PREPARATION AND QUALITY EVALUATION OF WHEY BASED MINT (*Mentha arvensis*) FLAVORED WATERMELON BEVERAGE AND ITS STORAGE STABILITY

by

Sandeep Lamichhane

Department of Food Technology

Central Campus of Technology

Institute of Science and Technology

Tribhuvan University, Nepal

2022

Preparation and quality evaluation of whey based mint (*Mentha arvensis*) flavored watermelon beverage and its storage stability

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

by

Sandeep Lamichhane

Department of Food Technology

Central Campus of Technology, Dharan

Institute of Science and Technology

Tribhuvan University, Nepal

March, 2022

Tribhuvan University

Institute of Science and Technology

Department of Food Technology

Central Campus of Technology, Dharan

Approval Letter

This ***dissertation*** entitled ***Preparation and Quality Evaluation of Whey Based Mint* (*Mentha arvensis*) *Flavored Watermelon Beverage and its Storage Stability*** presented by **Sandeep Lamichhane** has been accepted as the partial fulfillment of the requirement for the **B. Tech. degree in Food Technology**

Dissertation Committee

1. **Head of Department** _____
   (Asst. Prof. Navin Gautam)

2. **External Examiner** _____
   (Mr. Anup Halwai, FTQCDO, Dhankuta)

3. **Supervisor** _____
   (Asst. Prof. Navin Gautam)

4. **Internal examiner** _____
   (Asst. Prof. Mahalaxmi Pradhananga)

March, 2022

Acknowledgements

I am deeply indebted to my respected supervisor and Head of Department of Food Technology, Central Campus of Technology, Asst. Prof. Navin Gautam, for his excellent guidance, frequent inspiration, encouragement and valuable suggestions throughout the endeavor.

I am eternally thankful to Prof. Basanta Kumar Rai (Head of Central Department of Food Technology, Tribhuvan University), Asst. Prof. Kabindra Bhattarai (Head of Department of Nutrition and Dietetics, Central Campus of Technology) and Asst. Prof. Bunty Maskey sir for their generosity and co-operation in providing an opportunity and facilities to perform this work successfully.

I cannot stay without thanking all my classmates for their help in preparing the final work. Special thanks must be bestowed to my friends Nabindra Shrestha, Bishesh Vishwokarma, Sudip Bhattarai, Sudip Adhikari, Jitendra Shahi and Anjana Shrestha, my juniors Subash Sigdel and Diwash Acharya for their co-operation to make this work successful. I am deeply indebted to all the teachers, all the technical and non-technical staffs of Central Campus of Technology. I salute all those whose perceptions, observation and inputs have helped me directly or indirectly.

Above all, I am indebted to my parents without whose constant encouragements, inspirations and affection, I would never have reached this far, and this work would have never seen the light of the day.

Date of submission: March, 2022

Sandeep Lamichhane

Abstract

The aim of this study was to optimize watermelon juice and mint extract to formulate whey based mint flavored watermelon beverage by using DOE (Design of expert) v 7.1.5. under mixed condition. Five formulations of beverages were obtained with varying levels of whey, watermelon juice and mint extract in the range of 50-100%, 0-50% and 0-5% respectively. The sensory evaluation of prepared samples of whey based mint flavored watermelon beverage were carried out. The prepared beverage was pasteurized at 60^0C for 30 min and stored in 180 ml plastic bottles at room temperature (25±5°C) and refrigeration temperature (7±1°C) for 28 days. The effects of storage time and temperature on physicochemical (TSS, pH, acidity, reducing sugar) and microbial (TPC, yeast & mold count) properties were evaluated.

The beverage sample with 50% watermelon, 2.5% mint extract and 50% whey were found to be superior. However, sensory parameters viz. appearance, color, flavor, taste and overall acceptability of different formulations were significantly difference at 5% level of significance. Considering microbiological aspect, it was safe to consume upto 28 days of its initial packaging while stored at refrigeration temperature (7±1°C). Total soluble solids, acidity and reducing sugar contents increased while pH decreased with progressing storage time in both storage condition. During storage TSS, pH, acidity and reducing sugar were found to be in the range 12.93-14.10%, 5.18-5.05, 0.2-0.33%, 3.98-4.36% respectively under refrigerated condition and from 12.93-14.65%, 5.18-4.97, 0.2-0.4%, 3.98-4.36% respectively under ambient storage condition (25±5°C).

Table of contents

List of Tables

List of Figures

List of abbreviation

Abbreviation	Full form
BU	Biological unit
CCT Lab	Central Campus of Technology, Laboratory
CW	Cheese whey
DDC	Dairy development corporation
LC	Least count
LSD	Least significance difference
MT	Metric ton
NT	Normal temperature (25 ± 5^0C)
PET	Polyethylene tetracycline
RT	Refrigerated temperature (7 ± 1^0C)
RTS	Ready to serve
SNF	Solid not fat
TSS	Total soluble solid
WBWB	Whey based watermelon beverage
WBWHB	Whey based watermelon herbal beverage
WP	Whey protein
WPC	Whey Protein Concentrate
WPI	Whey protein isolate

Part I

Introduction

1.1 General introduction

Whey is a nutritious by product of cheese, chhana and paneer industry containing valuable nutrients like lactose, protein, minerals and vitamin etc., which has indispensable value as human food. Regulation for preventing disposal of untreated whey and recognition of the value of whey components accelerated in the late 20th century. Whey constitute 45-50% of total milk solids, 70% of milk sugar (lactose), 20% of milk protein and 70-90% of milk minerals and most importantly, almost all the water soluble vitamins originally present in milk (Yadav *et al.,* 2010). Traditionally, whey was defined as a byproduct of cheese-making and regarded by cheese producers as waste with little or no commercial value. This view changed radically as increasing numbers of technical and nutritional applications were discovered for whey or whey components, and whey is now considered as a co-product of cheese-making. The exploitation of the diverse properties of whey in a variety of food systems has firmly established that it is possible to transfer functionalities originally identified in whey to novel products. The recognition of this principle led to the use of whey proteins as physiologically functional food ingredients. Whey protein concentrates, produced commercially for a number of years, are used to improve the protein quality and content of many food products (Walzem *et al.,* 2002).

Before 1970, the dairy industry used to consider whey as a waste product, and it was drained if not used as animal feed or applied to fields as liquid fertilizer. It was the stringent environmental regulations implemented worldwide that made the cheese industry find ways of utilizing cheese whey for different purposes. Concerted research efforts focused particularly on ultrafiltration technology for whey processing. As a result, dairy plants started to process whey into a variety of products such as whey powder, sweet whey, demineralized whey, de-proteinized whey, non-hygroscopic demineralized whey, reduced lactose whey, lactose, whey protein concentrate (WPC), and whey protein isolates (WPI). These products have been highly accepted as important ingredients in many foods, feeds, and pharmaceutical and other industrial applications, thereby enhancing the reputation of whey as an important dairy co-product and receiving very good monetary returns, which influenced positively the overall milk income system (Anand *et al.,* 2013).

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

A tendency to use substitutes of ingredients in recipes of many products has been observed for several years in the food processing industry. It pertains to foods with reduced fat and sugar, or food products for vegetarians and people with lactose intolerance (Bolumer *et al.,* 2015; Serna *et al.,* 2014). Whey and its preparations may serve as substitutes having a positive impact not only on the consumers' health, but also on the finances of many companies by reducing the costs of raw materials, and thus lowering production costs (bozanic *et al.,* 2014). The conversion of whey into beverages through fermentation or without fermentation is one of the most attractive avenues for the utilization of whey for human consumption. Beverages based on fruit and milk products are currently receiving considerable attention as their market potential is growing. Besides being delicious these beverages are highly nutritious. In terms of functionality, whey protein enhances protein content of beverage while improving its quality (Yadav *et al.,* 2010).

1.2 Statement of the problem

Whey has been considered as a by-product in the preparation of different milk product and it is disposed off in the environment without any treatment and it may lead to environment pollution, so utilization of whey by the preparation of whey-based beverage can be a good solution and a source of income too. In Nepal number of large- and small-scale industries produce cheese and *paneer* products and whey so produced is dumped in streams.

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. Increased awareness in health issues leads to increase the consumption of fruit juices and other natural products as an alternate to the traditional caffeine containing beverages such as tea, coffee or other soft drinks. Accompanying the increase in quantity of consumption, there has been a parallel increase in the variety of fruit juices and beverages offered for sale in the market (Dhamsaniya and Varshney, 2013a).

1.3 Objectives

1.3.1 General objective

The general objective of this work is production of whey (paneer whey) based mint flavored watermelon beverage and its quality evaluation.

1.3.2 Specific objectives

1. To prepare and carry out the nutritive analysis of fresh whey and watermelon juice.
2. To formulate the proportion of whey, watermelon juice and mint extract.
3. To conduct sensory analysis.
4. To analyze physico- chemical analysis of best formulation.
5. To study Shelf life of the prepared beverage with respect to its microbial (TPC & yeast and mold) and chemical properties.

1.4 Significance of study

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

Utilization of whey as an excellent beverages base has been recognized as it is genuine thirst quencher. Whey drinks are light, refreshing, healthful and nutritious but less acidic than fruit juices, and offers good profit margins. Whey improves the flavors, texture, appearance and shelf-life of beverages. Soft beverage industry has made significant progress during the last two decades in terms of rise in production and consumption; however, there is a limited range of fruit juice based RTS beverages available in the Nepalese market. Many types of syrups and soft drinks containing artificial fruit flavors are

well known throughout the world. The basic factor considered is the nutritive and therapeutic values, which make them popular and acceptable. At present fruit beverages are generally synthetic flavored, bottled and sold in the market. If this could be substituted with fruit juice and dairy whey, it will be more beneficial to the consumer, dairy industries and beverage manufacturers as well as fruit growers.

1.5 Limitations of the study

1. Due to the time constraints only single variety of watermelon juice was taken and analyzed.
2. Only the paneer whey was utilized
3. For the microbial analysis TPC, yeast and mold count was done for pasteurized product only.
4. Shelf life of product was studied only for 28 days.

Part II

Literature review

2.1 Whey

Milk whey is one of the highly nutritious by-products obtained from the dairy industry producing cheese, chhanna and paneer. It constitutes almost 45-50% of total milk solids, 70% of milk sugar mainly lactose, 20% of milk proteins, 70-90% of milk minerals and almost all the water soluble vitamins originally present in milk (Punnagaiarasi *et al.*, 2017). It resulted into unraveling the secrets of whey proteins and other components and established a sound basis for their nutritional and functional value (Smithers, 2008).

There are two basic types of whey: acid whey and sweet whey or rennet whey. Acid whey is produced from cottage cheese or acid casein manufacture, where milk is coagulated by direct addition of acid as a coagulant. Sweet whey or rennet whey is obtained from the manufacture of cheese products, which involve rennet treatment for milk coagulation (Anand *et al.*, 2013). Whey can be conveniently classed into groups:
Acid whey: Titratable acidity greater than 0.40%, pH > 5.0.
Medium acid whey: Titratable acidity 0.20- 0.40%, pH typically 5.0- 5.8.
Sweet whey: Titratable acidity 0.10- 0.20%, pH typically 5.8- 6.6 (Zadow, 1994).

2.2 Whey production

Whey is the largest byproduct of dairy industry obtained during manufacture of casein, cheese, *paneer*, chhana etc. Whey had been considered as the milk by products for years and it has been mainly dumped into lands, sewage, waterways, and oceans while partly used as animal feed. Dumping of whey globally pushed the environmental pollution to further increment which leads searching the new possibility for the utilization of whey (Affertsholt, 2015). On the basis of whey type, cheese whey (mostly sweet whey) accounted for 95% of the total whey production, whereas casein whey accounted for 5%. The total global market volume of whey ingredients was 770,000 MT in 2008, and from 2005 to 2008 the market increased by 6%. The EU and the USA are the largest markets for whey ingredients, sharing 40% and 31% of the global total, respectively. Regarding the utilization of whey ingredients in food consumption, 190,000 MT of whey powder, 85,000 MT of WPC, and 14,500 MT of WPI were used in food consumption in 2008, the major

food sectors being dairy, bakery, dry/ wet blending, functional food application, infant formula, and confectionary (Anand *et al.*, 2013). Global production of liquid whey from cheese and casein amounted to 192 million MT in 2015 and annual average growth of ~3-4%. The European Union and United state produce ~70% of whey in the world (Affertsholt, 2015). Production of whey by the different projects associated with DDC in the year 2073/2074 was estimated to be more than 3.5 million liters.

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

2.3 Background of whey utilization

Whey, for years, had been viewed as nothing more than a waste product. Whey was once taken back to the farms for disposal on land, as a component in animal feeds, or was disposed of down drains and into waterways and oceans. Each year worldwide whey production is estimated at over 80 billion liters (Smithers *et al.*, 1996). Utilization and/or disposal of whey have been major concerns to dairy specialists all over the world as it contains valuable constituents that should not be wasted. Furthermore, whey represents an important environmental pollutant (Abd el-salam *et al.*, 2009).

With the advent of industrial ultrafiltration and chromatographic methods, recovery and fractionation of whey proteins in their native forms has become possible. A wide variety of commercial whey protein concentrates (WPC) and isolates (WPI), whey protein fractions (α-lactalbumin and β-lactoglobulin rich fractions, casein glycomacropeptide, lactoferrin, and lactoperoxidase) and protein hydrolysates (WPH) are available in the market. Whey protein products are valued as excellent food ingredients because of their unique functional characteristics. As foodstuffs, they are not used only because of their functional properties, but also because of their high nutritive value and GRAS status (Abd el-salam *et al.*, 2009). In its most sophisticated and totally profitable sense, whey utilization implies that whey as a concentrate or fraction will be utilized by man or animal in the form of a nutritious food or some essential component of a food. Such whey or whey product has a monetary value to the cheesemaker, and the market place becomes important (Kosikowski, 1979).

2.4 Whey components

2.4.1 Whey proteins

Whey portion contains various proteins, peptides, amino acids, lactose, minerals, vitamins, and varying quantities of lipids. The major whey proteins are β-lactoglobulin (65%) and α-lactalbumin (25%). Whey is also abundant in other proteins, including bovine serum albumin (8%), 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 (Walzem *et al.*, 2002).

Whey proteins have a biological value of 110, which is higher than the value for casein, soy protein, beef, or wheat gluten and have a high content of sulfur-containing amino acids such as cysteine and methionine (Fox *et al.*, 2015).

2.4.1.1 β –lactoglobulin

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

2.4.1.2 α- lactalbumin

α-lactalbumin (α–La) is the second most important protein in whey and milk with a molecular weight of ~ 14 kDa. It comprises 20 % of total whey protein and also 3.5% of total protein in milk. It has dependency on calcium ($Ca^{2+)}$ ions and it is known as a metallo-protein. 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 (Walzem *et al.*, 2002).

2.4.1.3 Bovine serum albumin

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

2.4.1.4 Lactoferrin

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

2.4.1.5 Glycomacropeptide

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

2.4.1.6 Immunoglobulin

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

2.4.2 Lactose

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

2.4.3 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 Zn ions, which even in trace amount helps in better diarrheal management (Jelen, 2002).

2.4.4 Vitamins

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

2.5 Composition of whey

Whey is a multi-component solution of various water-soluble milk constituents in water, the dry matter of whey consists primary of carbohydrate (lactose), protein (several chemically different whey proteins) and various minerals.

Table 2.1 Chemical composition of whey

Constituents	Acid whey	Rennet Whey
Total solids (%)	6.3- 7.0	6.3- 7.0
Lactose (%)	5.03	5.01
Protein (%)	0.38	0.98
Fat (%)	0.13	0.34
Ash (%)	0.60	0.54
Lactic Acid (%)	0.21	0.14
Calcium (ppm)	710.65	501.50
Phosphorous (ppm)	560.50	441.50

Source: Darade and Ghodake (2012)

2.6 Nutritional and therapeutic value of whey

Whey protein actually consists of several component proteins including β-lactoglobulin, α-lactalbumin, glycomacropeptide, bovine serum albumin, immunoglobulins, and lactoferrin (Canning, 2004). Whey proteins are high quality proteins due to their high cysteine content, and significant quantities of B vitamins (Varnum and Sutherland, 1994). They are also rich in calcium, phosphorous, magnesium and zinc (Hazen, 2005). Whey protein concentrate and whey protein isolate typically contain 500-700 mg calcium per 100 g powder (Direinzo, 2004). Whey proteins are one of the most bio-available proteins, having an amino-acid profile that is easily digested and allowing the body to utilize the protein faster than rice or soy protein (Canning, 2004; Hazen, 2005). The amino acid profile of sweet whey is very balanced, and each amino acid present exceeds the intake recommendations for both children ages 2-5 and adults as set by the Food and Agricultural Organization and World Health Organization (Sindayikengera and Xia, 2005).

Whey is a complex mixture of proteins that enhances a range of innate and acquired immune responses. The proliferation of lymphocytes is increased when they are incubated with whey. Whey is thought to boost intracellular levels of glutathione, a molecule known to increase proliferation of lymphocytes. In contrast, whey reduces some functions of neutrophils. Diets containing whey have been tested extensively for effects on innate and acquired immune responses. For example, a diet containing whey protein concentrate (WPC) elicited higher antibody responses to specific antigens than diets containing other protein sources. Studies have demonstrated that diets containing WPC also increased phagocytic cell function and mucosal antibody responses to oral immunizations. Whey-based diets also increased the proliferation of murine lymphocytes to mitogens. The immunity-enhancing activities of WPC are also affected by the conformational state of the proteins. Whey improved liver function tests in people with chronic hepatitis B infections, but had no effect on those with hepatitis C infections (Anand *et al.*, 2013).

