and Kc, 2007).

3.3.2 Recipe formulation of Prepared RTS beverage

Different recipe formulation was formulated using similar research conducted by (Panghal *et al.*, 2007).

Table:3.1 Different recipe samples

S.N	Code (water replacement with whey)	Whey (ml)	Water (ml)	Juice content (ml)
1.	A (0%)	0	85	15
2.	B (25%)	21.25	63.75	15
3.	C (50%)	42.5	42.5	15
4.	D (75%)	63.75	21.25	15
5.	E (100%)	85	0	15

The table 3.1 outlines the formulation of whey pomegranate RTS beverage with varying levels of whey as a substitute for water, maintaining an equal amount of juice content of 15 ml across all samples. The formulations are identified by codes A through E, each representing a different percentage of water replacement with whey. Sample A contains no whey, using 85 ml of water. As the percentage of whey increases in subsequent samples B (25% whey), C (50% whey), D (75% whey), and E (100% whey). The water content decreases correspondingly, with the final formulation consisting entirely of whey.

3.3.3 Sensory evaluation

Among 5 prepared samples of RTS, the best one was determined by the physiochemical analysis and sensory evaluation method. For sensory evaluation, 9-point hedonic scale as per Rangana (1986) was used. The panelist member consisted of research students and teachers of CCT, Hattisar who had some previous experience (semi-trained) in sensory evaluation. The parameters for sensory evaluation were taken to be appearance/color, smell/flavor, teste and overall acceptability. Panelist were requested to give the points 1 to 9, 1 for extremely disliked and 9 for extremely liked sample. Each panelist was provided with coded samples with random numbers and evaluation card (Appendix A.)

3.3.4 Statistical analysis

Analysis of variance (ANOVA) was carried out for data from sensory evaluation. The data were analyzed for two-way ANOVA, mean ANOVA (No blocking at 5% level of significance), LSD and interaction effects using GenStat (GenStat Discovery Edition 12, 2009) at 5% significance level were obtained to determine whether the sample were significantly different from each other and to determine which one is superior among them. The specimen evaluation card used for the sensory test appears in Appendix A. The mean was compared using LSD method. Standard deviation and means were also analyzed from the same statistical tool.

3.3.5 Micro biological analysis

Total Plate Count (TPC) was determined by pour plate technique on Plate Count Agar (PCA) medium (incubated at 30°C/48 h). Coliform count was determined by pour plate technique on MacConkey medium (incubated at 37°C/48h) (Chemists and Chemists, 1925).

3.3.6 Storage studies

Prepared RTS juice was filled in sterilized plastic of 250 ml capacity which were further subjected to two different conditions i.e., room temperature and refrigeration temperature for 28 days. The samples were drawn at interval of 7 days and evaluated for physio-chemical properties (TSS, acidity, pH, reducing sugar) and microbiological qualities (TPC and coliform).

Part IV

Results and discussion

Whey pomegranate RTS beverage was prepared at the laboratory of Central Campus of Technology for the present study. The Pomegranate juice, Paneer whey and water were the major ingredient. Pomegranate juice was extracted using mixer and muslin cloth while the paneer whey was obtained by 2% citric acid coagulation. The extracted raw materials were filtered and different formulated sample was analyzed through physio-chemical analysis and sensory evaluation and best scored sample was stored for 28 days at refrigerated and normal condition for study of storage stability.

4.1 Analysis of potable water

The drinking water we used for the dilution and preparation of RTS was analyzed before the use and the results are shown in the Table 4.1 Chemical composition of water shows pH 6.7, hardness 54 ppm, alkalinity 87 ppm and coliform was absent, was under the specification of Indian standard for drinking water as mention by Haldar *et al.* (2016).

Table 4.1 Analysis of drinking water

Parameter	value
pH	6.7
Alkalinity	87 ppm
Hardness	54 ppm
Coliform	Not detected

4.2 Analysis of raw material

In the preparation of whey pomegranate RTS beverage, whey and pomegranate juice were the major ingredient which were analyzed for their chemical composition presented as in table 4.2.

The chemical composition of pomegranate juice was analyzed and result revealed that moisture content was 85.64%, TSS was 13.6 °Bx, acidity was 0.396% and pH was 3.76. This chemical composition data for the pomegranate juice was found to be a bit different than the data obtained by (Vučić *et al.*, 2019) and (El-Nemr *et al.*, 1990) for the pomegranate juice. This may be due to the variation of species, variety of the pomegranate produced. Similarly, chemical composition of paneer whey was observed as moisture 92.62%, TSS 5.8 °Bx, total acidity 0.28% and pH 5.14 respectively. The results reported were in close agreement with (Webb and Whittier, 1948). Also, variation on the whey composition may due to the variation in the milk constitute obtained and produced in the different parts of the world.

Table 4.2 Chemical composition of Raw materials

Parameter	Pomegranate juice	Whey
T.S.S (°Bx)	13.6 (0.15)	5.8 (0.1)
pH	3.76(0.03)	5.14 (0.015)
Moisture (%)	85.64 (0.032)	92.62 (0.018)
Acidity	0.396 (0.016)	0.28 (0.026)
Reducing sugar (%)	6.76 (0.021)	2.92 (0.022)
Non- reducing sugar (%)	5.32 (0.02)	2.68 (0.04)
Total sugar (%)	11.54 (0.043)	5.6 (0.01)
Protein (%)	0.22 (0.005)	0.46 (0.01)
Ash (%)	0.32 (0.005)	0.55 (0.018)
Fat (%)	0.35 (0.023)	0.42 (0.01)
Calcium (mg/100ml)	-	47 (0.6)
Total solids (%)	14.2 (0.01)	7.05 (0.018)

* Values are the means of three determinations. Figures in the parentheses are the standard deviations.

4.3 Effect of formulation on sensory characteristics

Five different samples of varying proportion of whey and water with 15% juice constant was taken and coded as A, B, C, D and E respectively. The TSS and acidity of the sample was kept constant i.e. 13 °Bx and 0.3 % respectively. Then the formulation having different proportion of whey and water was subject to sensory evaluation.

4.3.1 Color

The mean sensory score for color were found to be 8, 7.5, 6.6, 5.9, and 5.4 for the RTS formulations A, B, C, D and E respectively. Statistical analysis showed that whey utilized pomegranate RTS beverage had significant effect ($p>0.05$) on the color of the different RTS formulations. Sample C and D were significantly different to each other which is shown graphically in Fig. 4.1. Similarly, samples A&B and D&E were not significantly different at 5% level of significance (Appendix C). The sample A in sensory evaluation (color) was scored the highest i.e. 8.

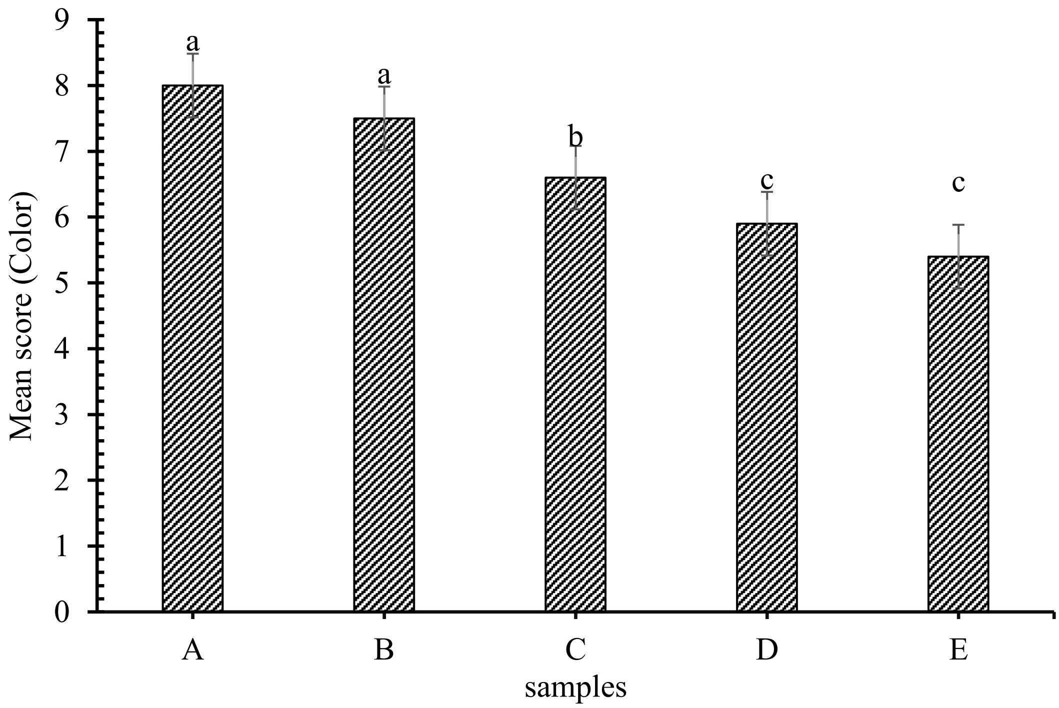

Fig. 4.1 Mean sensory score for color

*A, B, C, D and E denotes whey-pomegranate RTS beverage with 0, 25, 50, 75 and 100% whey replacement with water respectively. Fig 4.1 represents the mean sensory scores for color of whey-pomegranate RTS 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 panelist.