Whey based beverages target a large scale of consumers - from old people to little children. Because of its health benefits, it was used to treat some illnesses, such as tuberculosis and skin and digestive tract diseases, since the time of Ancient Greece. Whey was also successfully applied for treatments of diarrhea, bile illness, skin problems, scales in the urinary tract and some intoxication. Due to high amount of whey proteins with

nutritional value these beverages are ideal source of energy and nutrients for athletes. Whey proteins are a rich source of branched chain amino acids (BCAA) like isoleucine, leucine and valine. BCAAs unlike other essential amino acids are metabolized directly into the muscle tissue and are first amino acids used during periods of exercise and resistance trainings (Shukla *et al.,* 2004). Due to presence of lactoferrin whey beverages can be used as functional food intended to improve iron absorption from food and/or help to keep pathogens from attaching to the intestinal walls. That is very important for nutrition of little children and babies. Furthermore, these beverages may improve absorption of calcium important for older population which is often suffering from osteoporosis (Miller, 2005).

2.7 Whey protein digestion and absorption

Whey contains most of the water-soluble components of milk except casein-bound minerals. It contains all the water-soluble milk protein fragments such as lactoglobulin and lactalbumin, serum albumin, lactoferrin, lactoperoxidase. Whey proteins are also called “fast proteins.” They are not coagulated by acid in the stomach, and rapidly reach the intestine, where they remain for a long time for a sustained absorption. They are absorbed slowly and completely in the small intestine. The structure of lactoferrin is abnormally resistant to proteolytic enzymes. Extreme stability is the reason that lactoferrin passes through the gastrointestinal tract. This can be monitored, for substantial quantities of lactoferrin are found in the stool of subjects administered lactoferrin. Lactoferrin also improves iron intake in human subjects. Reports suggest that iron status (hematocrit) of infants fed formula with bovine lactoferrin was better than that of infants fed formula with the same amount of iron from other sources but no lactoferrin. In a study conducted with female long-distance runners, subjects given iron only showed significantly lower serum iron, ferritin and red blood cell counts after the intervention, whereas the iron level was maintained in the group given lactoferrin (Anand *et al.*, 2013).

2.8 Some whey products

Depending on the technology used and the whey constituents in the final products, there are different types of processed whey products available on the market. Different types of whey products manufactured through the utilization of membrane technology include WPCs, WPI, demineralized whey, reduced lactose whey, deproteinized whey, etc. Lactose and milk minerals are important byproducts of whey filtration (Anand *et al.*, 2013).

2.8.1 Whey Powder

Whey powder is manufactured by concentrating whey to 40-70% total solids, and spray or roller drying to a moisture content of less than 5%. It is necessary to convert a high percentage of the lactose to its a-monohydrate crystalline form to minimize water absorption and concomitant defects during storage. Lactose crystallization is either completed in the concentrate prior to drying or, as is most commonly done, a two-stage drier is employed that allows for crystallization prior to final drying. Lactose crystallization is also promoted in this hygroscopic product by rapidly cooling it to less than 100°C as it is removed from the drier (Macwan *et al.*, 2016). Some of the health benefits of whey powder are:

- Increased lean muscle mass
- Increased fat burning
- Powerful detoxifying properties
- Helps return blood pressure to normal
- Controls cholesterol
- Maintaining health of all major organs – heart, liver, kidneys
- Promotes healthy skin
- Can help certain digestive disorders (Anonymous, 2011).

2.8.2 Whey protein products

Developments in several solid-liquid separation technologies have been utilized by the dairy industry to produce large number of whey products with good functional, nutritional and flavor properties. The main feature of these products is that proteins are largely present in their native form. There are at least two reasons that account for the large number of the developed whey protein products. First, no single product can be utilized in a wide spectrum of food products as each requires a whey protein product of specific properties. Second, production of whey protein products with more protein content utilizes only small portion of whey solids and there will be a need to utilize or discard the remaining effluent stream, which increases the overall cost of production. Generally, whey protein products differ in their composition mainly in their protein, lactose, fat and mineral contents. Also, differences in the relative whey protein concentrations will be found in the different preparations (Abd el-salam *et al.*, 2009).

2.8.2.1 Whey protein concentrate (WPC)

WPCs with protein contents ranging from 35–80% are available. The basic steps in the manufacture of these products are whey pretreatment, ultrafiltration concentration by evaporation under reduced pressure, and spray drying (Abd el-salam *et al.*, 2009).

2.8.2.2 Whey protein isolates (WPI)

WPIs, with a minimum protein content of 90%, are either manufactured by ion exchange chromatography or microfiltration. The ion exchange method is based on the retention of major whey proteins by binding to the ion exchanger and subsequent elution by changing pH. In the microfiltration (MF) method, whey is micro filtered using a suitable membrane (pores < 1 *m*m) to remove lipid and protein aggregates and microbial debris. The MF permeate is ultra-filtered, concentrated and spray dried. WPI prepared by ion exchangers has less casein glycomacropeptide content than that prepared by microfiltration (Abd el-salam *et al.*, 2009).

2.8.2.3 β-Lactoglobulin and α-Lactalbumin Rich Fractions

A growing demand for *α*-lactalbumin for use in infant food formulation encouraged processors to fractionate whey proteins. Several methods have been developed to separate the two major whey proteins, *β*-lactoglobulin and *α*-lactalbumin in relatively rich fractions. These methods are based on differential solubility at different pH, temperature and ionic strength (Abd el-salam *et al.*, 2009).

2.8.2.4 Glycomacropeptide (GMP)

Glycomacropeptide is a peptide arises from the action of chymosin on casein and more specifically *k*-casein during cheese making. It represents the C-terminal part of *k*-casein from residue 106(Met) to 169. This fraction has unique composition, biological activity and nutritional significance. Ultrafiltration and chromatographic methods are used to separate GMP from other whey proteins. Almost pure commercial GMP products are now available (Abd el-salam *et al.*, 2009).

2.8.2.5 Whey protein hydrolysates (WPH)

Enzymatic hydrolysis with a wide range of proteolytic enzymes is generally used to produce WPH of different level of hydrolysis depending on the purpose of its use (Abd el-salam *et al.*, 2009).

2.8.2.6 Modified whey proteins (MWP)

Whey protein polymers have been developed as a cold set ingredient. This product is made by heat polymerization of whey proteins at conditions that do not yield a gel (low ionic strength and high pH). The product forms a gel under cold conditions (20–37ºC) by changing the solvent quality (i.e., addition of Ca). Another product is prepared by heat denaturation of WPI in acidic conditions (pH 3.5) and in the presence of Ca (Abd el-salam *et al.*, 2009).

2.8.2.7 Minor whey proteins (Lactoferrin, Lactoperoxidase, Basic Milk Proteins)

These are prepared mainly by chromatographic methods. These products are used mainly as antimicrobial agents or in pharmaceutical preparations. The most widely produced whey protein products as food ingredients are WPC and WPI. Therefore, discussion will be focused mainly on the functional properties of these products (Abd el-salam *et al.*, 2009).

2.8.3 Lactose

Lactose is the main component of whey and is recovered by crystallization from sweet and/or acid whey. There are two basic methods for the recovery of lactose, depending on the composition of the source i.e., Crystallization in concentrated whey and crystallization in concentrated deproteinated whey (e.g. ultrafiltration permeate from whey). The whey is pasteurized and concentrated by evaporation to 60 – 65% total solids. The concentrated whey is then transferred to crystallization tanks at a temperature of about 50°C. The concentrate is, during gentle stirring, subjected to a predetermined time/temperature programme for cooling down to 10°C. The concentration of lactose in the concentrated whey amounts to 40-45%, while its solubility decreases from 17.5% at 50°C to about 6% at 10°C. Because of supersaturating the lactose will crystallize mainly to so-called _-monohydrate lactose crystals, containing one molecule of crystallization water. Adding

lactose seed crystals accelerates this crystallization process. After crystallization, the slurry proceeds to a decanter centrifuge for separation of the crystals (De Wit, 2004).

2.8.4 Yoghurt

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

Whey products used in the production of yoghurt includes:

- Sweet whey powder, which may replace skimmed milk powder at the level 2–5.2%.
- WPCs, which are most often used by manufacturers of yoghurt. Addition of WPC34 at the level 0.7–2.0% or WPC80 at 0.5–0.8% is sufficient in the case of mixed yoghurt (a greater amount of the additive may adversely affect some quality characteristics). Replacing skimmed milk powder with WPCs causes, among other effects, an increased gel strength in solid yoghurt, increasing the viscosity of mixed yoghurt, and reduces the risk of syneresis in both types of yoghurt.
- WPI which, due to the low content of lactose and milk fat, is used in yoghurts with reduced lactose content.
- Demineralized whey powder, with reduced mineral content, which accelerates the fermentation process. On the other hand, low mineral content weakens the structure of the gel, so it is necessary to add milk protein hydrolysates when this type of formulation is used (DeWit, 2002).

Furthermore, the addition of whey protein gives yoghurt a smooth and creamy texture; it also increases its nutritional value. The bioactive components present in whey and whey protein can stimulate the growth of probiotic bacterial cultures (both in the finished product, and in the human digestive tract (Hugunin, 2009).

2.8.5 Whey confectionery products

Whey products, including, demineralized whey powders, low-lactose whey powders, WPCs and isolates, and lactose have been used in the following confectionery: chocolates and chocolate chips, candies, jellies and chewing gums. Lactose – milk sugar – can serve

as a bulking agent. It is slightly sweet, less soluble than sucrose, and has a low hygroscopicity level; however, it influences the color, the taste, and the texture of the finished product and takes part in the Maillard reaction. For these reasons, use of lactose can be more or less reduced, which depends on many characteristics of the confectionery. Another use of derivatives of WPCs is the production of the so-called aerated confectionery and chocolate. The foaming properties of concentrates are used in this case. In addition, WPI and concentrates of high protein content (WPC80) can be successfully used in the production of protein bars for athletes (Pernot, 2007; Stoliar, 2009).

2.8.6 Whey beverages

2.8.6.1 Nutritious beverages

Dietetic beverages, beverages with hydrolyzed lactose, are prepared by addition of some sweetening agent (most often saccharin and cyclamate), fruit bases of apple or some tropical fruits and stabilizing agent. These beverages have very low energy value (104-113 kJ/100 ml) what makes them suitable for consumption by large group of consumers. Lactose hydrolyzation results in production of glucose and galactose - monosaccharides with much higher sweetness, better solubility and better absorption ability than lactose (Chavan *et al.*, 2015).

Besides fruits, other flavoring agents like chocolate, cocoa, vanilla, cereals (mostly rice, oat and barley), honey, etc. are added. Addition of cereals (especially bran), seems to be very interesting which results in production of a beverage fortified with dietary fibers, essential fatty acids (with addition of oat) and hypoallergenic proteins what makes these beverages suitable for consumption by allergic population and children. In order to prepare a hypoallergenic beverage, the addition of other vegetable sources of proteins like isolates of potato or soy proteins may be used. Addition of honey to such beverage instead of sugar or other sweeteners results in fortifying it with numerous other nutrients like vitamins, minerals and phytochemicals which are not naturally present in whey (Chavan *et al.*, 2015).

Suitable flavorings included peach puree (20%), strawberry (10%), and red raspberry (10%). The preparation of the flavored whey drinks involved concentration to 18-20°Brix and addition of citric acid to bring the pH down to 3.6. At low pH, the proteins in these

whole whey drinks may tend to precipitate and form deposits in the container. Drinks from deproteinized whey or whey permeate avoid this problem if the market is sensitive to the residue (Kravchenko, 1988).

Whey based mango herbal beverage (water 48% and panner whey 32%) with 12 per cent mango pulp, 8% sugar and lemon grass 1.5% obtained highest sensory score (Sahu *et al.*, 2005). Whey based mango RTS beverage with 70% whey and 30% mango juice, scored at maximum for all sensory attributes viz., color, flavour, taste and overall acceptability (Sakhale *et al.*, 2007a). Custard apple whey beverage with combination of whey: pulp 90:10, 85:15, and 80:20. Out of these combinations, 85:15 (whey: pulp) with 10% sugar was the best combination and obtained maximum scored (Ingale *et al.*, 2009). Whey based watermelon beverage with combination of (whey: juice: sugar) 83:5:12, 78:10:12, 73:15:12. Out of these combinations, 78:10:12 (whey: juice: sugar) was the best combination and obtained maximum scored (Punnagaiarasi *et al.*, 2017). Organoleptic evaluation showed that whey based Pineapple fruit juice beverage prepared by using whey & water (70:30) and 20 % fruit juice was found to be more acceptable as compared to sample prepared by using different combination of whey, water and fruit juice concentration, as it gave good flavor & taste and overall acceptability (Devi *et al.*, 2017).

2.8.6.2 Thirst-quenching carbonated beverages

The most typical product representing this type of whey beverage is the Swiss ‘Rivella’. Rivella, a sparkling, crystal clear infusion of alpine herbs, first, appeared in Switzerland in 1952. Rivella was prepared by fermenting deproteinized whey with lactic acid bacteria, filtering, condensing to a 7:1 concentrate, adding sugar and flavoring, refiltering, diluting and carbonating, after which the product was bottled and pasteurized. The finished beverage contained 9.7% total solids, 0.125% total nitrogen and the pH was about 3.7 (Chavan *et al.*, 2015).

2.8.6.3 Alcoholic whey beverages

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

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

2.8.6.3.1 Low alcohol beverage

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

The whey was first fermented with kefir culture to obtain 1% lactic acid and 3.5% lactose. An equal volume of a 3% extract of leaves and herbs was added; the tannins precipitated the whey proteins (Lactannid process). After filtration the aromatic flavored whey was end fermented with lactose-fermenting yeast and sweetened with saccharine. The final beverage contained 0.8% alcohol. It was bottled under carbon dioxide and was stable for one year (Holsinger *et al.*, 1974).

2.8.6.3.2 Whey beer

Whey has many properties which make it suitable for the manufacture of beer-like beverages. Because whey contains material similar to the colloids of beer wort, it has a great capacity for binding carbonic acid. Whey, like beer wort, has a high salt content. Some constituents in whey, after prolonged heating under pressure, develop caramel-like flavors which are similar to the taste and odor of cured malt. Lactose is only slightly sweet so it does not alter the taste of the finished beverage. Dietrich developed a beer substitute by mixing 5.4% malt wort with 2.5% deproteinized whey. The malt-whey mixture was fermented by *Saccharomyces lactis,* after 5 to 7 days, the product had developed a true beer flavor and character (Holsinger *et al.*, 1974).

2.8.6.3.3 Whey wine

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

Under proper processing circumstances some wine-like flavor develops from whey. However, liquid fresh whey does not contain sufficient lactose for conversion into alcohol to make table wine. Fortifying whey with glucose, sucrose, or with grape juice to create a richer fermentable substrate has been a basis for some published whey wine processes. In other instances, the lactose in whey was hydrolyzed prior to fermentation with lactase. A recent interesting experimental observation, shows that food grade fungal acid lactase remains highly active during the alcohol fermentation of whey wine by *Saccharomyces cerevisiae* yeast (Kosikowski, 1979).

As the basis of a new whey wine process, a demineralized, deproteinized whey concentrate containing 24% lactose is used as a substrate. It is fermented by adapted *Kluyveromyces fragilis* to table wine. The resulting white whey wine can be baked to give an acceptable whey sherry. In this process an acid whey concentrate of 28% total solids is prepared then ultra-filtrated, demineralized, and fermented with *K. fragilis* in 5 to 6 days at 30 C. No added lactase is required, and all of the alcohol of the basic wine (12 to 14% v/v) is derived totally and directly from lactose (Kosikowski, 1979).