Pomegranate RTS beverages are valued for their vibrant red color. the pH of a whey-pomegranate RTS beverage plays a crucial role in determining its color. Maintaining an acidic pH will likely preserve the bright red color of pomegranate anthocyanins, while interactions with whey proteins and other factors could influence the final appearance of the beverage. Depending on the concentration of whey and its interaction with pomegranate pigments, the color intensity may be affected. Higher levels of whey could potentially dilute or alter the color of the beverage (McDougall *et al.*, 2005). (Panghal *et al.*, 2017b) also reported similar result in the preparation of whey utilized papaya RTS beverage.

4.3.2 Taste

The mean sensory score for taste were found to be 8.2, 7.5, 7.1, 6.2 and 5.9 for the RTS formulations A, B, C, D and E respectively. Statistical analysis showed that whey utilized pomegranate RTS beverage had significant effect ($p>0.05$) on the test of the different RTS formulations. Sample A and B and, sample D and E were not significantly different to each other which is shown graphically in Fig. 4.2. Similarly, sample B and C were also not significantly different at $p>0.05$ but significantly different to others. The sample A got highest score than other samples. The components in whey that influence the taste of a pomegranate RTS beverage include proteins (such as β-LG, α-LA, IgGs, BSA, and GMP), lactose, minerals (including calcium, phosphorus, magnesium, and sodium), and lipids. These elements contribute to different taste qualities such as sweetness, bitterness, umami, saltiness, and mouthfeel, all of which shape the beverage's overall sensory profile (Tunick, 2008).

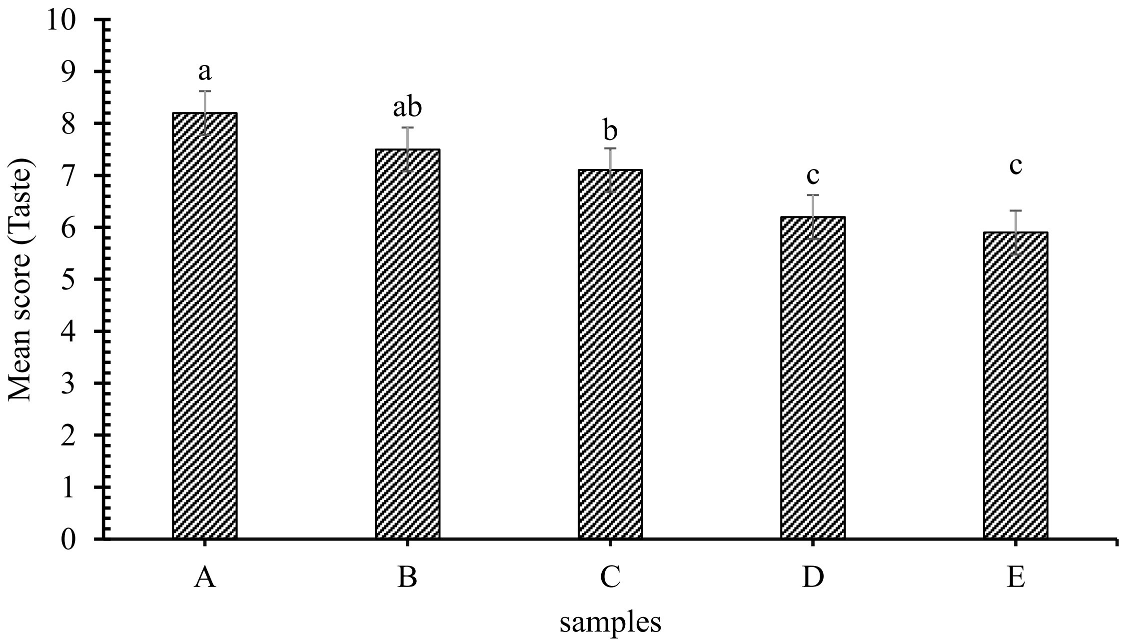

Fig. 4.2 Mean sensory score for Taste

*A, B, C, D and E denotes whey-pomegranate RTS beverage with 0, 25, 50, 75 and 100% whey replacement with water respectively. Fig 4.3 represents the mean sensory scores for taste of whey-pomegranate RTS 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 panelist. On increasing whey level, decrease in sensory score for the taste of the beverage. Similar result was observed by (Panghal *et al.*, 2017b) .

4.3.3 Flavor

The mean sensory score for flavor were found to be 8.1, 7.5, 6.6, 5.9 and 5.5 for the RTS formulations A, B, C, D and E respectively. Statistical analysis showed that whey utilized pomegranate RTS beverage had significant effect ($p>0.05$) on the flavor of the different RTS formulations. Sample A& B and C&D were not significantly different to each other which is shown graphically in Fig. 4.3. Similarly, sample D and E were also not significantly different at $p>0.05$ but significantly different to others. The sample A got highest score than other samples.

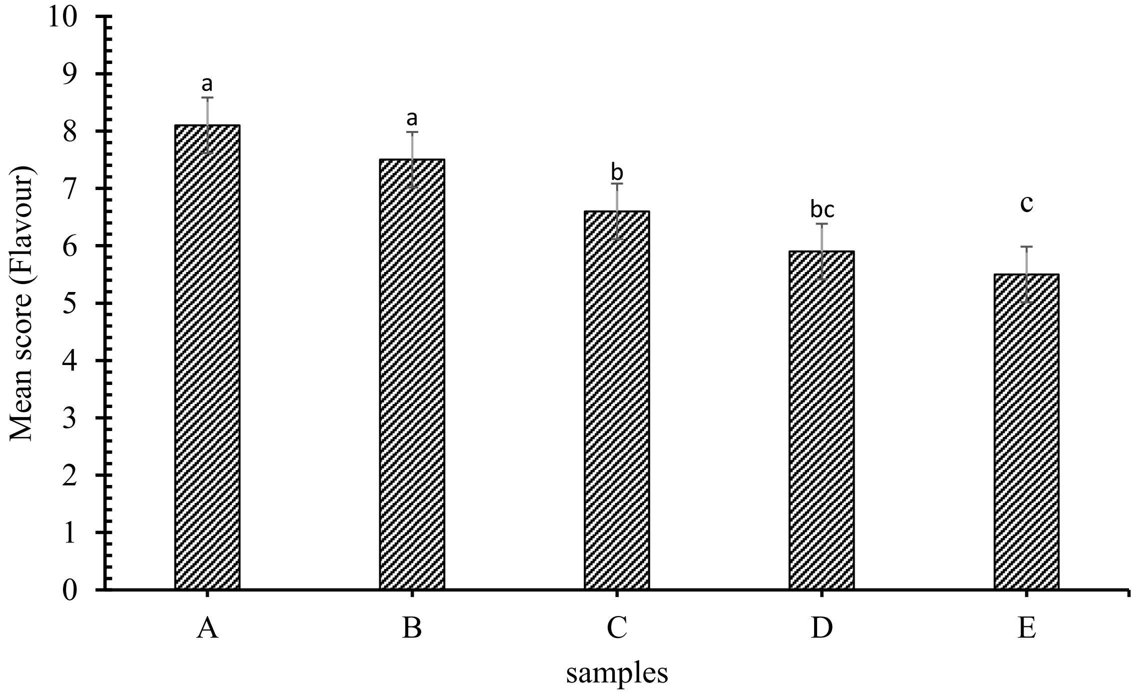

Fig. 4.3 Mean sensory score for flavor

*A, B, C, D and E denotes whey-pomegranate RTS beverage with 0, 25, 50, 75 and 100% whey replacement with water respectively. Fig 4.4 represents the mean sensory scores for flavor of whey-pomegranate RTS 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 panelist.

The incorporation of whey into pomegranate RTS beverages tends to result in a decrease in the intensity of the pomegranate flavor due to dilution effects, protein-flavor interactions, and changes in the balance of sweetness and tartness. The chemical interactions between whey proteins and polyphenolic compounds in fruit juices and their effects on flavor profiles (Vojnović *et al.*, 1993). Tornambé *et al.* (2006) stated that whey proteins can affect the volatility of aroma compounds, leading to a decrease in the aromatic profile of the pomegranate juice.

4.3.4 Overall Acceptability

The mean sensory score for overall acceptability were found to be 8.3, 8, 6.7, 6 and 5.6 for the RTS formulations A, B, C, D and E respectively. Statistical analysis showed that whey utilized pomegranate RTS beverage had significant effect (p>0.05) on the overall acceptability of the different RTS formulations.

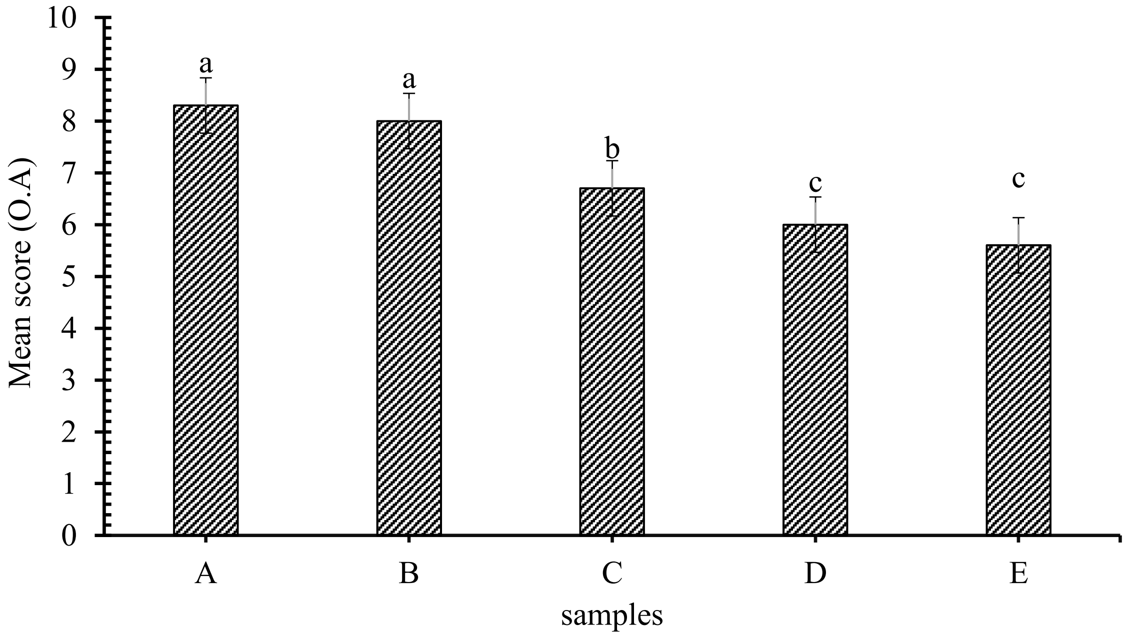

Fig. 4.4 Mean sensory score for overall acceptability

*A, B, C, D and E denotes whey-pomegranate RTS beverage with 0, 25, 50, 75 and 100% whey replacement with water respectively. Fig 4.2 represents the mean sensory scores for color of whey-pomegranate RTS 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 panelist.

Sample A and B were not significantly different to each other which is shown graphically in Fig. 4.4. Similarly, sample D and E were also not significantly different at $p>0.05$ but significantly different to others. The sample A got highest score than other samples. (Maheswarlu *et al.*, 2010a) Found that increase in whey incorporation in the manufacture of whey-pomegranate RTS decrease in sensory score for the overall acceptability of the beverage. According to (De Wit, 1998) and (Hernández-Ledesma *et al.*, 2011) the decrease in overall acceptability of pomegranate RTS beverages with increased whey incorporation can be attributed to several sensory changes, including flavor dilution, altered mouthfeel, and reduced aroma. These changes can make the beverage less appealing to consumers who expect a certain flavor profile and sensory experience from pomegranate juice. The combined effects of flavor masking, altered mouthfeel, and reduced aroma can lead to lower overall acceptability. These above findings were supported the result obtained.

4.4 Chemical composition of final product

The sample B with 25% (V/V) water replacement with whey is rated superior in the sensory evaluation. Pomegranate bland flavor and pomegranate test on the RTS 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 optimized product

S. N	Parameter	Final product
1.	TSS (°Bx)	12.9±0.07
2.	Moisture (%)	86.43±0.05
3.	pH	3.21±0.01
4.	Acidity (%)	0.3±0.02
5.	Reducing sugar (%)	7.27±0.04
6.	Non-reducing sugar (%)	6.23±0.03
7.	Total sugar (%)	13.43±0.1
8.	Proteins (%)	0.36±0.02
9.	Ash (%)	0.47±0.01
10.	Fat (%	0.21±0.03
11.	Calsium (mg/L)	148±0.6
12.	Total solids (%)	13.44±0.01

*Values are the means of three determinations. Figures in the parentheses are the standard deviations.

4.5 Storage study

Prepared whey-pomegranate RTS beverage was stored for 28 days' time period at refrigerated (R) condition and ambient (A) temperature. On every 7 days interval microbiological and chemical compositions were determined.

4.5.1 Microbiological analysis

In microbiological analysis TPC and coliform were performed and change in microbial counts during storage are given in fig 4.5. There was no any colony observed during initial storage period. During preparation of beverage heat treatment at 80°C for 10 min was done.

At this temperature treatment destruction or elimination of all viable organisms along with enzymes inactivation occurs in/on a food product. Due to storage condition in air-tight caped product, growth of microorganism was found to be slow.

Here, A= Ambient temperature R= Refrigeration temperature

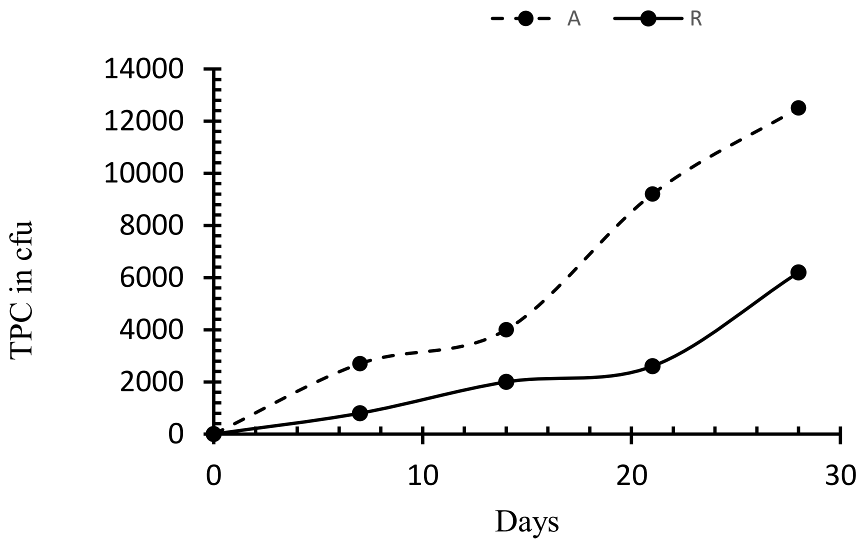

Fig 4.5. Change in TPC during storage of whey-pomegranate RTS

The result shows that no coliform was seen till 28 days but total plate count was found to be increasing with time of storage. Also, temperature had slight effect in increasing number of microbial load with time. Growth of microbial load during storage depends upon the packaging material, storage temperature and use of preservatives (Rahman *et al.*, 2011). (Rahman *et al.*, 2011) had reported maximum permitted count in juice is 1×10^4 cfu/ml thus, sample at room temperature exceed the limit after 28 days.

During the initial phase of storage, the Total Plate Count (TPC) in the RTS beverage increases steadily at ambient temperature, reflecting ongoing microbial growth, while refrigeration significantly slows this increase, demonstrating its effectiveness in inhibiting microbial activity. In the middle phase microbial growth accelerates under ambient conditions, bringing the TPC close to the maximum acceptable limit . In contrast, under refrigeration, the TPC continues to rise, but at a much slower pace, remaining well below the safety threshold. Finally, in the last phase , the TPC at ambient temperature surpasses the 10,000 cfu limit, indicating that the product is no longer microbiologically safe for consumption under these conditions. However, the TPC in refrigerated samples, although still increasing, remains below the maximum limit, suggesting that refrigeration is crucial in maintaining the microbiological safety of the RTS beverage throughout the 30-day storage period.

4.5.2 Chemical analysis

Effects of storage period on chemical composition (TSS, pH, titratable acidity and reducing sugar) of whey pomegranate beverage were analyzed. The data in the table compares the changes in Total Soluble Solids (TSS), acidity, reducing sugar , and pH of a beverage stored under ambient and refrigerated conditions over 28 days. The changes in physicochemical properties during storage is presented in Table 4.5.

Initially, both storage conditions start with a TSS of 13°Bx, acidity at 0.29%, reducing sugar at 7.06%, and pH at 3.21. Over time, under ambient conditions, TSS gradually increases from 13°Bx to 14.2°Bx, indicating a concentration of solids as the beverage likely loses moisture. Acidity also rises significantly, from 0.29% to 0.68%, showing a marked increase in sourness, while reducing sugar content increases slightly from 7.06% to 7.97%, likely due to the breakdown of complex sugars. The pH drops from 3.21 to 3.05, indicating an increase in acidity.

In contrast, the refrigerated storage shows a slower progression in these changes. TSS increases more gradually from 13°Bx to 13.7°Bx, and acidity rises from 0.29% to 0.45%, which is less dramatic than in ambient conditions. The reducing sugar content increases modestly from 7.06% to 7.59%, and the pH decreases slightly from 3.21 to 3.12. This indicates that refrigeration helps maintain the beverage's stability better, slowing down the rate of change in its physicochemical properties compared to ambient storage.