2.8.7 Whey protein based edible films and coating

Native whey proteins are globular proteins containing most of the hydrophobic and SH groups hidden in the interior of the molecule. Formation of whey protein films has mainly involved heat denaturation of whey proteins in aqueous solutions. Heating modifies the three-dimensional structure of the protein, exposing internal SH and hydrophobic groups, which promote intermolecular S-S bonding and hydrophobic interactions upon drying (Perez-Gago and Krochta, 2002). Films are formed principally by cohesive forces between the polymer molecules and by adhesive forces between the film and the substrate. Cohesion and adhesion are related to the polymer structure and chemistry. Denaturing and crosslinking additives promote molecular order and, therefore, increase cohesion and

rigidity of films (Banerjee and Chen, 1995). McHugh et al. (1994) produced plasticized whey protein isolate films by heat-treating 8–12% (w/w) solutions of whey proteins at temperatures between 75^0 C and 100^0C. WPI solution concentrations greater than 12% (w/w) gelled upon heating. Optimal conditions for preparing these films included the heating of 10% (w/w) WPI solutions at 90^oC for 30 min. These conditions formed films with a consistent structure, and X-ray diffraction indicated that whey proteins were irreversibly denatured (Perez-Gago and Krochta, 2002)

2.9 Use of fruits in preparation of whey beverage

Mixtures of fruit juices and unprocessed or deproteinated whey or UF permeates are the most common types of whey drinks to be manufactured. The main two basic ingredients are typically liquid whey and liquid fruit juice or, more likely, fruit juice concentrate. The flavors used in these beverages most often include citrus fruits (mainly orange, followed by lemon, rarely grapefruit), as well as mango, passion fruit, pear, apple, strawberry, raspberry or fruit juice combinations, since they have proved to be very efficient in covering up the undesirable odor of cooked milk and salty-sour flavor of fresh whey (Chavan *et al.*, 2015)

Singh *et al*. (2014) prepared guava blended beverage by using whey and guava pulp in the proportion 67.5:20 and was found to be more acceptable and of good quality in terms of protein and vitamin C content. A delicious and nutritious RTS beverage from the ripe banana juice and milk whey using *Mentha arvensis (*mint*)* extract as a natural flavoring agent was prepared by (Dhamsaniya and Varshney, 2013a). The proportion of banana juice, *M. arvensis* extract and milk whey was varied from 5-15 ml, 1-5 ml and 72-86 ml per 100 ml of the prepared beverage, respectively. The screening of beverage samples was done on the basis of their physicochemical and sensory characteristics. Whey-banana-RTS beverage prepared having 77 ml milk whey+15 ml banana juices+3 ml *Mentha arvensis* extract+8 g sugar powder per 100 ml of the prepared beverage was found to be acceptable and recommended for large scale production.

2.10 Use of watermelon in beverage

2.10.1 Description

Watermelon which belongs to the family Cucurbitaceae and species *Citrullus lanatus,* is a major fruit widely distributed in the tropics. It refers to both fruit and plant of a vinelike (climber and trailer) herb. It is believed to originate from southern Africa and is one of the most widely cultivated crops in the world (Huh *et al.*, 2008). Its global consumption is greater than that of any other cucurbit. It accounts for 6.8% of the world area compared to vegetable production (Guner and Wehner, 2004). China is the leading country in production of watermelon followed by Turkey, Iran and Brazil (Anon., 2018). This flowering plant produces a special type of fruit, which have a thick rind and fleshy center. Its fruit has a smooth exterior rind (green and yellow) and a juicy, sweet, usually red, but sometimes orange, yellow, or pink interior flesh (Marr and Tisserant, 1998). The fruit serves as a thirst quencher owing to its high-water content (92%). It is an excellent source of minerals, lycopene, vitamin C and A. However, the juice or pulp of the watermelons is consumed whereas the rind and seed are mainly discarded as agricultural food wastes which constitute solid waste to the environment (Egbounu, 2015).

2.10.2 Origin

David Livingstone, an explorer of Africa, described watermelon as abundant in the Kalahari Desert, where it is believed to have been originated. There, the ancestral melon grows wild and is known as the Tsamma melon (*Citrullus lanatus var tastius*). Originating in Africa, watermelons were first cultivated in Egypt and there, the fruit was held is such regarding, that it was placed in the tombs of many Egyptian kings. It is not surprising that watermelon played such an important role in Egypt, and subsequently in the countries of Mediterranean region, since water was often in short supply in these areas, and people could depend upon watermelon for its thirst-quenching properties (Bates and Robinson, 1995).

2.10.3 Cultivation

Watermelon is believed to be domesticated at least 4,000 years ago, and the plant was grown as a crop in the Nile valley (Bates and Robinson, 1995). The indigenous people of the Kalahari, in their search for water-containing foods, selected varieties with low

glycoside content. From there, several varieties of melon spread to the Mediterranean areas and in an eastern direction to India. These days, cultivated varieties are a popular crop, which can be cultivated in any climate that has warm summer, and are best suited to those climates that have long hot summers (Victor, 2009). Even in some part of terai belt of eastern Nepal, it is cultivated in large scale.

2.10.4 Varieties

There are more than 50 varieties of watermelon. Most have red flesh, but there are orange and yellow-fleshed varieties. Of the 50 varieties of watermelon, there are four general categories: All sweet, Ice-Box, Seedless and Yellow Flesh (Langer and Hill, 1991). They vary in fruit size, fruit number, fruit shape, flesh color, rind color and seed color (Zohary and Hopf, 2000). Although modern breed watermelons produce very large fruits up to 10 kg in weight, there are a number of hard-fleshed smaller and more primitive genotypes that are cultivated for their fruit which is used for the production of jam and pickles (Langer and Hill, 1991).

2.10.5 Composition

As the watermelon is a rich source of water its moisture content is more than 91%. Fat content in watermelon lies in the seeds. The composition of watermelon is shown in Table 2.2.

Table 2.2 Chemical composition of watermelon and watermelon juice. (Per 100 g)

Proximate Composition	Watermelon	Watermelon Juice
Moisture (g)	91.45	91.8
Protein (g)	0.61	0.44
Reducing Sugar	4.99	4.5
Fat (g)	0.15	0.04
Ash	0.25	0.29
Total Dietary fiber	0.4	0.07
Potassium (mg)	112	80
Vitamin C	8.1	3.2

Source**:** Ozcelik and Yavuz (2016)

2.10.6 Nutritional and medicinal importance of watermelon

Watermelon is not only great on a hot summer day, this delectable thirst quencher may also help to quench the inflammation that contributes to conditions like asthma, atherosclerosis, diabetes, colon cancer and arthritis (Chiej, 1984). Some importance of watermelon are listed below;

- **Medicinal importance**

The seed is demulcent, diuretic, pectoral and tonic. It is sometimes used in the treatment of the urinary passage and has been used to treat bed wetting. The fruit is also diuretic, being effective in the treatment of dropsy and renal stones (Duke and Ayensu, 1985).

- **Increases blood amino acid arginine level**

Eating watermelon increases blood amino acid arginine concentration. It is essential for the synthesis of nitric oxide, proline and creatine, required for various biological processes. For example, nitric oxide helps relax vascular vessels and improves immune function. Proline is essential for joint function and wound healing. Creatine plays important roles in neurologic and muscular functions. The nutrient responsible for the increased plasma

arginine level is called Citrulline. On average, a gram of watermelon flesh contains 1.5 mg citrulline (Collins, 2007).

- **Rich in citrulline**

Watermelon is exceptionally high in citrulline, an amino acid; our body utilizes it to produce another amino acid arginine that is used by in the urea cycle to remove ammonia from the body (Collins, 2007).

- **Rich in Lycopene**

Watermelon is a rich natural source of lycopene and β- carotene, these are of great interest because of their antioxidant capacity and potential health benefits. Lycopene is a red pigment and occurs naturally only in plant and algal tissue. It is highly effective antioxidant owing to its ability to act as a free radical scavenger (Veazie *et al.*, 2001). This powerful antioxidant travel through the body neutralizing free radicals which prevent cholesterol sticking to blood vessel walls or else that lead to heart attack or stroke. They reduce the airway spam that occurs in asthma, they also reduce colon cancer by neutralizing free radicals that damage the cells lining the colon. Besides, fruit are also used as a face mask for the delicate skin to reduce signs of ageing (Chiej, 1984). Watermelon and tomatoes are the most familiar sources of lycopene in the Western diet, the lycopene content in commercial watermelons has been reported to be 45.1-53.2 µg/g fresh weight while those reported for fresh tomato was 30.2 µg/g fresh weight (Veazie *et al.*, 2001).

2.11 Use of mint (*Mentha arvensis*) in beverage

Mentha arvensis L. *(Lamiaceae*), popularly known as Japanese mint or menthol mint and locally known as *pudina*. Which is belongs to the family *Libeaceae* and it is a common edible and aromatic perennial herb cultivated in India, China, Brazil, Japan, USA, France, Australia and in Nepal as well for its menthol rich essential oil. Menthol is widely used in food, beverage, pharmaceutical, cosmetic, perfumery industries, etc. throughout the world. The major components identified in the oil of *M. arvensis* were menthol (71.40%), *p*-menthone (8.04%), *iso*-menthone (5.42%) and *neo*-menthol (3.18%) (Pandey *et al.*, 2003). The physico-chemical properties of menthol are melting point 43°C (106-109°F), freezing point is 27-28°C, boiling point is 212°C (414°F). Molecular formula $C_{10}H_{20}O$ and

molecular weight is 156.27 g/mol. It has an antioxidant, antimicrobial, cytotoxic and analgesic activities of *Mentha arvensis* extract (Satpute *et al.*, 2018b).

Herbal extract of *Mentha arvensis* has preventive and curative value. It is used to treat sour throat, gastric problems and other problems related to gastrointestinal tract (Satpute *et al.*, 2018b). The aromatic leaves of mint widely used for flavoring foods and beverages. Whey based mango herbal beverage prepared with 2% *Mentha* extract has been found to be highest overall acceptability on the day of preparation as well as after 30 days of storage. In beverages menthol is used for the cooling effect and flavoring (Satpute *et al.*, 2018b). According to Yadav *et al.* (2010) Beverage prepared from banana juice and whey in combination with edible herbal medicinal plant extract of *Mentha arvensis* will not have only excellent nutritional properties but will also possess therapeutic, prophylactic, antibacterial and organoleptic properties. It also acts as a good appetizer, acceptable to consumer and at the same time make the product more palatable. The menthe extract not only modifies taste and flavor characteristics of the beverage, but also act as natural preservative, therefore minimizing the need of chemical or artificial preservatives (Yadav *et al.*, 2010).

2.12 Preservation of whey

Fluid milk and whey are perishable dairy products that require proper cooling and handling to maintain their freshness and quality. However, milk and whey solids may be preserved for future use by various methods, the most common of which is concentration by removing water, using either heat or membrane methodology, followed by drying. Dairy products commonly manufactured through the use of one or more of these processes are evaporated milks, condensed and sweetened condensed milks, dry milks, condensed whey products, and dry whey products (Warren and Clark, 2001).

2.13 Preservation of beverage

Preservation by heat, cold refrigeration, freezing temperature, or other means and application of the preservation techniques are mostly used. Storage conditions and preservation processes are subject to Food and Drug Administration (FDA) inspection and enforcement. Preservation from microbial, chemical, and physical contamination, as well as enzymatic activity, is necessary for preserving and extending the shelf life (the time a

product can be stored without significant change in quality) of food. Adequate packaging is important in preserving food. Preservation and processing of foods make food last look good, and taste good (Vacklavik and Elizabeth, 2008). Here is only describing about heat preservation.

2.14 Heat preservation

Heating or cooking foods as a means of preserving them or making them more palatable has been important for centuries. Heating is a vital form of food preservation and there are many different methods of heating processes available today. Foods are heat processed for four main reasons.

- To eliminate pathogens (organisms that cause disease)
- To eliminate or reduce spoilage organisms
- To extend the shelf life of the food
- To improve palatability of the food (Vacklavik and Elizabeth, 2008).

2.15 Heat treatment methods

Heat treatment methods can be divided into two categories, depending on the amount of heat applied: The heat processing method may be mild or severe. The aims, advantages, and disadvantages of these two types of heat treatment are different. Depending on the objectives, a food processor may choose to use either a mild or a severe form of heat treatment to preserve a food product. Consumers rely on cooking to uphold conditions of food safety in the home. Examples of mild heat treatment include pasteurization and blanching. Pasteurization is a mild heat treatment used for milk, liquid egg, fruit juices, and beer (Vacklavik and Elizabeth, 2008). The main purpose of pasteurization is to achieve the following

- Destroy pathogens
- Reduce bacterial count
- Inactivate enzymes
- Extend shelf life (Vacklavik and Elizabeth, 2008).

2.16 Effect of heat on whey beverage

Heat processing of whey beverages is required to eliminate microorganisms, making the beverages suitable for human consumption. However, whey components undergo a number of heat-induced physical and chemical changes, the magnitude of which are linked to reaction conditions (pH, concentration, temperature, ionic strength). Whey proteins aggregate at temperatures above 60°C due to the heat sensitivity of β-lactoglobulin, α-lactalbumin and bovine serum albumin (BSA), all of which are ordered globular proteins (Wong *et al.*, 1996). Aggregation of whey proteins produces large particles that settle in whey drinks. Other components of the fruit also interact with proteins to induce sediments in protein-fortified beverages. The failure of whey-based beverages to perform well on the market is related to sedimentation problems (Jelen *et al.*, 1987).

To overcome sedimentation problems, high methoxyl pectin are used in acidified milk beverages. Processing parameters, including pH, temperature and homogenization, affect the stability of milk beverage (Dickinson, 1998). Steric stabilization by pectin of acid milk beverages at low pH is due to complexation of pectin with casein particles. Glahn and Rolin (1994) reported that the production of small casein particles along with the presence of pectin above a critical pectin level prevent whey separation. The high content of lactic acid in acid whey is associated with an undesirable flavor and protein aggregation in whey drinks; therefore, researchers incorporate fruits such as citrus and lemon to ameliorate the acid flavor note. Various other fruits are used, including mango, pineapple, guava, peach, passion fruit, apricot and kiwi (Jelen, 2002).

2.17 Effect of heat on watermelon juice

Watermelon juice is sensitive to heat, oxygen, light, and ions. Several techniques have been applied to maintain the original quality and aroma of watermelon juice. High pressure treatment less influences the aroma and color of watermelon juice, high pressure carbon dioxide treatment inactivates polyphenol oxidase, peroxidase, and pectin methyl esterase of watermelon juice, thus maintaining their original properties, pulsed electric field treatment maintains the typical aroma of watermelon juice for 21 days and also maintains the total antioxidant capacity of watermelon juice. However, the mentioned techniques were intermittent, which maintained qualities and aroma of watermelon juice at the expense of high cost and limited production capacity. Hence, Conventional and continuous processing

techniques are more feasible for the production of watermelon juice, such as ultrahigh temperature (UHT), low temperature long time (LTLT), and high temperature short time (HTST) pasteurization. LTLT and HTST are the most conventional ways to inactivate bacteria for centuries (Wang *et al.*, 2018).

Wang *et al.* (2018) Evaluate the effect of thermal treatments on the quality and aroma of watermelon juice by pasteurized the juice via ultrahigh temperature (UHT, pasteurized at 135°C for 2 s), low temperature long time (LTLT, pasteurized at 60°C for 30 min), and high temperature short time (HTST, pasteurized at 100°C for 5 min), respectively. UHT and LTLT reduced the total flora count and maintained the color of the pasteurized juice, while the HTST led to a significant color difference. The aroma of the LTLT treated juice was similar to that of unpasteurized juice. Moreover, the shelf life of the LTLT reached 101 days and 14 days at 4°C and 25°C, respectively. Hence, the LTLT was the best way to maintain the quality and aroma of watermelon juice.

Part III

Materials and methods

3.1 Materials

3.1.1 Milk

Standardized pasteurized milk (Product of Kamdhenu Dairy, Itahari) brought from the local market of Dharan having 3% fat and 8% SNF was taken for the preparation of whey.

3.1.2 Table sugar

Table sugar was obtained from local store of Dharan.

3.1.3 Watermelon

Watermelon was obtained from the Bhanuchowk vegetable and fruit store of Dharan. It was washed thoroughly with water to remove foreign materials. Juice was extracted by a juice extractor.

3.1.4 Mint *(Mentha arvensis)*

Fresh matured leaves of mint were collected from the local area of Dharan.

3.1.5 Bottle

Plastic bottles (HDPE) of 180 ml capacity were purchased from the local market of Dharan.

3.1.6 Aluminium foil seal

Aluminium foil seal was bought from JBS, Itahari.

3.1.7 Chemicals and equipments

All chemicals, glassware and equipments were required were used from the laboratory of Central Campus of Technology, Dharan. Details of chemicals and equipments are given in Appendix G.

3.2 Methodology

3.2.1 Preparation of whey

The milk was first heated to 80°C and cooled to 72°C. It was coagulated at that temperature by using 2% citric acid solution (40-50 ml per liter of milk). Complete coagulation was affected within one minute. The whey thus obtained was strained using muslin cloth to get clear liquid.

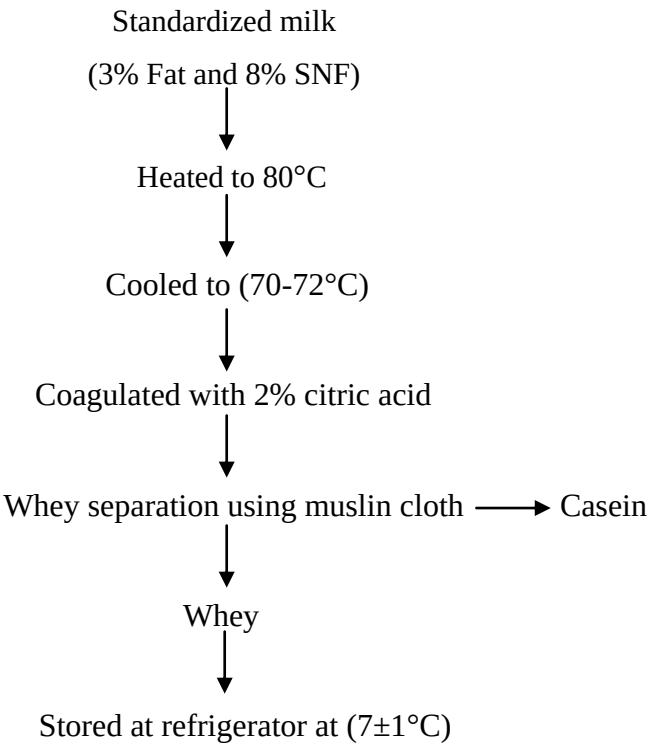

Fig. 3.1 Flowchart for preparation of whey

Source: Awsi and Dorcus (2012).

3.2.2 Preparation of watermelon juice

Ripe watermelon was selected and washed with water to remove the adhered dust particles. It was cut into the pieces and only red fleshy part of watermelon was taken and all the seeds were removed manually. Then extraction of juice was done by juice extractor and the juice was filtered by muslin cloth.

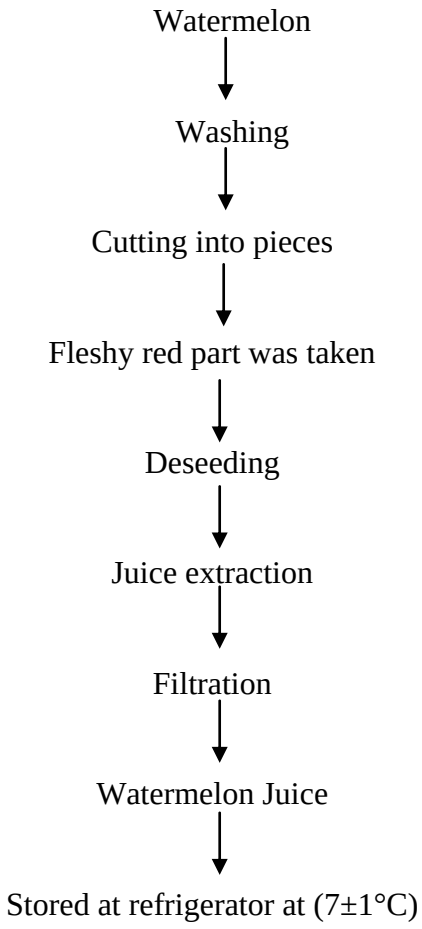

Fig. 3.2 Flowchart for preparation of watermelon juice

3.2.3 Preparation of mint (*Mentha arvensis*) extract

Fresh matured leaves of mint were collected and thoroughly washed with potable water and cut into small pieces then ground in a mixer grinder followed by filtering through muslin cloth. The extract thus obtained was stored in the refrigerator for further use.

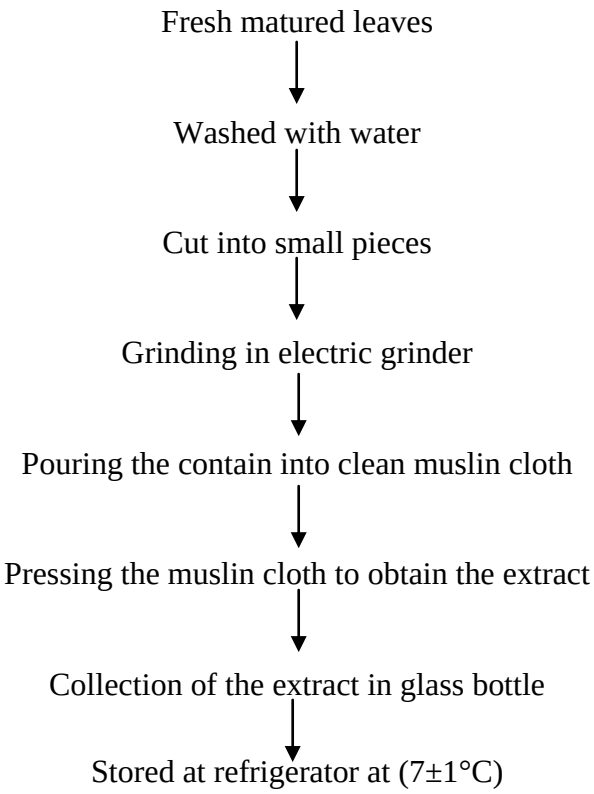

Fig. 3.3 Flow chart for Mint extract preparation

3.2.4 Experimental design

Watermelon juice used at the levels of 0%, 12.5%, 25%, 37.5% and 50%. Optimum amount of juice was determined by sensory evaluation. After the optimization of watermelon juice, mint extract was optimized by using 0%, 1.5%, 2.5%, 3.75% and 5% mint extract on whey watermelon beverage. Optimum amount of mint extract was determined by sensory evaluation. Different recipe formulation was formulated using Design expert 11 as shown in table 3.1 and table 3.2 for the optimization of watermelon juice and for the optimization of mint extract respectively. Rotatable central composite design is used to formulate the recipe.

Table 3.1 Different recipe samples to optimize watermelon juice.

S.No.	Code	Whey (ml)	Watermelon Juice (ml)
1.	A (control)	100	0
2.	B	87.5	12.5
3.	C	75	25
4.	D	62.5	37.5
5.	E	50	50

Table 3.2 Different recipe samples to optimize mint extract.

S. No.	Code	Optimized whey- watermelon beverage (ml)	Mint extract (ml)
1.	MA (control)	100	0
2.	MB	98.75	1.25
3.	MC	97.5	2.5
4.	MD	96.25	3.75
5.	ME	95	5

3.2.5 Preparation of whey based mint flavored watermelon beverage

Different formulation of whey beverage was prepared by heating whey at 45°C with addition watermelon juice and mint extract as shown in Fig. 3.4. Sugar was added to make TSS 13°Bx. After proper mixing it was filtered through clean muslin cloth to obtain whey based mint flavored watermelon beverage. Pasteurization of juice was performed in a stainless-steel vessel at 60°C. When the temperature reached 60°C, holding of beverage was done for 30 min in the vessel.

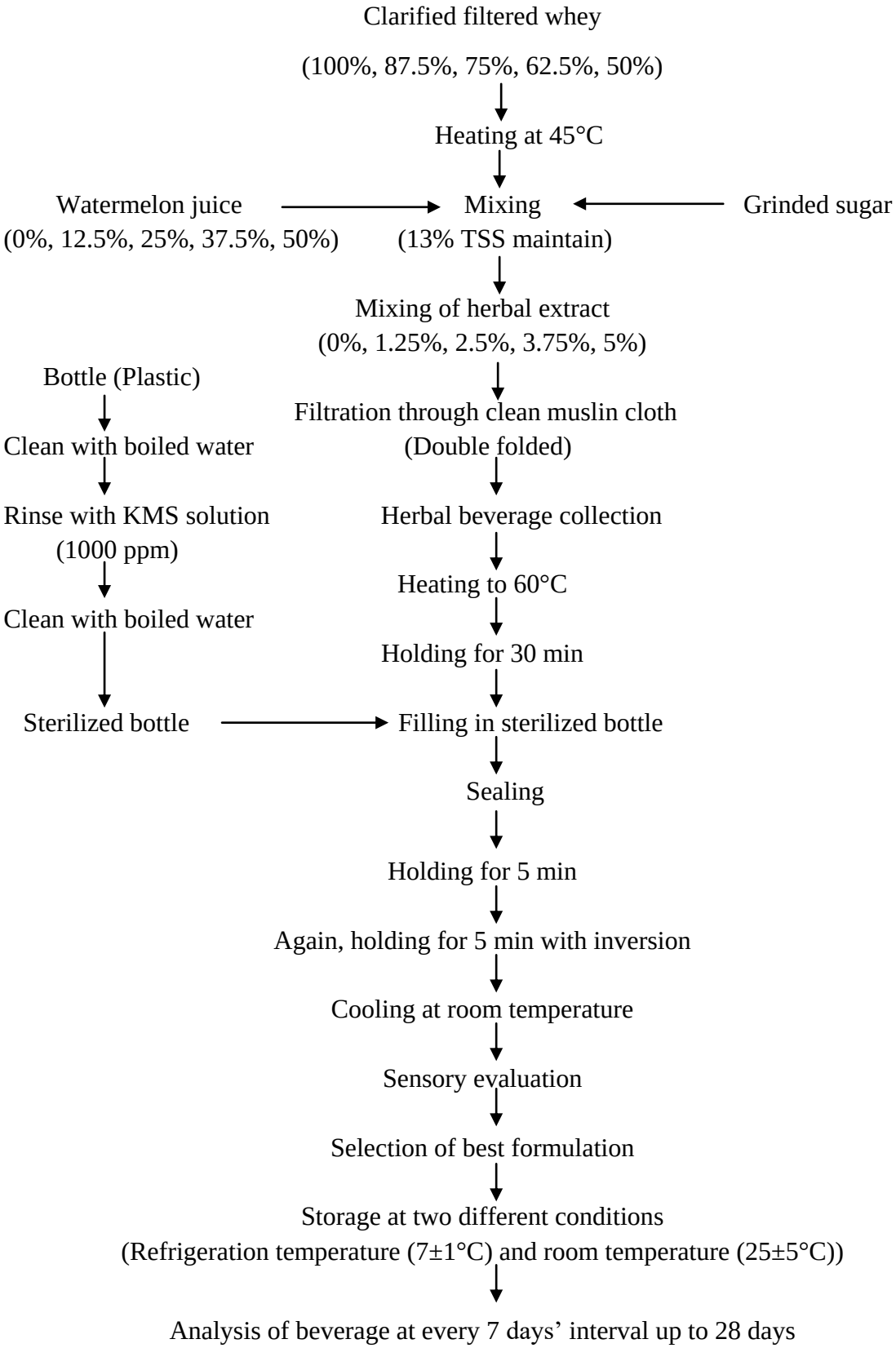

Fig. 3.4 Flowchart for preparation of whey based mint flavored watermelon beverage

Soon after the first holding, beverage was then filled into the sterilized plastic bottle and again holding for 5 min in the bottle. Sealing of bottle was done by using aluminium seal along with electric iron i.e., heat sealing. Then the bottles were inverted and holding for 5 min. Later the bottles were cooled and subjected to sensory evaluation to find out best formulation which was stored in refrigeration and ambient temperature for further study. The complete process flowchart for the preparation of beverage is presented in Fig. 3.4

3.3 Analytical procedure

3.3.1 Physical and chemical examination

Different parameter like total soluble solid, fat content, total and reducing sugar content, protein content, ash content, acidity, pH, and ascorbic acid was done for raw whey, watermelon juice and prepared beverage.

3.3.1.1 Total soluble solid (TSS)

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

3.3.1.2 Fat content

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

3.3.1.3 Total and reducing sugar content

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

3.3.1.4 Titratable acidity

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

3.3.1.5 pH

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

3.3.1.6 Ascorbic acid

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

3.3.1.7 Protein content

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

3.3.1.8 Ash content

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

3.3.1.9 Total solids

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

3.3.1.10 Moisture content

It was determined according to Ranganna (1986).

3.3.2 Sensory evaluation

The coded sample of the beverage were sensorially evaluated for appearance, color, flavor, taste and overall acceptance on 9-point hedonic scale as described by Ranganna (1986). The panelists were given instruction to give 9 points to extremely liked and 1 point to the extremely disliked point sample. The coded samples were randomly presented. For the sensory analysis, 10 semi trained panelist Central Campus of Technology were taken. The specimen card for sensory evaluation is shown in Appendix A.

3.3.3 Statistical analysis

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.

3.3.4 Microbiological analysis

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

3.3.5 Storage studies

Prepared beverage was aseptically filled in plastic bottles and glass bottles. The bottles containing beverages were stored at refrigeration temperature (7±1°C) and room temperature (25±5°C) for 28 days. Samples were drawn at intervals of 7 days and evaluated for physico-chemical properties (TSS, acidity, pH and reducing sugar) and microbiological qualities (TPC and yeast and mold count).

Part IV

Results and discussion

Whey based mint flavored watermelon beverage was prepared at the laboratory of Central Campus of Technology for the present study. The Watermelon juice and paneer whey were the major ingredient. Watermelon juice was extracted using juice extractor, paneer whey was obtained by 2% citric acid coagulation while the extract of mint was obtained by grinding and filtering of mature mint leaves. The extracted raw materials were filtered and different formulated sample was prepared and pasteurized at 60°C for about 30 min. The samples were analyzed through sensory evaluation and best scored sample was stored for 28 days at refrigerated (7±1°C) and normal (25±1°C) for study of storage stability.

4.1 Analysis of raw material

In the preparation of whey-based mint flavored watermelon beverage, whey and watermelon juice were the major raw materials. They were analyzed for their composition. The chemical composition of whey is presented in the Table 4.1.

Table 4.1 Chemical Composition of the whey

S.N.	Parameter	Value
1.	TSS (^{o}Bx)	5.87 (0.047)
2.	Moisture (%)	92.53 (0.067)
3.	pH	5.26 (0.045)
4.	Acidity as lactic acid (%)	0.23 (0.016)
5.	Reducing Sugar (%)	4.81 (0.026)
7.	Total Sugar (%)	8.70 (0.081)
8.	Protein (%)	0.55 (0.049)
9.	Ash (%)	0.57 (0.024)
10.	Fat (%)	0.46 (0.028)
11.	Total Solids (%)	7.58 (0.075)

Values are means of triplicate, figures in the parentheses are the standard deviations.

Table 4.2 Chemical composition of watermelon juice

S.N.	Parameter	Value
1.	TSS (^{0}Bx)	8.60 (0.04)
2.	Moisture (%)	93.33 (0.12)
3.	pH	5.23 (0.02)
4.	Acidity as citric acid (%)	0.07 (0.008)
5.	Reducing Sugar (%)	2.34 (0.029)
6.	Non- Reducing Sugar (%)	3.63 (0.04)
7.	Total Sugar (%)	5.97 (0.03)
8.	Protein (%)	0.35 (0.03)
9.	Ash (%)	0.21 (0.01)
10.	Vitamin C as ascorbic acid (mg/100gm)	2.78 (0.14)

Values are means of triplicate, figures in the parentheses are the standard deviations.

This chemical composition data for the fresh whey and watermelon juice was found to be a bit different than the data obtained by (Rupnar, 2006) and (Ozcelik and Yavuz, 2016) for the *paneer* whey and watermelon respectively. This may be due to the variation of species, variety of the watermelon produced. Also, variation on the whey composition may be due to the variation in the milk constitute obtained and produced in the different parts of the world.

4.2 Effect of watermelon juice on sensory characteristics

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

4.2.1 Appearance

The mean sensory score for color of beverage samples of different formulation is shown in Fig. 4.1.

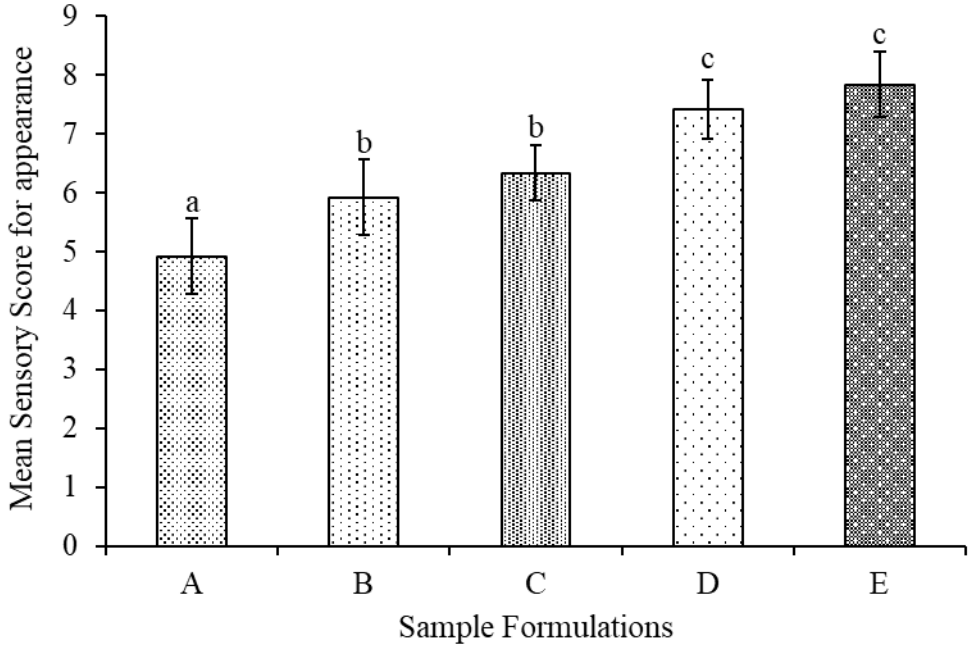

Fig. 4.1 The mean sensory scores for appearance of beverage of different formulations. Bars with similar alphabets at the top are not significantly different.

The mean sensory score for the appearance of the beverage of five samples A, B, C, D and E were determined to be 4.9, 5.9, 6.3, 7.4 and 7.8 respectively. Statistical analysis showed that there was significant effect of proportion variation of whey and watermelon juice on the appearance of beverage at 5% level of significance (Appendix C). LSD shows that product B and C and D and E were not significantly different while the other products sample were found to be significantly different to each other. The mean sensory score for appearance of sample E was found to be 7.8 and was highest of all other formulations.

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

4.2.2 Color

The mean sensory score for color of beverage samples of different formulation is shown in Fig. 4.2.