Table 4.5 Physicochemical properties during storage

Days/ parameter	Day-0 A/R		Day-7		Day-14		Day-21		Day-28	
	A	R	A	R	A	R	A	R	A	R
TSS(°Bx)	12.9	12.9	13.2	13.1	13.5	13.3	13.7	13.5	14.2	13.7
	(0.07)	(0.05)	(0.05)	(0.05)	(0.05)	(0.05)	(0.05)	(0.05)	(0.05)	(0.05)
pH	3.21	3.21	3.19	3.20	3.14	3.17	3.09	3.14	3.05	3.12
	(0.01)	(0.02)	(0.01)	(0.01)	(0.04)	(0.01)	(0.01)	(0.03)	(0.01)	(0.06)
Acidity (%)	0.29	0.29	0.34	0.31	0.41	0.35	0.51	0.40	0.68	0.45
	(0.04)	(0.01)	(0.01)	(0.03)	(0.06)	(0.01)	(0.02)	(0.01)	(0.01)	(0.07)
Reducing Sugar (%)	7.06	7.06	7.30	7.20	7.52	7.31	7.85	7.46	7.97	7.59
	(0.05)	(0.02)	(0.01)	(0.03)	(0.01)	(0.01)	(0.08)	(0.04)	(0.01)	(0.05)

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

4.5.2.1 Effect on TSS

A slight increase in TSS of whey-pomegranate RTS during 28 days of storage at different storage condition was observed (Fig 4.6). The two-way ANOVA analysis reveals that both the condition and the number of days significantly influence the levels of Total Soluble Solids (TSS). The condition has a notable impact, as evidenced by a high F-value of 15.59 and a p-value of less than 0.001, indicating that different conditions lead to statistically significant differences in TSS. Similarly, the storage days shows an even stronger effect on TSS, with an F-value of 37.46 and a p-value of less than 0.001, suggesting that the duration over which the TSS is measured significantly affects its levels. (Appendix C).

Retention or minimum increase in TSS content of juice during storage is desirable for preservation of good juice quality (Bhardwaj *et al.*, 2011). Similar results were observed in the preparation of mixed fruit RTS by Bull *et al.* (2004). A gradual rise in TSS value during storage of fruit juice has been reported under all storage condition which might be associated with continuous increase in hydrolysis of polysaccharides and acids. The gradual passage of storage time as function of increase in TSS may be due to greater hydrolysis of polysaccharides. However, this rise in TSS was functional to storage temperature. This might be correlated with lower rate of hydrolysis of sugars, polysaccharides and organic acids at lower temperature following the La Chatelier Principle of chemical reaction (Sharma *et al.*, 2011).

Here, A= Ambient temperature R= Refrigeration temperature

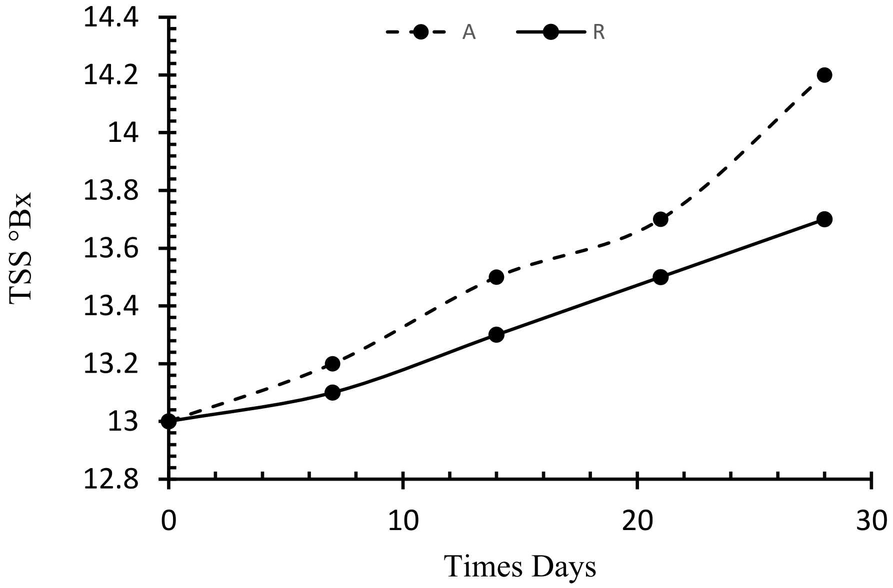

Fig.4.6 Effect of storage time and temperature on TSS of prepared beverage

4.5.2.2 Effect on acidity

A slight increase in acidity of whey-pomegranate RTS during 28 days of storage at different storage condition was observed (Fig 4.7).

The effect of storage on the acidity of beverage is shown in fig 4.7. During 28 days of storage the acidity was found to have gradually increased from 0.29 to 0.68 (Ambient temperature) and 0.29 to 0.45 (refrigeration temperature). The two-way ANOVA results reveal significant effects of both storage conditions and storage days on Total Titratable acidity. The storage condition has an F-value of 25.91 with a p-value < 0.001, indicating a highly significant difference in acidity between ambient and refrigeration temperatures. Similarly, the number of storage days has an F-value of 33.86 with a p-value < 0.001, showing a significant variation in TSS over time. (Appendix D).

Here, A= Ambient temperature R= Refrigeration temperature

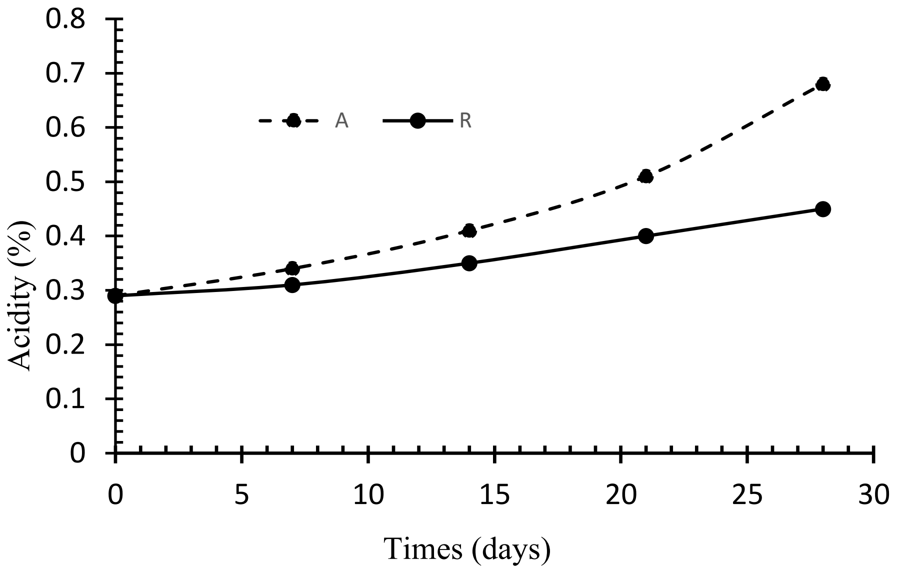

Fig.4.7 Effect of storage time and temperature on acidity of prepared RTS beverage

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 finding reported in whey based banana herbal beverage (Yadav *et al.*, 2010) and also in whey based RTS from mango (Shukla *et al.*, 2000).

4.5.2.3 Effect on reducing sugar (RS)

During 28 days of storage, cc. The analysis reveals that the type of condition has a substantial impact on reducing sugar with an F-value of 42.79 and a p-value less than 0.001, indicating that the sugar levels differ significantly between ambient and refrigeration conditions. Similarly, the effect of time is also significant, with an F-value of 61.73 and a p-value less than 0.001, suggesting that reducing sugar levels change considerably over the days. (Appendix D) Overall, these results suggest that both the storage condition and the duration of storage play crucial roles in influencing the reducing sugar levels.

Here, A= Ambient temperature   R= Refrigeration temperature

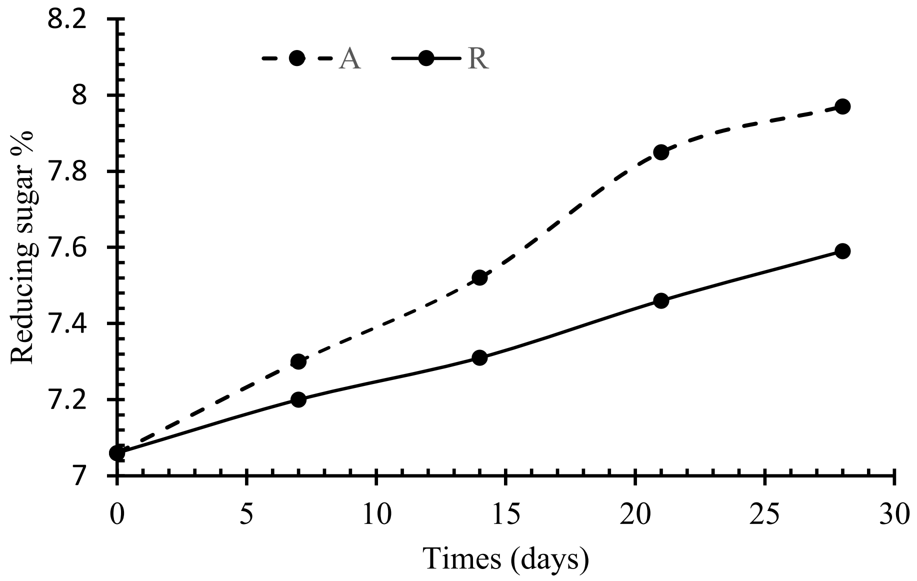

Fig.4.8 Effect of storage time and temperature on acidity of prepared RTS beverage

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).