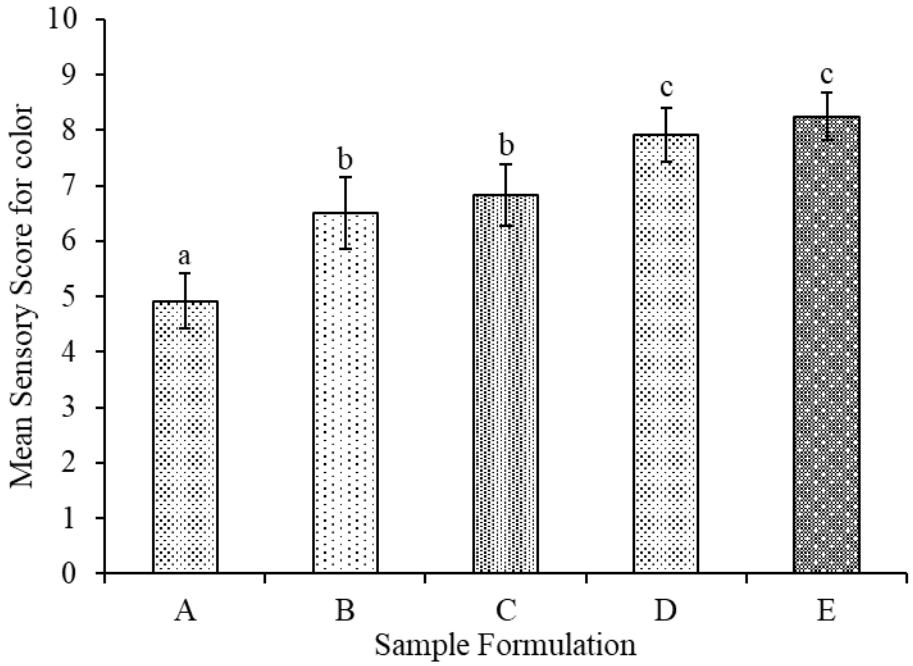

Fig 4.2 The mean sensory scores for color of beverage of different formulations. Bars with similar alphabets at the top are not significantly different.

The mean sensory score for the color of the beverage of five samples A, B, C, D & E were determined to be 4.9, 6.5, 6.8, 7.9 & 8.3 respectively. Statistical analysis showed that there was significant effect of proportion variation of whey & watermelon juice on the color of beverage at 5 % level of significance (Appendix C). LSD shows that product B&C and D&E were not significantly different while the other products sample were found to be significantly different to each other. The mean sensory score for color of sample E was found to be 8.3 and was highest of all other formulation. Sample E got highest score may be due to appealing red color of watermelon juice over whey.

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

4.2.3 Flavor

The mean sensory score for flavor of beverage samples of different formulation is shown in Fig. 4.3

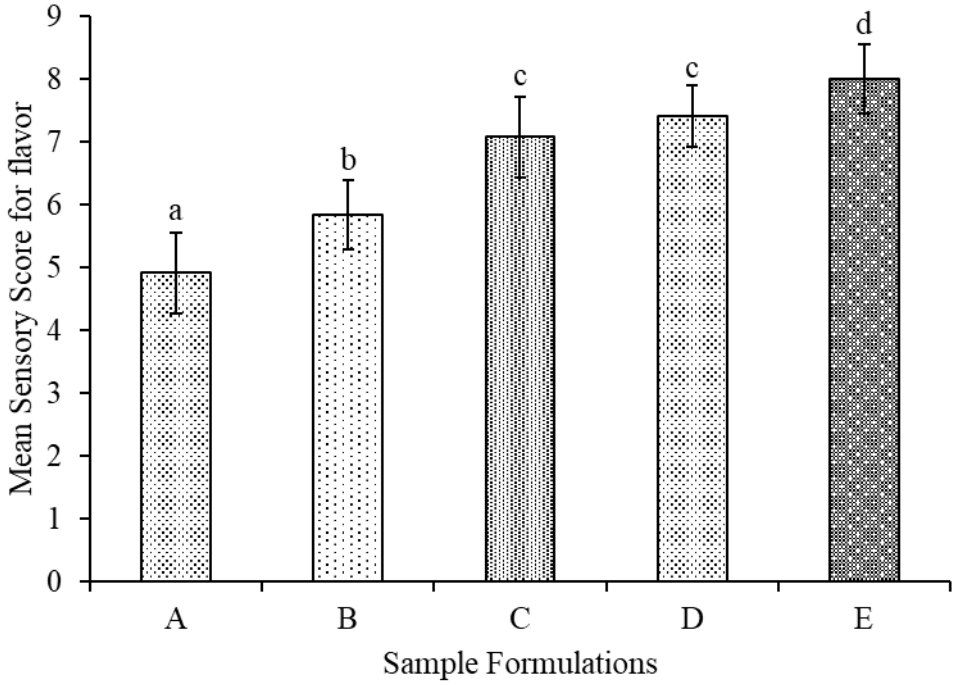

Fig. 4.3 The mean sensory scores for flavor of beverage of different formulations. Bars with similar alphabets at the top are not significantly different.

The mean sensory score for the flavor of the beverage of five samples A, B, C, D and E were determined to be 4.9, 5.8, 7.1, 7.4 and 8.0 respectively. Statistical analysis showed that there was significant effect of proportion variation of whey and watermelon juice on the flavor of beverage at 5% level of significance (Appendix C). LSD shows that product C and D were not significantly different to each other but significantly different with other formulations.

Saxena *et al.* (2013) reported that the mean sensory score for the flavour decreased with increase in quantity of whey. This is because the blends contain low total solids in the beverage as the level of whey increased and also owing to the strong flavour of whey. Same result obtained by Nagadevi and Puraikalan (2015) and Zaman *et al.* (2016) who found flavor score increased on increasing the fruit juice content.

4.2.4 Taste

The mean sensory score for taste of beverage samples of different formulations is shown in Fig. 4.4

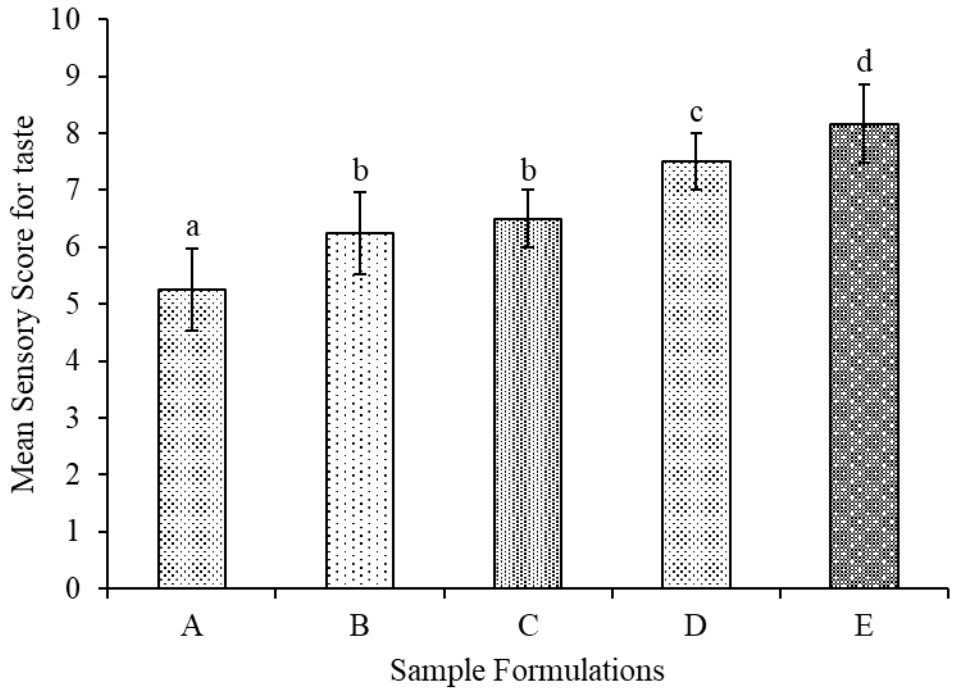

Fig. 4.4 The mean sensory scores for taste of beverage of different formulations. Bars with similar alphabets at the top are not significantly different.

The mean sensory score for the taste of the beverage of five samples A, B, C, D and E were determined to be 5.3, 6.3, 6.5, 7.5 and 8.2 respectively. Statistical analysis showed that there was significant effect of proportion variation of whey and watermelon juice on the Taste of beverage at 5% level of significance (Appendix C). LSD shows that product B and C were not significantly different to each other but significantly different with other formulations. Products A, D and E were significantly different to each other among which the mean sensory score for taste of sample E was found to be 8.2 and was highest of all other formulations.

Saxena *et al.* (2013) reported increase in the sensory score for the taste of beverage while increasing the watermelon juice content in the preparation of whey based

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

4.2.5 Overall Acceptability

The mean sensory score for overall acceptability of beverage samples of different formulation is shown in Fig. 4.5.

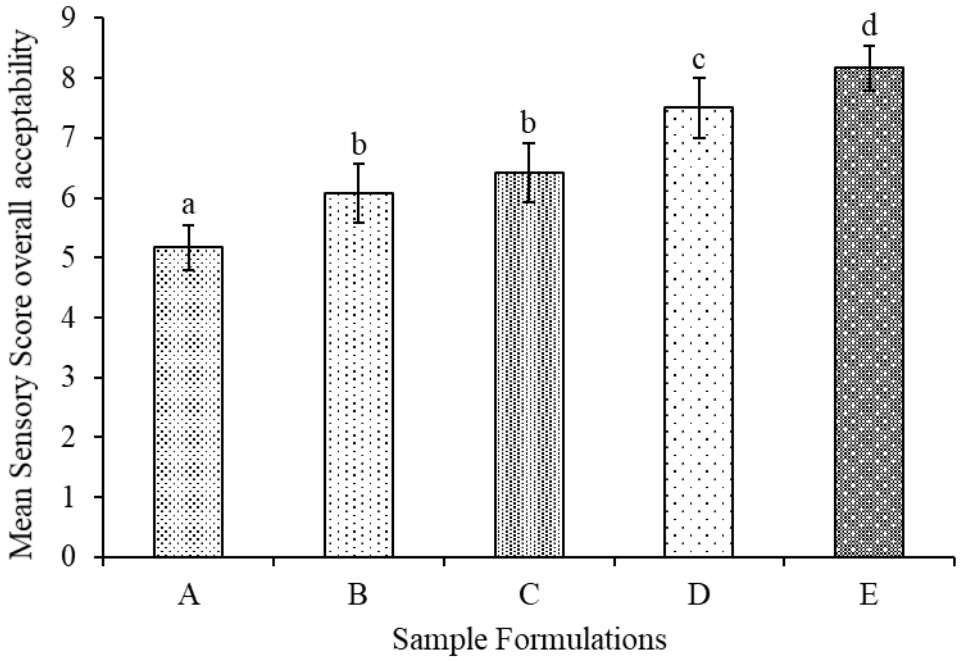

Fig. 4.5 The mean sensory scores for overall acceptability of beverage of different formulations. Bars with similar alphabets at the top are not significantly different.

The mean sensory score for the overall acceptability of the beverage of five samples A, B, C, D and E were determined to be 5.2, 6.1, 6.4, 7.5 and 8.2 respectively. Statistical analysis showed that there was significant effect of proportion variation of whey and watermelon juice on the overall acceptability of beverage at 5% level of significance (Appendix C). LSD shows that product B and C were not significantly different to each other but significantly different with other formulations. Products A, D and E were significantly different to each other among which the mean sensory score for taste of sample E was found to be 8.2 and was highest of all other formulations.

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

4.3 Effect of mint *(Mentha arvensis)* on sensory quality of beverage.

Five different samples of varying proportion of whey- watermelon beverage & mint extract were taken and coded as MA, MB, MC, MD and ME respectively. The TSS of the samples was kept constant *viz.* 13°Bx. Then the formulations having different proportion of whey based mint flavored watermelon beverage was subjected to sensory evaluation.

4.3.1 Appearance

The effect of mint extract on appearance of beverage samples of different formulation are shown in Fig. 4.6

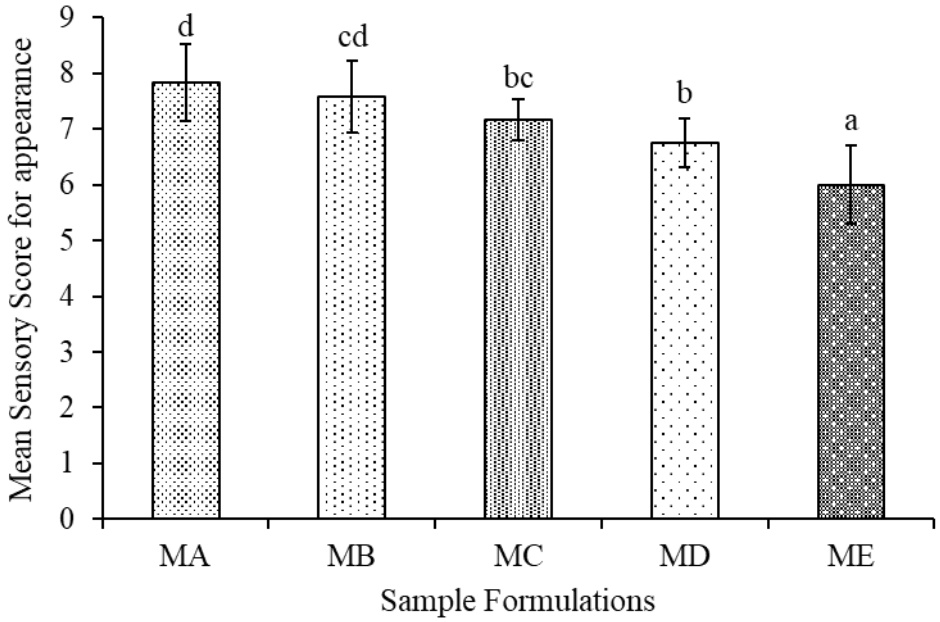

Fig. 4.6 The mean sensory scores for appearance of beverage of different formulations. Bars with similar alphabets at the top are not significantly different.

The mean sensory score for the appearance of the beverage of five samples MA, MB, MC, MD and ME were determined to be 7.8, 7.6, 7.2, 6.8 and 6.0 respectively. Statistical analysis showed that there was significant effect of proportion variation of mint extract on the appearance of beverage at 5% level of significance (Appendix D). LSD shows that product MA and MB and MB and MC and MC and MD were not significantly different while the other products sample were found to be significantly different to each other. The mean sensory score for appearance of sample MA was found to be 7.8 and was highest of all other formulations. This may due to increase in dusty appearance of beverage by increasing mint extract.

Satpute *et al.* (2018a) reported decrease in the sensory score for the appearance of beverage while increasing the *mentha* extract content in the preparation of whey-based beetroot and menthol beverage.

4.3.2 Color

The effect of mint extract on color of beverage samples of different formulations are shown in Fig. 4.7

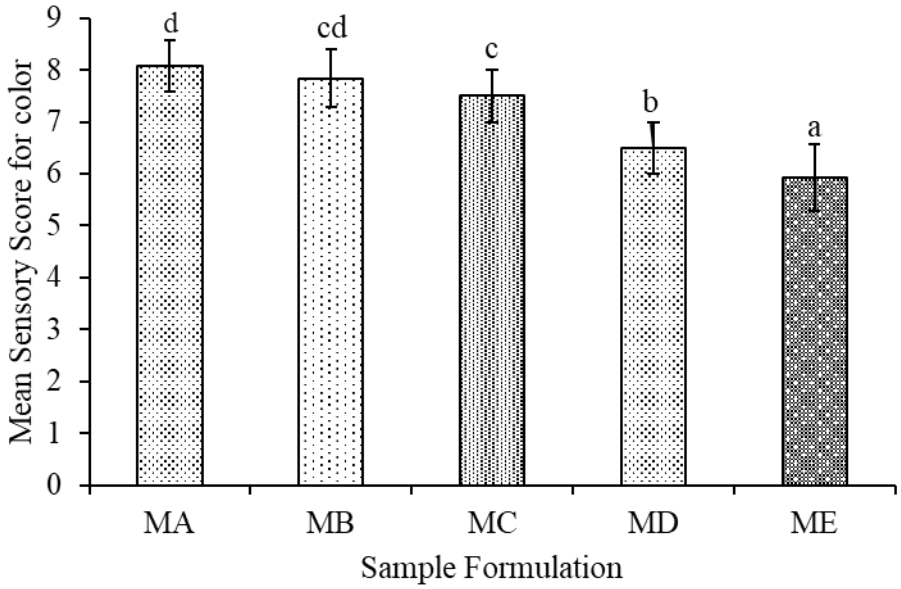

Fig. 4.7 The mean sensory scores for color of beverage of different formulations. Bars with similar alphabets at the top are not significantly different.

The mean sensory score for the color of the beverage of five samples MA, MB, MC, MD and ME were determined to be 8.1, 7.8, 7.5, 6.5 and 5.9 respectively. Statistical analysis showed that there was significant effect of proportion variation of mint extract on the color of beverage at 5 % level of significance (Appendix D). LSD shows that product MA and MB and MB and MC were not significantly different while the other products sample were found to be significantly different to each other. The mean sensory score for color of sample MA was found to be 8.1 and was highest of all other formulations. Sample MA got highest score among the other samples may be due to attractive red color of beverage. The mean sensory score decreases with increase in mint extract content, this may due to addition of green color of mint extract to the beverage.