4.5.2.4 Effect on pH

The effect of storage on the pH of beverage is shown in fig 4.9. During storage pH was found to be gradually decreased from 3.21 to 3.02 (Ambient temperature) and 3.21 to 3.12 (Refrigeration temperature) that affects the organoleptic quality of juice. This may be due to the production of organic acids which results from the action of ascorbic acid on sugar and protein content of the beverage. Lactose and protein are converted into lactic acid and amino acids leading to decrease in pH of the beverage. Similar results were reported for bottle gourd blended juice and basil leaves juice by (Majumdar *et al.*, 2011) and for pineapple juice blend with carrot and banana Jan and Masih (2012).

Here, A= Ambient temperature   R= Refrigeration temperature

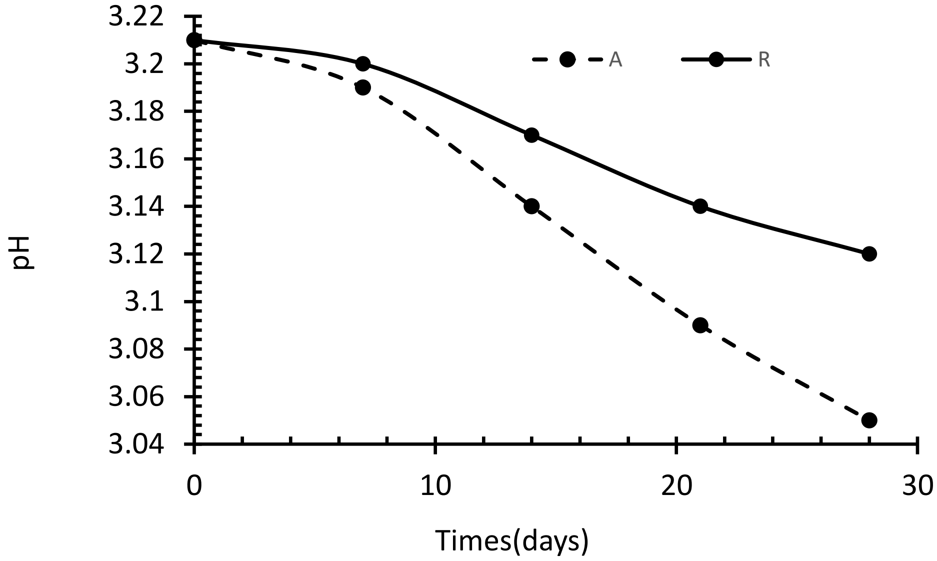

Fig.4.9 Effect of storage time and temperature on pH of prepared beverage

The analysis of pH for the whey pomegranate RTS under different storage conditions reveals that both the storage condition and the duration of storage have a significant impact on the product's stability and quality. The two-way ANOVA results demonstrate that variations in both storage conditions and storage days lead to statistically significant differences in the PH of RTS. The high F-values and extremely low p-values (<.001) for both factors indicate that the differences observed are not due to random chance but are indeed influenced by the specific storage conditions and the length of time the product was store.

Part V

Conclusions and recommendations

5.1 Conclusion

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

1. From sensory analysis, whey utilized pomegranate RTS with 25 % water replacement and 12.9°Bx TSS was found superior with respect to color, flavor, taste and overall acceptance.
2. During 28 days of storage TSS, acidity and reducing sugar content increased while pH content decreased. These change in refrigerated storage condition were found to be minor with respect to normal storage condition.
3. Production cost (NRs 30.44) of prepared beverage was reasonable, within the reach of general population and much lower than commercial fruit juices production cost for the year 2023.

5.2 Recommendations

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

1. Shelf life of beverage (storage stability) can be studied using different chemical preservatives, different packaging materials and with different storage conditions.
2. Formulation of such type of RTS beverage can be done with varying proportion of different fruit juices.

PART VI

Summary

Milk whey is highly nutritious by-products obtained from the diary industry producing cheese, chhana 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. For utilization of whey by the preparation of whey-pomegranate RTS beverage can be a good solution.

For the study, pomegranate, Milk and sugar were purchased from the local market of Dharan. Whey was separated by coagulating with 2% citric acid solution during paneer making while pomegranate juice was extracted using juice extractor and the separated juice stored in refrigerated conditions for further analysis. Different formulated beverage were evaluated on a 9-point hedonic rating (1=dislike extremely, 9=like extremely) be ten semi-trained panelist. Prepared RTS (whey-pomegranate) only with 25% whey as water replacement and TSS 12.9 (°Bx) was found to be best in overall sensory evaluation.

The superior beverage from the sensory analysis was analyzed for chemical composition and storage stability under refrigerated and normal storage condition. The analyzed chemical composition of best sample was found to be TSS (12.9 °Bx), Moisture (86.43%), pH(3.21), Acidity (0.3), Reducing sugar (7.27%), Non-reducing sugar (6.23%), Total sugar(13.43%), and Total solids (13.44%). During the storage study TSS, pH and reducing sugar increased while acidity decreased under both storage conditions.

The study concluded that the formulated whey-Pomegranate RTS beverage was both nutritionally beneficial and appealing in taste. Most fruit beverages available today are synthetic, flavored, and commercially bottled. Replacing these with whey beverages would be environmentally friendly and cost-effective for consumers while aiding the dairy industry in managing whey disposal issues.

References

Al-Maiman, S. A. and Ahmad, D. J. F. C. (2002). Changes in physical and chemical properties during pomegranate (Punica granatum L.) fruit maturation. *"In: Food Chemistry"* **76** (4), 437-441. [doi:10.1016/S0308-8146(01)00301-6].

Ali, S. and Clark, A. J. J. J. o. M. B. (1988). Characterization of the gene encoding ovine beta-lactoglobulin: similarity to the genes for retinol binding protein and other secretory proteins. *J. Mol. Biol*. **199** (3), 415-426. [doi:10.1016/0022-2836(88)90614-6].

Anirudh, J., Dhineshkumar, V. and Sangeetha, T. J. J. o. P. T. (2022). Whey as crucial component in rejuvenating athlete health-a review. *J. post harvest technol*. **10** (4), 135-155.

Atreya, P., Shrestha, C., Suvedi, B. and Pandey, S. (2020). Emerging fruits of Nepal: pomegranate, kiwifruit, avocado, dragon fruit and grape; opportunities, challenges and ways forward. *Proceeding of 11th National Horticulture Seminar*. 6-7.

Ayoub, A., Sofi, S., Mohite, A. J. I. J. o. A., Environment and Biotechnology. (2017). Physico-chemical Analysis of Fruit Juices by Using Self-Made Low Cost Portable Ohmic Heater. *Int. J. Environ. Agric. Biotech*. **10** (6), 739-748. [doi:10.5958/2230-732X.2017.00091.2].

Begum, T., Islam, Z., Siddiki, M. S. R., Habib, R. and Rashid, H.-u. (2019). Preparation of fermented beverage from whey-based watermelon (Citrullus lanatus) juice. *Asian J. Dairy Food Res*. . **38** (4), 301-306. [doi:10.18805/ajdfr.DR-150].

Bhardwaj, R. L., Pandey, S. J. C. r. i. f. s. and nutrition. (2011). Juice blends—a way of utilization of under-utilized fruits, vegetables, and spices: a review. *Crit. Rev. Food Sci. Nutr.* **51** (6), 563-570. [doi:10.1080/10408391003710654].

Blomstrand, E. and Newsholme, E. J. A. p. s. (1992). Effect of branched-chain amino acid supplementation on the exercise-induced change in aromatic amino acid concentration in human muscle. *Acta Physiol. Scand.* **146** (3), 293-298. [doi:10.1111/j.1748-1716.1992.tb09422.x].

Bohora, G. (2018). Preparation of whey based pineapple beverage and its storage quality evaluation

Bounous, G. J. A. r. (2000). Whey protein concentrate (WPC) and glutathione modulation in cancer treatment. *Anticancer Res.* **20** (6), 4785-4792. [doi:10.1006/mcbr.2000.0238.].