Satpute *et al.* (2018a) reported decrease in the sensory score for the color of beverage while increasing the mentha extract content in the preparation of whey-based beetroot and menthol beverage. Baljeet *et al.* (2013) also concluded the similar result for the color of mixed herbal beverage.

4.3.3 Flavor

The effect of mint extract on flavor of beverage samples of different formulation are shown in Fig. 4.8

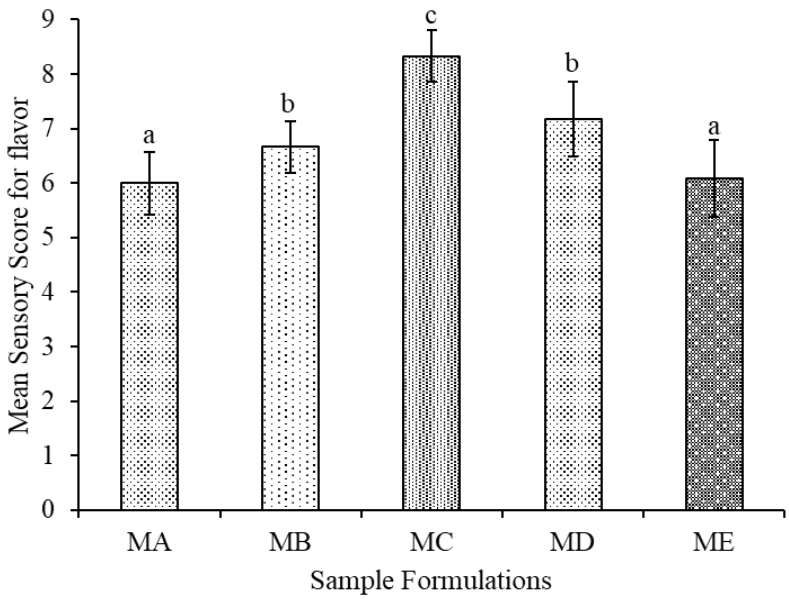

Fig. 4.8 The mean sensory scores for flavor of beverage of different formulations. Bars with similar alphabets at the top are not significantly different.

The mean sensory score for the flavor of the beverage of five samples MA, MB, MC, MD and ME were determined to be 6.0, 6.7, 8.3, 7.2 and 6.1 respectively. Statistical analysis showed that there was significant effect of proportion variation of mint extract on the flavor of beverage at 5% level of significance (Appendix D). LSD shows that product MA and ME and MB and MD were not significantly different to each other while the sample MC was found to be significantly different with other samples and was got highest score than other samples.

According to Dhamsaniya and Varshney (2013b) the value of mean sensory score for the flavor of whey based banana herbal juice was increased only up to 3% level of mint extract.

4.3.4 Taste

The effect of mint extract on taste of beverage samples of different formulation are shown in Fig. 4.9

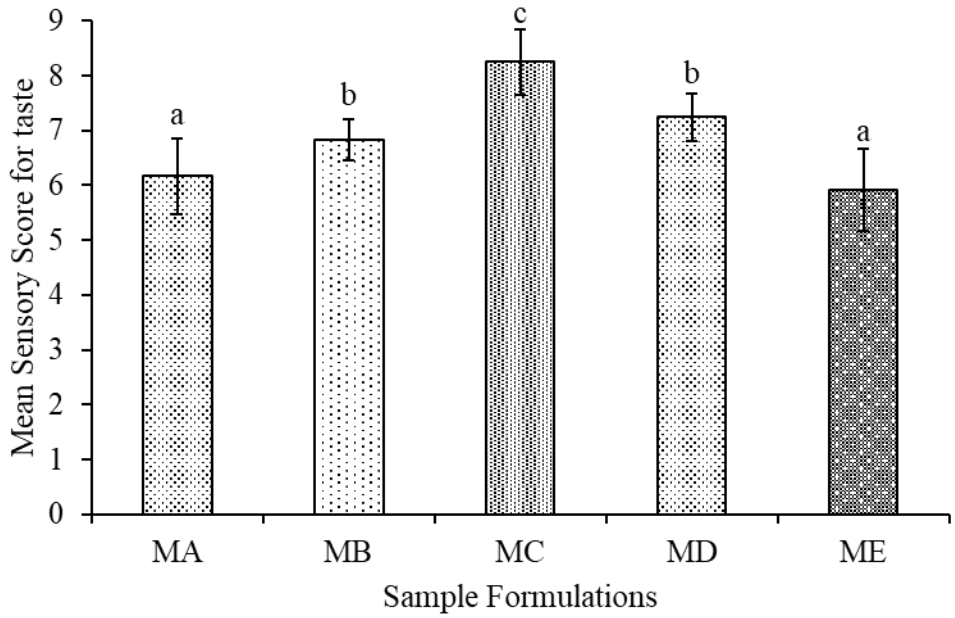

Fig. 4.9 The mean sensory scores for taste of beverage of different formulations. Bars with similar alphabets at the top are not significantly different.

The mean sensory score for the taste of the beverage of five samples MA, MB, MC, MD and ME were determined to be 6.2, 6.8, 8.3, 7.3 and 5.9 respectively. Statistical

analysis showed that there was significant effect of proportion variation of mint extract on the taste of beverage at 5% level of significance (Appendix D). LSD shows that product MA and ME and MB and MD were not significantly different to each other while the sample MC was found to be significantly different with other samples and was got highest score than other samples.

Satpute *et al.* (2018a) reported similar result in the sensory score for the taste of beverage while increasing the mentha extract content in the preparation of whey based beetroot and menthol beverage.

4.3.5 Overall Acceptability

The effect of mint extract on overall acceptability of beverage samples of different formulation are shown in Fig. 4.10

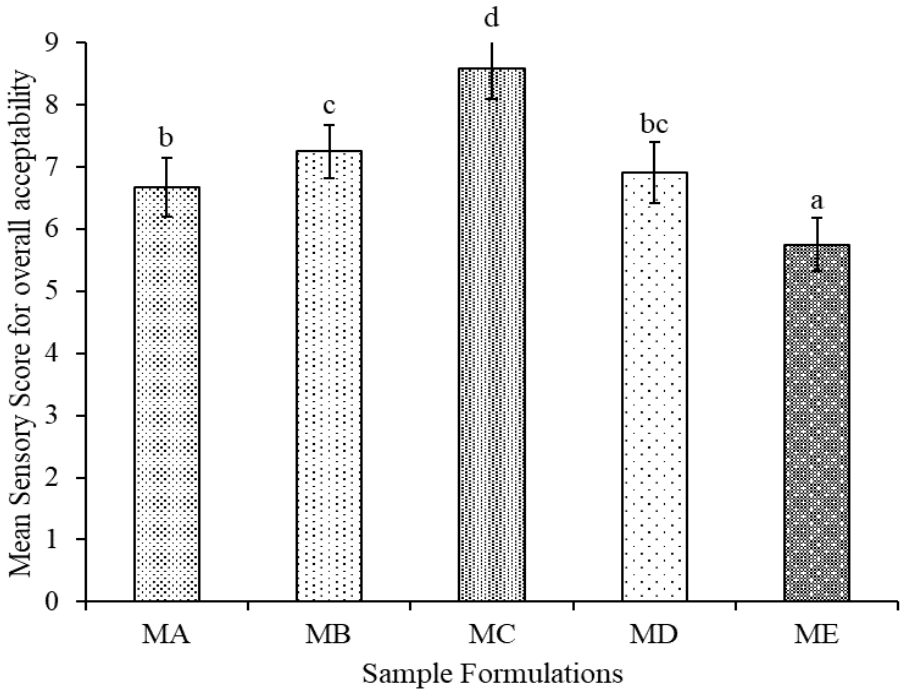

Fig. 4.10 The mean sensory scores for overall acceptability of beverage of different formulations. Bars with similar alphabets at the top are not significantly different.

The mean sensory score for the overall acceptability of the beverage of five samples MA, MB, MC, MD and ME were determined to be 6.7, 7.3, 8.6, 6.9 and 5.8 respectively. Statistical analysis showed that there was significant effect of proportion variation of mint

extract on the overall acceptability of beverage at 5% level of significance (Appendix D). LSD shows that product MA and MD and MB and MD were not significantly different to each other while the other samples were found to be significantly different with other samples.

From the statistical sensory analysis sample E with watermelon juice 50% and whey 50% was found to be best for the optimization of watermelon juice, and for the optimization of mint extract the sample MC with mint extract 2.5% was found to be best.

4.4 Chemical composition of final product.

The sample with whey 50% (V/V), water-melon juice 50% (V/V) and mint extract 2.5% (V/V) is rated superior in the sensory evaluation. Water-melon and mint extract blend flavor and mint extract taste on the beverage was accepted and rated superior. The chemical composition of the final product (sensorily scored superior) by the sensory panelist is presented in Table 4.3.

Table 4.3 Chemical composition of final product

S.N.	Parameter	Value
1.	TSS (^{0}Bx)	12.93 (0.047)
2.	Moisture (%)	93.60 (0.081)
3.	pH	5.18 (0.062)
4.	Acidity as citric acid (%)	0.20 (0.008)
5.	Reducing Sugar (%)	3.98 (0.124)
6.	Non- Reducing Sugar (%)	8.72 (0.084)
7.	Total Sugar (%)	12.70 (0.040)
8.	Protein (%)	0.43 (0.020)
9.	Ash (%)	0.38 (0.004)
10.	Fat (%)	0.24 (0.020)
11.	Total Solid	6.47 (0.124)
10.	Vitamin C as ascorbic acid (mg/100gm)	1.77 (0.024)

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

4.5 Storage study

Prepared whey watermelon herbal beverage was stored for 28 days' time period at refrigerated condition (7±1°C) and room temperature (25±5°C). On every 7 days' interval microbiological and chemical compositions were determined.

4.5.1 Microbiological analysis

In microbiological analysis TPC, yeast and mold count was done. There was no any colony observed during initial storage period. During preparation of beverage heat treatment at 60°C for 30 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 sealed product, growth of microorganism was found to be slow. The microbial analysis is shown in fig. 4.11 and fig. 4.12.

4.5.1.1 Total plate count

Increase in the total plate count of the whey based mint flavored watermelon beverage during 28 days of storage at both storage temperatures was observed (Fig. 4.11). Total plate count for the beverage ranged from 0 cfu/ml to 18×10^3 cfu/ml for room temperature (25±5°C) and 0 cfu/ml to 10×10^3 cfu/ml for refrigeration temperature (7±1°C) during the 28 days of storage.

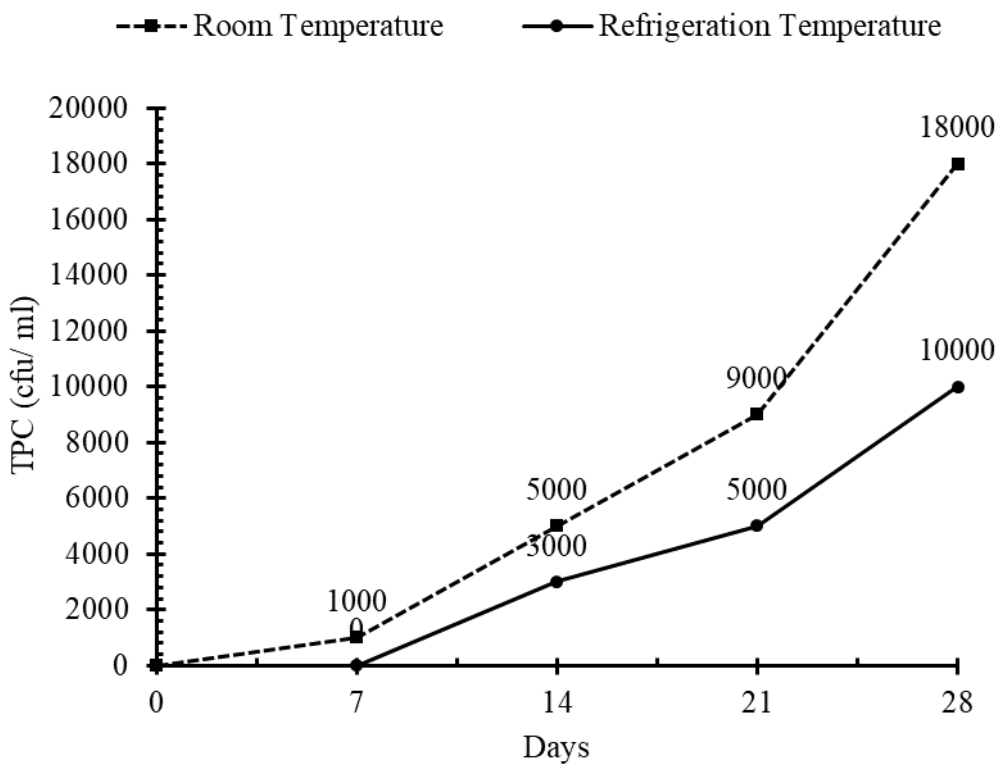

Fig. 4.11 Total plate count of product

4.5.1.2 Yeast and mold count

Increase in the yeast and mold count of the whey based mint flavored watermelon beverage during 28 days of storage at both storage temperatures was observed (Fig. 4.12). Yeast and mold count for the beverage ranged from 0 cfu/ml to 14×10^3 cfu/ml for room temperature (25±5°C) and 0 cfu/ml to 8×10^3 cfu/ml for refrigeration temperature (7±1°C) during the 28 days of storage.

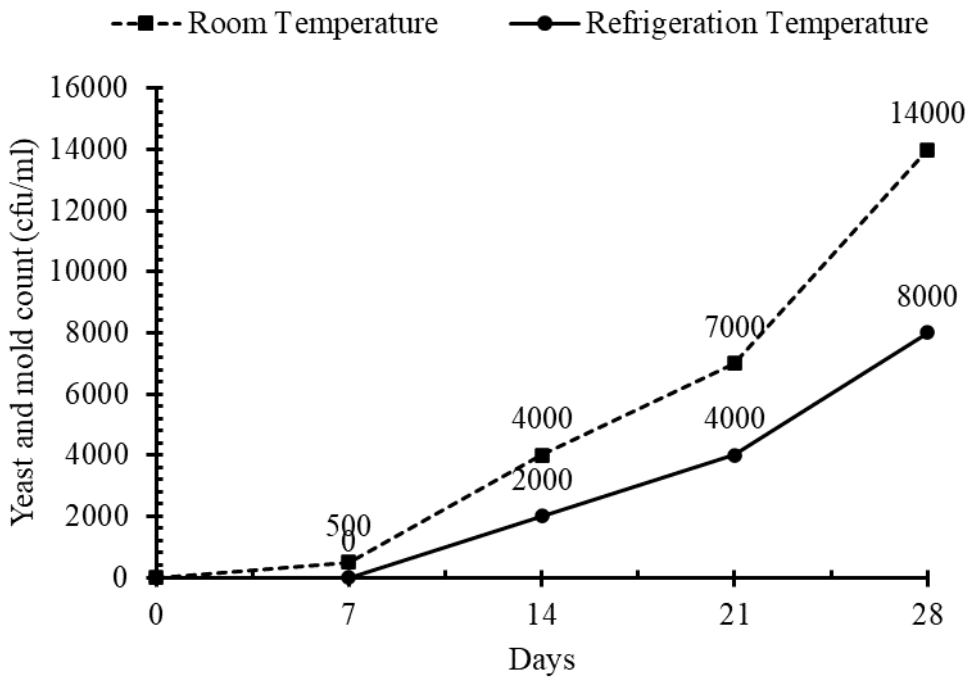

Fig. 4.12 Yeast and mold count of product

4.5.2 Chemical analysis

Effects of storage period on chemical composition (TSS, pH, titrable acidity and reducing sugar) of whey water-melon beverage were analyzed. The changes in physicochemical properties during storage are presented in graphs below.

4.5.2.1 Effect on TSS

A slight increase in the TSS of the whey based mint flavored watermelon beverage during 28 days of storage at both storage temperatures was observed (Fig. 4.13). TSS for the beverage ranged from 12.93 to 14.65°Bx (25±5°C) and 12.93–14.1°Bx (7±1°C) during the 28 days of storage. Retention or minimum increase in TSS content of juice during storage is desirable for preservation of good juice quality (Bhardwaj and Pandey, 2011). Similar results were observed in the preparation of mixed fruit RTS (Bull *et al.*, 2004; Deka and Sethi, 2001). LSD showed that there was significant difference on TSS at 5% level of significance (Appendix E).

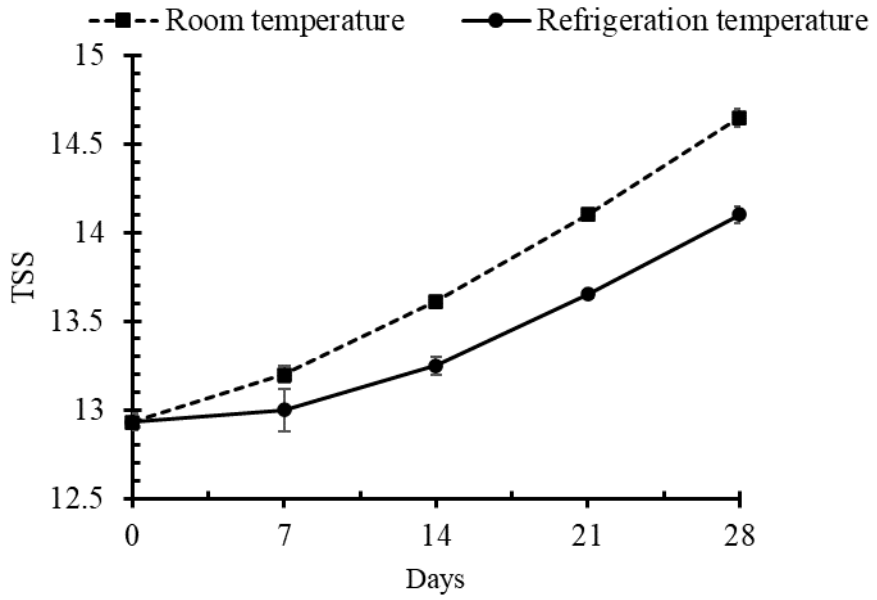

Fig. 4.13 Effect of storage time and temperature on TSS of prepared beverage

4.5.2.2 Effect on pH

There was a significant decrease in pH during storage. pH is one of the important quality characteristics that describes the stability of bioactive compounds in fruit juice (Bhardwaj and Pandey, 2011). There was a slight decrease in pH during 28 days of storage (Fig. 4.14) which ranged from 5.18 to 5.05 (7±1°C) and 5.18 to 4.97 (25±5◦C) that affects the organoleptic quality of juice. This might be due to increase in titrable acidity, as acidity and pH are inversely proportional to each other. Similar results were reported for a juice blend of bottle guard and basil leaves juice by Majumdar *et al.* (2011) and for pineapple juice blend with carrot and orange by Awsi and Dorcus (2012). LSD showed that there was significant difference on pH at 5% level of significance (Appendix E).

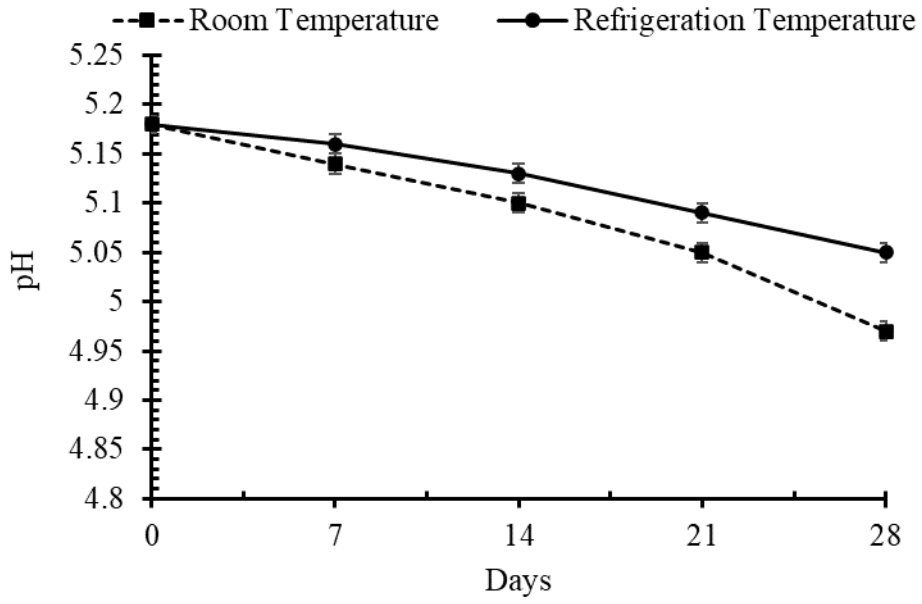

Fig. 4.14 Effect of storage time and temperature on pH of prepared beverage

4.5.2.3 Effect on titrable acidity

The effect of storage on the acidity of beverage is shown in (Fig. 4.15). During 28 days 0f storage the acidity was found to have gradually increased from 0.20 to 0.40 (25±5 °C) and 0.20 to 0.33 (7±1°C). The increase in acidity may be due to the production of organic acids and amino acids by the action of ascorbic acid on sugar and protein content of the beverages. Lactose and proteins are converted into lactic acid and amino acids leading to increase in acidity and decrease in pH of beverages (Kalra *et al.*, 1991). The results are in agreement with the findings reported in whey-based banana herbal beverage (Yadav *et al.*, 2010) and also in whey based RTS from mango (Sakhale *et al.*, 2007b). LSD showed that there was significant difference on acidity at 5% level of significance (Appendix E).

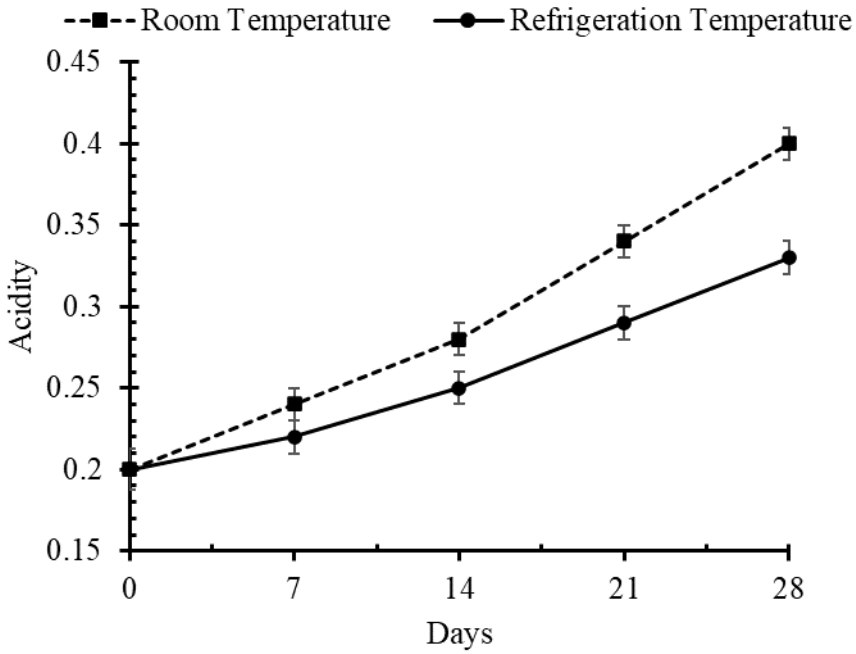

Fig. 4. 15 Effect of storage time and temperature on acidity of prepared beverage

4.5.2.4 Effect on reducing sugar (RS)

Sugars are one of the most important constituents of fruit products, essential for and also act as a natural food preservative (Bhardwaj and Pandey, 2011). The RS value for beverage ranged from 3.98 to 4.56 (25±5°C) and 3.98 to 4.36 (7±1 °C) during the 28 days of storage. The results showed gradual increase in reducing sugar with increasing storage period (Fig. 4.16). 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 (Kauser *et al.*, 2012) and bottled gourd–basil leave juice (Majumdar *et al.*, 2011). LSD showed that there was significant difference on reducing sugar at 5% level of significance (Appendix E).

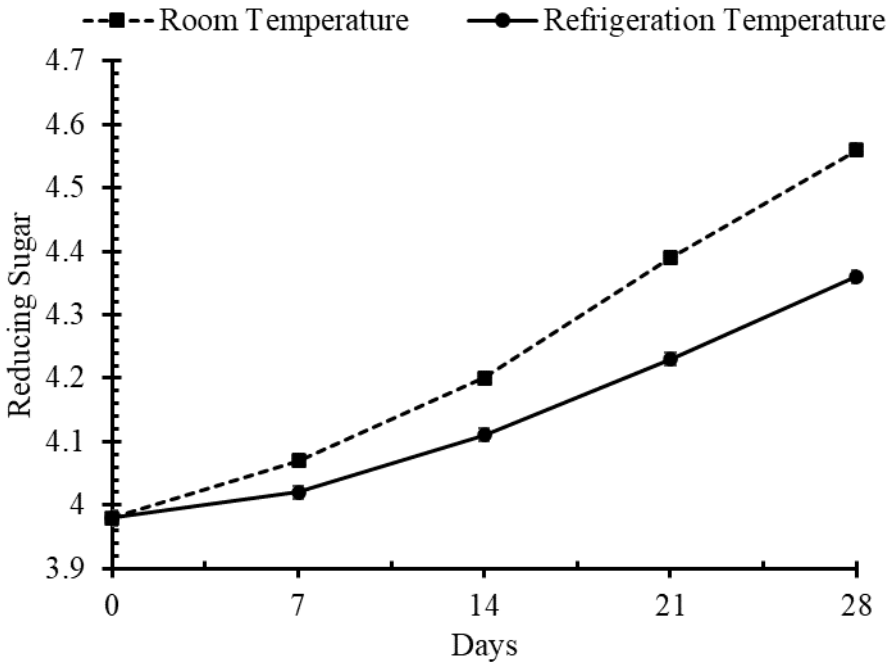

Fig. 4.16 Effect of storage time and temperature on reducing sugar content of prepared beverage.

Part V

Conclusions and recommendations

5.1 Conclusions

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

1. From sensory analysis, whey-watermelon herbal beverage with 50% watermelon juice (V/V), 2.5% mint extract (V/V), 50% whey (V/V) and 13°Bx TSS was found superior with respect to appearance, color, flavor, taste and overall acceptance.

2. During 28 days of storage TSS, acidity and reducing sugar contents increased while pH decreased. These changes in refrigerated storage condition (7±1°C) were found to be minor with respect to normal storage condition (25±5°C).

3. The beverage can be stored at refrigeration temperature without adding any chemical preservative with desirable acceptability up to 28 days.

4. The composition of the product was found to be 12.93^0 BX TSS, 6.47% total solids, 0.20 % acidity, pH 5.18, 3.98 % reducing sugar, 0.43% protein, 0.24% fat, 0.38% ash, and 1.77 mg vitamin C.

5.2 Recommendations

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

1. Formulation of such type of beverage can be done with varying proportion of fruit juices and with incorporation of variable herbal type and fruit type can be done.
2. Shelf life of beverage (storage stability) can be studied using different chemical preservatives and with different packaging materials.
3. Change in nutritional as well as phytochemical properties during storage can be done.

Part VI

Summary

Whey is a nutritious by product of cheese, chhana and *paneer* industry containing valuable nutrients like lactose, protein, minerals and vitamin etc. Whey constitutes 45-50% of total milk solids, 70% of milk sugar (lactose), 20% of milk protein and 70-90% of milk minerals and most importantly, almost all the water-soluble vitamins originally present in milk. present work was connected to study the consumer acceptance of whey beverage, its chemical and storage quality.

The whey was prepared from market milk having 3% fat and 8% SNF brought from local market of Dharan by coagulating with 2% citric acid at optimum temperature. While the watermelon juice was extracted using juice extractor and the separated juices were stored for the further analysis. In the preparation of whey-based mint flavored watermelon beverage, whey and watermelon juice were the major raw materials. They were analyzed for their chemical composition. Whey-watermelon herbal beverage samples formulation was designed by experimental design software under mixed condition. From the statistical sensory analysis best product was formulated from watermelon juice 50% (V/V), mint extract 2.5% (V/V) and 50% (V/V) whey. TSS was maintained at the levels 13^0 Bx. The prepared beverage was evaluated for their physio-chemical properties, microbiological and organoleptic qualities. The superior beverage from the sensory analysis was analyzed for chemical composition and storage stability under refrigerated (7±1°C) and normal (25±5°C) storage condition. The analyzed chemical composition of best sample was found to be T.S.S (12.83 °Bx), Moisture (86.67%), pH (4.20), Acidity (0.46%), Reducing sugar (5.17%), Non-reducing sugar (10.60%), Total sugar (15.77%), Proteins (0.42%), Ash (0.32%), Fat (0.22%), Ascorbic acid (26.70 mg/100 g), and Total solids (13.33%). During storage study TSS, pH and reducing sugar increased while acidity and ascorbic acid content decreased under both storage conditions. Use of whey as a substitute of water during beverage preparation, will be beneficial to the consumer, dairy industries and fruit growers also and finally not only economic loss minimize but also helps to minimize environmental pollution.

References

Abd el-salam, M. H., Shibiny, S. and Salem, A. (2009). Factors affecting the functional properties of whey protein products: A Review. *Food Reviews Int*. **25**, 251- 270.

Affertsholt, T. (2015). International whey market overview. Presented at 2015 ADPI/ABI Annual Conference. p. 36.

Anand, S., Khanal, S. M. and Marella, C. (2013). Whey and whey products. *In:* "Milk and Dairy Products in Human Nutrition: Production, Composition and Health." (1st ed.). (Y. W. Park and G. F. W. Haenlein, Eds.). pp. 477- 497. John Wiley & Sons, Ltd.

Anon. (2015). Utilisation of whey. Retrieved from http://dairy-technology.blogspot.com/2014/12/dairy-technology.html. [Accessed 25 March, 2018].

Anon. (2018). Top watermelon producing countries in the world. World Atlas. Retrieved from https://www.worldatlas.com/articles/top-watermelon-producing-countries-in-the-world.html. (Last update 19 September, 2018). [Accessed 21 june 2020].

Anonymous. (2011). Whey Powder. Retrieved from http://www.wheypowder.org/. [Accessed 20 January, 2018].

AOAC. (2005). "Official Methods of Analysis." (12th ed.). Association of Official Analytical Chemists. Washington D.C.

Awsi, J. and Dorcus, M. (2012). Development and quality evaluation of pineapple juice blend with carrot and orange juice. *Int. J. Sci. Res.* **8** (2), 1-8.

Baljeet, S. Y., Ritika, B. Y. and Sarita, R. (2013). Studies on development and storage of whey- based pineapple and bottle gourd mixed herbal beverage. *Int. Food Res. J.* **20**, 607-612.

Banerjee, R. and Chen, H. (1995). Functional properties of edible films using whey protein concentrate. *J. Dairy Sci.* **78**, 1673- 1683.

Bates, D. M. and Robinson, R. W. (1995). Cucumbers, melons and water-melons. *In:* "Evolution of Crop Plants.". (J. Smart and N. W. Simmonds, Eds.). Longmon, London.

Bhardwaj, R. J. and Pandey, S. (2011). Juice blends- a way of utilization of under- utilized fruits, vegetables, and spices. *J. Crit. Rev. Food Sci. Nutr.* **51** (6), 563-5700. 2360.

Bolumer, T., Toepfl, S. and Heinz, V. (2015). Fat reduction and replacement in dry-cured fermented sausage by using high pressure processing meat as fat replacer and olive oil. *J. Food Nutri. Sci.* **65**, 175-182.

bozanic, R., Barukcik, I., Jakopovic, K. L. and Tratnik, L. (2014). Possibilities of whey utilization. *J. Nutri. Food Sci.* **2**, 1036.

Bull, M. K., Zerdin, K., Howe, E., Goicoechea, D., Paramanandhan, P., Stockman, R., Sellahewa, J., Szabo, E. A., Johnson, R. L. and Stewart, C. M. (2004). The effect of high pressure processing on the microbial, physical and chemical properties of Valrncia and Navel orange juice. *J. Innovative Food Sci. and Emerging Technol.* **5**, 135-149.

Canning, K. (2004). Wonderous whey. Dairy Field. **187** (9), 55- 62.

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

Chiej, R. (1984). Health benefits. *In:* "Encyclopedia of Medicinal Plants". [ISBN 0-356-10541-5].

Collins, J. (2007). Watermelon consumption increases plasma arginine. *In:* "Nutrition" (Vol. 23). pp. 261-266.

Darade, R. V. and Ghodake, S. S. (2012). An overview of whey beverages. *J. Anim. Husb. & Dairy Sci.* **3** (1), 41-44.

De Wit, J. N. (2004). Nutrients in whey. *In:* "Lecture Handbook on Whey and Whey Product" (1st ed.) pp. 16-19. Rue Montoyer 1000 Brussels, Belgium. European Whey Products Association.

Deka, B. C. and Sethi, V. (2001). Preparation of mixed fruit juice beverages. *Int. J. Food Sci. Nutr.* **2** (5), 102-105.

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

DeWit, J. N. (2002). "Lecturer's Handbook on Whey and Whey Products." (1st ed.). Vol. 1. European Whey Products Association. Belgium.

Dhamsaniya, N. K. and Varshney, A. K. (2013a). Development and evaluation of whey based RTS beverage from ripe banana juice. *J. Food Process Technol.* **4** (2). [doi:10.4172/2157-7110.1000203].

Dhamsaniya, N. K. and Varshney, A. K. (2013b). Development and evaluation of whey based RTS beverage from ripe banana juice. *J. Food Process Technol.* **4** (2).

Dickinson, E. (1998). Stability and rheological implications of electrostatic milk protein–polysaccharide interactions. *Trends Food Sci. Technol.* **9**, 347-354.

Direinzo, D. (2004). Whey products, milk minerals and dairy calcium: new findings and benefits. Reference Manual for U.S. Whey and Lactose Products, Section 6.7. USDEC. Arlington, VA., 75-77.

Duke, J. A. and Ayensu, E. S. (1985). "Medicinal Plants of China". Vol. 2. Reference Publications.