Bull, M. K., Zerdin, K., Howe, E., Goicoechea, D., Paramanandhan, P., Stockman, R., Sellahewa, J., Szabo, E. A., Johnson, R. L., Stewart, C. M. J. I. F. S. and Technologies, E. (2004). The effect of high pressure processing on the microbial, physical and chemical properties of Valencia and Navel orange juice. *Innov Food Sci Emerg Technol.* **5** (2), 135-149. [doi:10.1016/j.ifset.2003.11.005].

Ceclu, L. and Nistor, O. J. J. N. M. D. C. (2020). Red beetroot: Composition and health effects—A review. *J. Nutr. Med. Diet Care.* **6** (1), 1-9. [doi:10.23937/2572-3278.1510043].

Chandra, R., Babu, K. D., Jadhav, V. T., Jaime, A., Silva, T. J. F., Vegetable, Science, C. and Biotechnology. (2010). Origin, history and domestication of pomegranate. *Fruit, Veg. Cereal Sci. Biotech.* **2**, 1-6.

Chatterjee, G., De Neve, J., Dutta, A. and Das, S. J. R. m. d. i. q. (2015). Formulation and statistical evaluation of a ready-to-drink whey based orange beverage and its storage stability. **14** (2), 253-264.

Chavan, R., Shraddha, R., Kumar, A., Nalawade, T. J. J. o. F. P. and Technology. (2015a). Whey based beverage: its functionality, formulations, health benefits and applications. **6** (10), 1.

Chavan, R., Shraddha, R., Kumar, A., Nalawade, T. J. J. o. F. P. and Technology. (2015b). Whey based beverage: its functionality, formulations, health benefits and applications. *J. Food Process. Technol.* **6** (10), 1. [doi::10.4172/2157-7110.1000495].

Chemists, A. o. O. A. and Chemists, A. o. O. A. (1925). "Official methods of analysis of the Association of Official Analytical Chemists". Vol. 2. The Association.

Clare, D. and Swaisgood, H. J. J. o. d. s. (2000). Bioactive milk peptides: a prospectus. *J. Dairy Sci.* **83** (6), 1187-1195. [doi:10.3168/jds.S0022-0302(00)74983-6].

De Wit, J. (1998). Nutritional and functional characteristics of whey proteins in food products. **81** (3), 597-608.

Dhamsaniya, N. and Varshney, A. (2013). Development and evaluation of whey based RTS beverage from ripe banana juice. *Int. J. Food Process. Technol.* [doi:10.4172/2157-7110.1000203].

Dissanayake, M. (2011). Modulation of functional properties of whey proteins by microparticulation. Victoria University,

El-Nemr, S., Ismail, I. and Ragab, M. (1990). Chemical composition of juice and seeds of pomegranate fruit. **34** (7), 601-606.

Fahmy, H., Hegazi, N., El-Shamy, S. and Farag, M. A. (2020). Pomegranate juice as a functional food: A comprehensive review of its polyphenols, therapeutic merits, and recent patents. **11** (7), 5768-5781.

Faria, A. and Calhau, C. (2010). Pomegranate in human health: An overview. 551-563.

Farrell, H. M., Wickham, E. D., Dower, H. J., Piotrowski, E. G., Hoagland, P. D., Cooke, P. H. and Groves, M. L. J. J. o. P. C. (1999). Characterization of the particles of purified κ-casein: Trypsin as a probe of surface-accessible residues. *J. Protein Chem.* **18**, 637-652.

Farrell Jr, H., Jimenez-Flores, R., Bleck, G., Brown, E., Butler, J., Creamer, L., Hicks, C., Hollar, C., Ng-Kwai-Hang, K. and Swaisgood, H. J. J. o. d. s. (2004). Nomenclature of the proteins of cows' milk—Sixth revision. *J. Dairy Sci.* **87** (6), 1641-1674. [10.3168/jds.S0022-0302(04)73319-6].

Fitzsimons, S. M., Mulvihill, D. M. and Morris, E. R. (2007). Denaturation and aggregation processes in thermal gelation of whey proteins resolved by differential scanning calorimetry. **21** (4), 638-644.

Frid, A. H., Nilsson, M., Holst, J. J. and Björck, I. M. J. T. A. j. o. c. n. (2005a). Effect of whey on blood glucose and insulin responses to composite breakfast and lunch meals in type 2 diabetic subjects. **82** (1), 69-75.

Frid, A. H., Nilsson, M., Holst, J. J. and Björck, I. M. J. T. A. j. o. c. n. (2005b). Effect of whey on blood glucose and insulin responses to composite breakfast and lunch meals in type 2 diabetic subjects–. **82** (1), 69-75.

Gabriel Michael, J., Ringenback, R. and Hottenstein, S. J. J. o. I. D. (1971). The antimicrobial activity of human colostral antibody in the newborn. **124** (5), 445-448.

Gangurde, H., Chordiya, M., Patil, P. and Baste, N. J. S. R. J. (2011). Whey protein. **1** (2).

Gil, M. I., García-Viguera, C., Artés, F., Tomás-Barberán, F. A. J. J. o. t. S. o. F. and Agriculture. (1995). Changes in pomegranate juice pigmentation during ripening. *J. Sci. Food Agric.* **68** (1), 77-81. [doi:10.1002/jsfa.2740680113].

Gil, M. I., Tomás-Barberán, F. A., Hess-Pierce, B., Holcroft, D. M., Kader, A. A. J. J. o. A. and chemistry, F. (2000a). Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. **48** (10), 4581-4589.

Gil, M. I., Tomás-Barberán, F. A., Hess-Pierce, B., Holcroft, D. M., Kader, A. A. J. J. o. A. and chemistry, F. (2000b). Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *J. Agric. Food Chem.* . **48** (10), 4581-4589.

Haldar, D., Halder, S., Das, P., Halder, G. J. D. and Treatment, W. (2016). Assessment of water quality of Damodar River in South Bengal region of India by Canadian Council of Ministers of Environment (CCME) Water Quality Index: a case study. *Sustain. Water Resour. Manag.* **57** (8), 3489-3502. [doi:10.1007/s40899-017-0159-7].

Hapase, P. (2004). Preparation of chakkawhey Beverage using Mango pulp. Mahatma Phule Krishi Vidyapeeth, Rahuri, Harde, H., Schumacher, W., Firbas, F. and Deffer, D. J. C., London. (1970). Strasburg's textbook of botany. **2**.

Harper, W. J. (2004). Biological properties of whey components.

Haug, A., Høstmark, A. T., Harstad, O. M. J. L. i. h. and disease. (2007). Bovine milk in human nutrition–a review. *Lipids Health Dis.* **6** (1), 1-16. [doi:10.1186/1476-511X-6-25].

Hernández-Ledesma, B., Ramos, M. and Gómez-Ruiz, J. Á. (2011). Bioactive components of ovine and caprine cheese whey. **101** (1-3), 196-204.

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

Ismail, A. E., Abdelgader, M. O., Ali, A. A. J. A. J. o. F. S. and Technology. (2011). Microbial and chemical evaluation of whey-based mango beverage. *Adv. J. Food Sci. Technol.* **3** (4), 250-253.

Jan, A., Masih, E. D. J. I. J. o. S. and Publications, R. (2012). Development and quality evaluation of pineapple juice blend with carrot and orange juice. *Int. J. Sci. Res.* **2** (8), 1-8.

Jbir, R., Hasnaoui, N., Mars, M., Marrakchi, M. and Trifi, M. J. S. H. (2008). Characterization of Tunisian pomegranate (Punica granatum L.) cultivars using amplified fragment length polymorphism analysis. *Sci. Hortic.* **115** (3), 231-237. [doi10.1016/j.scienta.2007.09.002].

Jelen, P. J. E. o. F. S. and Technology. (1992). Whey: composition, properties, processing and uses. *Int. Dairy J.* **4**, 2835-2845.

Jeličić, I., Božanić, R. and Tratnik, L. J. M. č. z. u. p. i. p. m. (2008a). Whey based beverages-new generation of dairy products. *J Dairy Sci Technol.* **58** (3), 257-274.

Jeličić, I., Božanić, R. and Tratnik, L. J. M. č. z. u. p. i. p. m. (2008b). Whey based beverages-new generation of dairy products. *J Dairy Sci Technol.*. **58** (3), 257-274.

Johnson, J. A. (2012). FDA regulation of medical devices: Congressional Research Service Washington, DC, USA.

Joshi, P., Waghmare, P., Zanjad, P. and Khedkar, D. (1985). Utilization of curd whey for the preparation of fruit jelly. *J. Food Sci. Technol.*

Kadam, B., Ambadkar, R., Rathod, K. and Landge, S. J. I. J. o. L. R. (2018). Health benefits of whey: A brief review. *Int. J. Livest. Res.* **8** (5), 31-49.

Kalra, S., Tandon, D., Garg, N. and Singh, B. J. I. F. P. (1991). Quality evaluation of some market drinks. **45**, 40-53.

Kandylis, P. and Kokkinomagoulos, E. J. F. (2020a). Food applications and potential health benefits of pomegranate and its derivatives. **9** (2), 122.