Egbounu, A. C. (2015). Comparative investigation of the proximate and functional properties of watermelon (*Citrullus lanatus*) rind and seed. *Res. J. Environ. Toxicol.* **9** (3), 160-167.

Fox, P. F., Uniacke-Lowe, T., McSweeny, P. L. H. and O'Mahony, J. A. (2015). "Dairy Chemistry and Biochemistry" (2nd ed.). Springer International. Switzerland.

Guner, N. and Wehner, T. C. (2004). The genes of watermelon. *J. Am. Soc. Hort. Sci.* **39**, 1175-1182.

Hazen, C. (2005). Packing that Protein Punch. Food Product Design. **15** (4), 32-55.

Heino, A. (2010). Microfiltration in cheese and whey processing. *J. Dairy chem.* **56**, 456-461.

Holsinger, V. H., Posati, L. P. and Devilbiss, E. D. (1974). Whey beverages: A review. *J. Dairy Sci.* **57**, 849- 859.

Hugunin, A. (2009). Whey products in yogurt and fermented dairy products. Retrieved from http://usdec.files.cmsplus.com/PDFs/2008ReferenceManuals/Whey_Lactose_Reference_Manual_Complete2_Optimized.pdf. [Accessed 1 January, 2019].

Huh, Y. C., Solmaz, I. and N., S. (2008). Morphological characterization of Korean and Turkish watermelon germplasm. Presented at Proc. Genetics and Breeding of Cucurbitaceae (M. Pitrat, ed.). Avignon (France). May 21- 24.

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

Jelen, P. (2002). Whey: composition, properties, processing and uses. *J. Encyclopedia Food Sci. Technol.* **4**, 2652-2661.

Jelen, P., Currie, R. and Kadis, V. W. (1987). Compositional analysis of commercial whey drinks. *J. Dairy Sci.* **70**, 892-895.

Jelicic, I., Bozanic, R. and Tratnik, L. (2008). Whey-based beverages- a new generation of diary products. *J. Food Technol. Biotechnol.* **58** (3), 257-274.

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

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

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

Kosikowski, F. V. (1979). Whey utilization and whey products. *J. Dairy sci.* **62** (7), 1151.

Koziol, J., Gustaw, W., Wasko, A., Skrzypczak, K., Slawinska, A. and Solowiej, B. (2014). Effect of selected milk protein preparations on growth and survival of *Lactobacillus acidophilus* as well as on rheological properties of fermented milk beverages. [Abstract]. Nauka Technologia. **3**, 41-55. Retrieved from https://doi.org/10.1515/ pjfns-2015-0052. [Accessed 1 january, 2019].

Kravchenko, E. F. (1988). Whey beverages. Trends in Utilization of Whey and Whey Derivatives, IDF Bulletin 233. pp. 61-67. Brussels, Belgium. International Dairy Federation.

Liu, K., Tian, Y., Stieger, M. and Van, V. F. (2016). Evidence for ball-bearing mechanism of microparticulated whey protein as fat replacer in liquid and semi-solid multi-component. *J. Food Hydrocolloid.* **52**, 403-414.

Macwan, S. R., Dabhi, B. K., Parmer, S. C. and Aparnathi, K. D. (2016). Whey and its utilization. *Int. J. Curr. Microbiol. App. Sci.* **5** (8), 134-155.

Majumdar, T. K., Wadikar, D. D. and Bawa, A. S. (2011). Effect of storage on physicochemical, microbiological and sensory quality of bottleguard basil juice. *Am. J. Food Technol.* **6**, 226-234.

Marr, C. W. and Tisserant, N. (1998). Commercial vegetable production of watermelon. *J. Agr. Sci. Exp.*

Miller, G. (2005). Healthy growth ahead for welness drinks. *J. Food Dairy Technol.* **59**, 21-26.

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

Ozcelik, B. and Yavuz, M. (2016). Watermelon juice. *In:* "Handbook of Functional Beverages And Human Health." (4th ed., Vol. 53). (R. Balasasirekha, Ed.). pp. 553-559. Taylor & Francis Group.

Pandey, A. K., Rai, M. K. and Acharya, D. (2003). Chemical composition and antimycotic activity of the essential oils of corn mint (*Mentha arvensis*) and lemon grass (*Cymbopogon flexuosus*) against human pathogenic fungi. *Pharmaceut. Biology.* **41** (6), 421-425.

Perez-Gago, M. B. and Krochta, J. M. (2002). Formation and properties of whey protein films and coatings. *In:* "Protein Based Films and Coating". (A. Gennadios, Ed.). pp. 159- 170. USA. CRC press LLC.

Pernot, R. (2007). Importance of whey ingredients in confectionery products. Presented at International Whey Conference. Paris.

Punnagaiarasi, A., Elango, A. and Karthikeyan, N. (2017). Sensory analysis of whey based watermelon beverage. *Int. J. Sci. Environ. and Technol.* **6** (5), 3191-3195.

Ranganna, S. (1986). "Handbook of Analysis and Quality Control of Fruit and Vegetables". Tata McGerw Hill Pub. Co. Ltd. New Delhi.

Rupnar, P. S. (2006). Studies on preparation of paneer whey beverage using kokam juice. M. Sc (Ag) Thesis. Mahatma Phule Krishi Vidyapeeth, India.

Sahu, C. P., Choudhari, L. and Patel, S. (2005). Technoeconomic feasibility of rts whey based mango herbal beverage. *J. Dairy Sci.* **58** (4), 258-263.

Sakhale, B. N., Pawar, V. N., Kapse, B. M. and Ranveer, R. C. (2007b). Studies on preparation of whey based mango RTS beverage. Presented at International Conference on Traditional Dairy Foods. Haryana, India. December 14-17. p. 80.

Satpute, D., Padghan, P., Gore, S. and Patil, Y. (2018a). Studies on preparation of herbal whey based beverage by using menthol. *Int. J. Chem. Studies.* **6** (2), 188-195.

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

Saxena, D., Chakraborty, S. K., Sabikhi, L. and Singh, D. (2013). Process optimization for a nutritious low- calorie high- fiber whey- based ready to serve watermelon beverage. *J. Food Sci. Technol.*

Serna, G., Martinez, N., Mesias, M., Morales, F. j. and Castillo, M. D. (2014). Use of coffee silverskin and stevia to improve the formulation of biscuits. *J. Food Nutr. Sci.* **64**, 243-251.

Sharma, R., Rajput, Y. S. and Mann, B. (2013). Chemical and functional properties of glycomacropeptide (GMP) and its role in the detection of cheese whey adulteration in milk: a review. *J. Dairy Sci. Technol.* **93** (1), 21-43.

Shukla, F. C., Sharma, A. and Singh, B. (2004). Studies on the preparation of fruit beverages using whey and buttermilk. *J. Food Sci. Technol.* **41**, 102-104.

Sindayikengera, S. and Xia, W. (2005). Nutritional evaluation of caseins and whey proteins and their hydrolysates from protamex. *J. Zhejiang University*. **7** (3), 90-98.

Smithers, G. W. (2008). Whey and whey proteins- from gutter-to-gold. *J. Int. Dairy. Sci.* **18**, 695-704.

Smithers, G. W., Ballard, F. J. and Copeland, A. D. (1996). New opportunities from the isolation and utilization of whey proteins. symposium: advances in dairy foods processing and engineering. *J. Dairy Sci.* **79**, 1454- 1459.

Stoliar, M. (2009). Whey ingredients in bakery products. [Brochure]. pp. 1-8. Retrieved from http://www.thinkusadairy.org/Documents/Customer%20Site/C3-Using%20Dairy /C3.7-Resources%20and%20Insights/03-Application%20and%20 Technical% 20Materials/BAKERY_ENG.pdf. [Accessed 15 january, 2019].

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

Vacklavik, V. A. and Elizabeth, W. C. (2008). Food preservation and processing. *In:* "Essential of Food Science." (3rd ed.) pp. 425-428. 233 Spring Street, New York, NY 10013, USA. Springer Science+Business Media, LLC.

Varnum, A. H. and Sutherland, J. P. (1994). *In:* "Milk and Milk Products". pp. 159-189. New York. Chapman & Hall.

Veazie, P. P., Collins, J., Pair, S. D. and Roberts, W. (2001). Lycopene content differs among red fleshed watermelon cultivars. *J. Sci. Food Agri.* **81**, 983-987.

Walzem, R. L., Dillard, C. J. and J.B., G. (2002). Whey components: millennia of evolution create functionalities for mammalian nutrition: what we know and what we may be overlooking. *Crit. Rev. Food Sci. Nutr.* **42**, 353-375. [doi:10.1080/10408690290825574].

Wang, Y., Li, W., Ma, Y., Zhao, X. and Zhang, C. (2018). Effect of thermal treatments on quality and aroma of watermelon juice. *J. Food Quality.*

Warren, S. and Clark, J. R. (2001). Concentrated and dry milks and whey's. *In:* "Applied Dairy Microboilogy." (2nd ed.). (H. Elmer, Marth. and L. James, Eds.). Basel, Switzerland. Steels Eastern Hemisphere Distribution Marcel Dekker AG Hutgasse 4, Postfach 812, CH-4001.

Wong, D. W. S., camirand, W. M. and Pavlath, A. E. (1996). Structural and functionalities of milk proteins. *Crit. Rev. Food Sci. Nutr.* **36**, 807- 844. [doi:10.1080/10408399609527751].

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

Yang, Y., Bu, D., Zhao, X., Sun, P., Wang, J. and Zhou, L. (2013). Proteomic analysis of cow, yak, buffalo, goat and camel milk whey proteins: quantitative differential expression patterns. *J. Proteome Res.* **12** (4), 1660-1667.

Zadow, J. G. (1994). Utilisation of milk componenets: whey. *In:* "Modern Dairy Technology" (2nd ed., Vol. 1). (R. K. Robinson, Ed.). pp. 313- 320. Chapman & Hall. [ISBN 978-1-4615-2057-3].

Zaman, K., Shamsudin, R. and Adzahan, N. (2016). Effect of blending ration on quality of fresh pineapple (*Ananas comosus L.*) and mango (*Mangifera indica L.*) juice blends. . *Int. J. Food Sci.* **23**, 101-106.

Zohary, D. and Hopf, M. (2000). "Domestication of Plants in the Old World." (3rd ed.). Oxford University Press.

Appendices

Appendix A

Specimen card for sensory evaluation

Hedonic rating test

Name of panelist: - **Date: -**

Product: - Whey based watermelon herbal (*Mentha arvensis*) beverage.

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

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

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

Comments: -……

……

Signature

…….

Appendix B

Table B.1 Cost evaluation (for every 180 ml bottle)

S.N.	Particulars	Quantity	Rate (Rs)	Amount (NRs)
1.	Whey	50 ml	8/L	0.4
2.	Watermelon juice	50 ml	120/L	6
3.	Mint	2.5 ml	150/50ml	7.5
4.	Sugar	6.45 gm	85/Kg	0.5
5.	Bottle (180 ml)	1 piece	5/Pieces	5
6.	Raw Material cost			19.44
7.	Processing and labour cost (10% of Total material cost)			1.94
8.	Profit (10%)			2.13
9.	Total Cost			23.53
10.	Total Volume	102.5 ml		
11.	Total cost of beverage (NRs/100 ml)			22.95
12.	Total cost of beverage (NRs/180 ml)			41.32

The price of 100 ml whey drink cost NRs. 22.95. Thus, the price of 180 ml of drink is Rs. 41.32.

Appendix C

ANOVA for juice optimization

Table C.1 Two way ANOVA (no blocking) for appearance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	65.9000	16.4750	56.19	<.001
Panelist	11	6.1833	0.5621	1.92	0.063
Residual	44	12.9000	0.2932		
Total	59	84.9833			

Since F pr. <0.05, there is significant difference between the samples.

Table C.2 Two way ANOVA (no blocking) for color

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	83.4333	20.8583	61.32	<.001
Panelist	11	1.7833	0.1621	0.48	0.908
Residual	44	14.9667	0.3402		
Total	59	100.1833			

Since F pr. <0.05, there is significant difference between the samples.

Table C.3 Two way ANOVA (no blocking) for flavor

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	75.2333	18.8083	54.56	<.001
Panelist	11	5.2500	0.4773	1.38	0.214
Residual	44	15.1667	0.3447		
Total	59	95.6500			

Since F pr. <0.05, there is significant difference between the samples.

Table C.4 Two way ANOVA (no blocking) for taste

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	61.5667	15.3917	48.26	<.001
Panelist	11	10.1333	0.9212	2.89	0.006
Residual	44	14.0333	0.3189		
Total	59	85.7333			

Since F pr. <0.05, there is significant difference between the samples.

Table C.5 Two way ANOVA (no blocking) for overall acceptability

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	67.1667	16.7917	87.61	<.001
Panelist	11	3.7333	0.3394	1.77	0.089
Residual	44	8.4333	0.1917		
Total	59	79.3333			

Since F pr. <0.05, there is significant difference between the samples.

Appendix D

ANOVA for mint extract optimization

Table D.1 Two way ANOVA (no blocking) for appearance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	25.2333	6.3083	15.11	<.001
Panelist	11	2.1333	0.1939	0.46	0.915
Residual	44	18.3667	0.4174		
Total	59	45.7333			

Since F pr. <0.05, there is significant difference between the samples.

Table D.2 Two way ANOVA (no blocking) for color

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	40.8333	10.2083	35.18	<.001
Panelist	11	4.7333	0.4303	1.48	0.172
Residual	44	12.7667	0.2902		
Total	59	58.3333			

Since F pr. <0.05, there is significant difference between the samples.

Table D.3 Two way ANOVA (no blocking) for flavor

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	43.7333	10.9333	27.54	<.001
Panelist	11	2.4500	0.2227	0.56	0.849
Residual	44	17.4667	0.3970		
Total	59	63.6500			

Since F pr. <0.05, there is significant difference between the samples.

Table D.4 Two way ANOVA (no blocking) for taste

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	41.4333	10.3583	30.45	<.001
Panelist	11	5.7833	0.5258	1.55	0.150
Residual	44	14.9667	0.3402		
Total	59	62.1833			

Since F pr. <0.05, there is significant difference between the samples.

Table D.5 Two way ANOVA (no blocking) for overall acceptability

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	50.9333	12.7333	48.86	<.001
Panelist	11	1.5333	0.1394	0.53	0.869
Residual	44	11.4667	0.2606		
Total	59	63.9333			

Since F pr. <0.05, there is significant difference between the samples.

Appendix E

ANOVA of chemical constituents of whey-watermelon herbal beverage

Table E.1 Two way ANOVA for TSS as variate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	4	7.743787	1.935947	206.39	<.001
Condition	1	0.356430	0.356430	38.00	<.001
Residual	24	0.225120	0.009380		
Total	29	8.325337			

Since F pr. <0.05, there is significant difference between the samples.

Table E.2 Two way ANOVA for pH as variate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	4	0.1080000	0.0270000	89.01	<.001
Condition	1	0.0086700	0.0086700	28.58	<.001
Residual	24	0.0072800	0.0003033		
Total	29	0.1239500			

Since F pr. <0.05, there is significant difference between the samples.

Table E.3 Two way ANOVA for titratable acidity as variate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	4	0.1024333	0.0256083	81.37	<.001
Condition	1	0.0076800	0.0076800	24.40	<.001
Residual	24	0.0075533	0.0003147		
Total	29	0.1176667			

Since F pr. <0.05, there is significant difference between the samples.

Table E.4 Two way ANOVA for reducing sugar as variate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	4	0.927953	0.231988	91.71	<.001
Condition	1	0.076003	0.076003	30.04	<.001
Residual	24	0.060713	0.002530		
Total	29	1.064670			

Since F pr. <0.05, there is significant difference between the samples.

Appendix F

Table F.1 Summary of ANOVA of sensory evaluation for watermelon Juice optimization.

Sample	Appearance	Color	Flavor	Taste	Overall Acceptance
A	4.9± 0.64	4.9± 0.49	4.9±0.64	5.3±0.72	5.2±0.37
B	5.9±0.64	6.5±0.64	5.8±0.55	6.3±0.72	6.1±0.49
C	6.3±0.47	6.8±0.55	7.1±0.64	6.5±0.5	6.4±0.49
D	7.4±0.49	7.9±0.49	7.4±0.49	7.5±0.5	7.5±0.5
E	7.8±0.55	8.3±0.43	8.0±0.55	8.2±0.68	8.2±0.37
LSD (5%)	0.445	0.479	0.481	0.464	0.360

Table F.2 Summary of ANOVA of sensory evaluation for mint extract optimization.

Sample	Appearance	Color	Flavor	Taste	Overall Acceptance
A	7.8±0.68	8.1±0.49	6.0±0.57	6.2±0.68	6.7±0.47
B	7.5±0.64	7.8±0.55	6.7±0.47	6.8±0.37	7.3±0.43
C	7.2±0.37	7.5±0.50	8.3±0.47	8.3±0.59	8.6±0.43
D	6.8±0.43	6.5±0.50	7.2±0.68	7.3±0.43	6.9±0.43
E	6.0±0.70	5.9±0.64	6.1±0.70	5.9±0.73	5.8±0.43
LSD (5%)	0.531	0.443	0.518	0.479	0.420

Appendix G

Chemicals Used:

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 used:

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