Kandylis, P. and Kokkinomagoulos, E. J. F. (2020b). Food applications and potential health benefits of pomegranate and its derivatives. *Foods.* **9** (2), 122. [doi:10.3390/foods9020122].

Kausar, H., Saeed, S., Ahmad, M. M. and Salam, A. J. J. A. R. (2012). Studies on the development and storage stability of cucumber-melon functional drink. *J. Agric. Res.* **50** (2), 239-248.

Keri Marshall, N. J. A. m. r. (2004). Therapeutic applications of whey protein. **9** (2), 136-156.

Khamrui, K. and Rajorhia, G. (1998). Formulation of ready-to-serve whey based kinnow juice beverage. **51** (6), 413-419.

Khedkar, J., Choudhari, D., Pawar, B. and Kadam, V. (2014). Use of milk by-product (whey) for preparation of beverages. *Asian J. Anim. Sci.*

Kulkarni, A. P., Aradhya, S. M. and Divakar, S. J. F. c. (2004). Isolation and identification of a radical scavenging antioxidant–punicalagin from pith and carpellary membrane of pomegranate fruit. *Food Chem.* **87** (4), 551-557. [doi:10.1016/j.foodchem.2004.01.006].

Lawless, H. T. and Heymann, H. (2010). "Sensory evaluation of food: principles and practices". Springer Science & Business Media. [1441964886].

Lazeeza, S. J. I. j. o. a. s. (2021). Antioxidant activity of pomegranate. **52** (1), 196-203.

León-López, A., Pérez-Marroquín, X. A., Campos-Lozada, G., Campos-Montiel, R. G. and Aguirre-Álvarez, G. J. F. (2020). Characterization of whey-based fermented beverages supplemented with hydrolyzed collagen: Antioxidant activity and bioavailability. *J. Food Sci. Technol.*

. **9** (8), 1106. [doi:10.3390/foods9081106].

Macwan, S. R., Dabhi, B. K., Parmar, S., Aparnathi, K. J. I. J. o. C. M. and Sciences, A. (2016). Whey and its utilization. *Int. J. Curr. Microbiol. App. Sci.* **5** (8), 134-155.

Maheswarlu, M., Anitha, L., Sucharitha, K. and Leelavathi, M. (2010a). Qualitative changes in whey based pomegranate (Punica granatum) beverage during storage period. **3** (3), 669-672.

Maheswarlu, M., Anitha, L., Sucharitha, K. and Leelavathi, M. J. A. J. o. R. i. C. (2010b). Qualitative changes in whey based pomegranate (Punica granatum) beverage during storage period. *Asian J. Sci. Res.* **3** (3), 669-672.

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

Marcelo, P. A. and Rizvi, S. S. J. I. D. J. (2008). Physicochemical properties of liquid virgin whey protein isolate. **18** (3), 236-246.

McDougall, G., Fyffe, S., Dobson, P. and Stewart, D. (2005). Anthocyanins from red wine–their stability under simulated gastrointestinal digestion. **66** (21), 2540-2548.

McIntosh, G. H., Royle, P. J., Le Leu, R. K., Regester, G. O., Johnson, M. A., Grinsted, R. L., Kenward, R. S. and Smithers, G. W. (1998). Whey proteins as functional food ingredients? **8** (5-6), 425-434.

Mehmood, A. M. M. T., Iyer, A. B., Arif, S., Junaid, M., Khan, R. S., Nazir, W., Khalid, N. J. S. and Drinks, E. (2019). Sports and Energy Drinks.

Meilgaard, M. C., Carr, B. T. and Civille, G. V. (1999). "Sensory evaluation techniques". CRC press. [1003040721].

Meisel, H. and Bockelmann, W. J. A. V. L. (1999). Bioactive peptides encrypted in milk proteins: proteolytic activation and thropho-functional properties. **76**, 207-215.

Melnik, B. C., Schmitz, G., John, S. M., Carrera-Bastos, P., Lindeberg, S., Cordain, L. J. N. and metabolism. (2013). Bioactive peptides encrypted in milk proteins: proteolytic activation and thropho-functional properties

10, 1-6. [doi:10.1186/1743-7075-10-60].

Mohamed, A., Abbas, H. M. and Zayan, A. F. J. L. S. J. (2014). Physicochemical and microbiological properties of papaya functional beverages based on sweet cheese whey. *Life Sci.* **11** (3), 257-263.

Næs, T., Brockhoff, P. B. and Tomic, O. (2011). "Statistics for sensory and consumer science". John Wiley & Sons. [1119957249].

Nagar, S., Nagal, S. J. I. J. o. F. and Technology, F. (2013). Whey: Composition, role in human health and its utilization in preparation of value added products. **3** (2), 93.

Nilugin, S. and Mahendran, T. J. J. o. A. S. (2011). Preparation of ready-to-serve (RTS) beverage from palmyrah (Borassus flabellifer L.) fruit pulp. **5** (2).

Omole, J. O., Ighodaro, O. M. and Makanjuola, O. M. (2012). Proximate composition of whey from South West Nigeria.

Onwulata, C. and Huth, P. (2009). "Whey processing, functionality and health benefits". Vol. 82. John Wiley & Sons. [081380387X].

Panghal, A., Kumar, V., Dhull, S. B., Gat, Y. and Chhikara, N. (2017a). Utilization of dairy industry waste-whey in formulation of papaya RTS beverage. **5** (2), 168-174.

Panghal, A., Kumar, V., Dhull, S. B., Gat, Y., Chhikara, N. J. C. R. i. N. and Journal, F. S. (2017b). Utilization of dairy industry waste-whey in formulation of papaya RTS beverage. *Curr. res. nutr. food sci.* **5** (2), 168-174.

Panghal, A., Sharma, K., Setia, S. and Ray, A. (2007). Studies on utilization of whey for preparation of ready to serve (RTS) beverages. **34** (8), 77-79.

Pérez, M. D. and Calvo, M. J. J. o. d. s. (1995). Interaction of β-lactoglobulin with retinol and fatty acids and its role as a possible biological function for this protein: a review. *Int. J. Dairy Sci.* **78** (5), 978-988. [doi:10.3168/jds.S0022-0302(95)76713-3].

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

Poudel, K., Ansari, A. R. and Karki, S. (2017). Pomological characteristics of four pomegranate (Punica granatum L.) cultivars grown in eastern mid-hills of Nepal. *Proceedings of Ninth National Horticulture Workshop*.

Prakash, C. V. S. and Prakash, I. (2011). Bioactive chemical constituents from pomegranate (Punica granatum) juice, seed and peel-a review. **1** (1), 1-18.

Rahman, T., Hasan, S. and Noor, R. (2011). An assessment of microbiological quality of some commercially packed and fresh fruit juice available in Dhaka city: A comparative study. **1** (1), 13-18.

Rai, B. and Kc, J. (2007). Basic food analysis handbook.

Ranganna, S. (1986). "Handbook of analysis and quality control for fruit and vegetable products". Tata McGraw-Hill Education. [0074518518].

Said, H. M., Ong, D. E. and Shingleton, J. L. J. T. A. j. o. c. n. (1989). Intestinal uptake of retinol: enhancement by bovine milk beta-lactoglobulin. **49** (4), 690-694.

Seyfert, H.-M., Tuckoricz, A., Interthal, H., Koczan, D. and Hobom, G. J. G. (1994). Structure of the bovine lactoferrin-encoding gene and its promoter. *Gene.* **143** (2), 265-269. [doi:10.1016/0378].

Sharma, N., Tanwer, B. S. and Vijayvergia, R. (2011). Study of medicinal plants in Aravali regions of Rajasthan for treatment of kidney stone and urinary tract troubles. **3** (1), 110-113.

Shiby, V. K., Radhakrishna, K., Bawa, A. S. J. I. J. o. F. S. and Technology. (2013). Development of whey-fruit-based energy drink mixes using D-optimal mixture design. **48** (4), 742-748.

Shukla, F., Sharma, A. and Bakshi, A. (2000). Studies on the preparation offruit juice beverages using milk by-products. *International Conference on Processed Foods for 21st Century. Calcutta, India. January*. 14-16.

Silva e Alves, A. T., Spadoti, L. M., Zacarchenco, P. B. and Trento, F. K. J. B. (2018). Probiotic functional carbonated whey beverages: Development and quality evaluation. **4** (3), 49.

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

Singh, W., Kapoor, C. and Srivastava, D. (1999). Standardization of technology for the manufacture of guava whey beverage. (5).

Smithers, G. W., Ballard, F. J., Copeland, A. D., de Silva, K. J., Dionysius, D. A., Francis, G. L., Goddard, C., Grieve, P. A., McIntosh, G. H. and Mitchell, I. R. (1996). New opportunities from the isolation and utilization of whey proteins. **79** (8), 1454-1459.

Sreekumar, S., Sithul, H., Muraleedharan, P., Azeez, J. M. and Sreeharshan, S. J. B. r. i. (2014). Pomegranate fruit as a rich source of biologically active compounds. **2014**.

Stone, H. and Sidel, J. (2004). Sensory Evaluation Practices, Elsevier Academic Press.

Syed, D. N., Afaq, F. and Mukhtar, H. (2007). Pomegranate derived products for cancer chemoprevention. *Seminars in cancer biology*. **17**, 377-385.

Tanwar, T. K., Wagh, R., Mehta, N., Malav, O., Kour, S. and Kumar, P. J. P. I. J. (2022). Preparation of functional beverage from whey-based mango juice. **11** (7), 4710-4716.

www.thepharmajournal.com.

Tawa Jr, N. E., Goldberg, A. L. J. A. J. o. P.-E. and Metabolism. (1992). Suppression of muscle protein turnover and amino acid degradation by dietary protein deficiency. *Am J Physiol.* **263** (2), E317-E325. [doi:10.1152/ajpendo.1992.263.2.E317].

Tornambé, G., Cornu, A., Pradel, P., Kondjoyan, N., Carnat, A., Petit, M. and Martin, B. (2006). Changes in terpene content in milk from pasture-fed cows. **89** (6), 2309-2319.

Tunick, M. H. (2008). Whey protein production and utilization: a brief history. 1-13.

Vardin, H. and Fenercioğlu, H. J. F. n. (2003). Study on the development of pomegranate juice processing technology: clarification of pomegranate juice. *Mol. Nutr. Food Res.* **47** (5), 300-303. [doi:10.1002/food.200390070]

Vegara, S., Martí, N., Mena, P., Saura, D., Valero, M. J. L.-F. S. and Technology. (2013). Effect of pasteurization process and storage on color and shelf-life of pomegranate juices. *LWT - Food Sci. Technol.* **54** (2), 592-596. [doi:10.1016/j.lwt.2013.06.022].

Vilas-Boas, A. A., Magalhães, D., Campos, D. A., Porretta, S., Dellapina, G., Poli, G., Istanbullu, Y., Demir, S., San Martín, Á. M. and García-Gómez, P. (2022). Innovative processing technologies to develop a new segment of functional citrus-based beverages: current and future trends. **11** (23), 3859.

Villarreal, M. (2017). Value added products utilizing acid whey: development of a fruit yogurt beverage and a sports drink. *J. Undergrad. Res.* Cornell University,

Vojnović, V., Ritz, M. and Vahčić, N. (1993). Sensory evaluation of whey-based fruit beverages. **37** (3), 246-251.

Vučić, V., Grabež, M., Trchounian, A. and Arsić, A. (2019). Composition and potential health benefits of pomegranate: a review. **25** (16), 1817-1827.

Webb, B. H. and Whittier, E. O. (1948). The utilization of whey: a review. **31** (2), 139-164.

Wu, S.-Y., Perez, M. D., Puyol, P. and Sawyer, L. J. J. o. B. C. (1999). β-Lactoglobulin binds palmitate within its central cavity. *J. Biol. Chem.* **274** (1), 170-174. [doi:10.1074/jbc.274.1.170].

Yadav, R. B., Yadav, B. S. and Kalia, N. J. A. J. F. T. (2010). Development and storage studies on whey-based banana herbal (Mentha arvensis) beverage. *Am. J. Food Technol.* **5** (2), 121-129. [doi:10.3923/ajft.2010.121.129].

Yalcin, A. S. J. C. p. d. (2006). Emerging therapeutic potential of whey proteins and peptides. *Curr Pharm Des.* **12** (13), 1637-1643. [doi:10.2174/138161206776843296].

Yvon, M., Beucher, S., Guilloteau, P., Le Huerou-Luron, I. and Corring, T. J. R. N. D. (1994). Effects of caseinomacropeptide (CMP) on digestion regulation. *Reprod Nutr Dev.* **34** (6), 527-537. [doi: 10.1051/rnd:19940602].

Zarban, A., Malekaneh, M. and REZA, B. M. (2007). Antioxidant properties of pomegranate juice and its scavenging effect on free radicals. *J. Agric. Food Chem.*

Appendices

Appendix-A

Specimen card for sensory evaluation

Hedonic rating test

Name: …………………………………
Date……………………….

Product: Whey- Pomegranate ready to serve (RTS)

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

Quality Description:

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

S.N.	Samples	Appearance	Color	Flavor	Taste	Overall Acceptance
1.	A					
2.	B					
3.	C					
4.	D					
5.	E					

Comments……………………………………………………………………………………
…………………………………………………………………

Signature

Appendix B

ANOVA results of sensory analysis

Table B.1 ANOVA (Two-way no blocking) at 5% level of significance for Color

Source of variation	d.f	s.s.	m.s	v.r.	F pr.
RTS type	4	46.6800	11.6700	64.44	<.001
panelist	9	19.6800	2.1867	12.07	<.001
Residual	36	6.5200	0.1811		
Total	49	72.8800			

Table B.2 ANOVA (Two-way no blocking) at 5% level of significance for flavour

Source of variation	d.f	s.s.	m.s	v.r.	F pr.
RTS type	4	46.8800	11.7200	36.63	<.001
panelist	9	13.6800	1.5200	4.75	<.001
Residual	36	11.5200	0.3200		
Total	49	72.0800			

Table B.3 ANOVA (Two-way no blocking) at 5% level of significance for taste

Source of variation	d.f	s.s.	m.s	v.r.	F pr.
RTS type	4	35.4800	8.8700	18.87	<.001
panelist	9	22.5800	2.5089	5.34	<.001
Residual	36	16.9200	0.4700		
Total	49	74.9800			

Table B.4 ANOVA (Two-way no blocking) at 5% level of significance for overall acceptability

Source of variation	d.f	s.s.	m.s	v.r.	F pr.
RTS type	4	57.0800	14.2700	74.24	<.001
panelist	9	9.6800	1.0756	5.60	<.001
Residual	36	6.9200	0.1922		
Total	49	73.6800			

Appendix C

Table C.1 t-test (two-sample assuming unequal variance) for **protein content** of the best sample (sample B) with control (sample A).

	Control	best
Mean	0.15	0.35
Variance	1E-04	1E-04
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	-24.4949	
P(T<=t) one-tail	8.24E-06	
t Critical one-tail	2.131847	
P(T<=t) two-tail	1.65E-05	
t Critical two-tail	2.776445	

Table C.2 t-test (two-sample assuming unequal variance) for **reducing sugar** of the best sample (sample B) with control (sample A).

	Control	best
Mean	6.09	7.063333
Variance	0.0016	0.000633
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	-35.6735	
P(T<=t) one-tail	2.42E-05	
t Critical one-tail	2.353363	
P(T<=t) two-tail	4.84E-05	
t Critical two-tail	3.182446	

Table C.3 t-test (two-sample assuming unequal variance) for **calcium content** of the best sample (sample B) with control (sample A).

	Sample A (Control)	Sample B (Best)
Mean	52	148.3333
Variance	4	2.333333
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	-66.3011	
P(T<=t) one-tail	3.78E-06	
t Critical one-tail	2.353363	
P(T<=t) two-tail	7.56E-06	
t Critical two-tail	3.182446	

Table C.4 t-test (two-sample assuming unequal variance) for **Ash content** of the best sample (sample B) with control (sample A).

	Sample A (Control)	Sample B (Best)
Mean	0.12	0.47
Variance	0.0001	1E-04
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	-42.8661	
P(T<=t) one-tail	8.85E-07	
t Critical one-tail	2.131847	
P(T<=t) two-tail	1.77E-06	
t Critical two-tail	2.776445	

Appendix D

ANOVA of chemical constituents of whey-pomegranate RTS 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	0.36300	0.36300	15.59	<.001
Days	4	3.48800	0.87200	37.46	<.001
Residual	24	0.55867	0.02328		
Total	29	4.40967			

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	0.007680	0.007680	24.38	<.001
Days	4	0.066480	0.016620	27.81	<.001
Residual	24	0.070520	0.002938		
Total	29	0.144680			

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	0.055470	0.055470	25.91	<.001
Days	4	0.289980	0.072495	33.86	<.001
Residual	24	0.051380	0.002141		
Total	29	0.396830			

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	0.356430	0.356430	42.79	<.001
Days	4	2.056920	0.514230	61.73	<.001
Residual	24	0.199920	0.008330		
Total	29	2.613270			

Appendix E

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

S.N	Particulars	Quantity	Rate (Rs)	Amount (NRs)
1.	Whey	53 ml	10/L	0.53
2.	Pomegranate juice	37.5 ml	500/L	18.75
3.	Sugar	24 gm	90/kg	2.16
4.	Bottle(250ml)	1	9/piece	9
	Total			30.44

The price of 250 ml of drink is Rs 30.44.

Appendix F

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

Glassware and apparatus

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

Color Plates

P.1 Titration for reducing sugar determination

P.2 Raw Samples for analysis

P.3 Sensory Evaluations

P.4 Kjeldahl distillation unit