ADDIS ABABA UNIVERSITY

COLLEGE OF NATURAL SCIENCE

CENTER FOR FOOD SCIENCE AND NUTRITION

DESIGN AND DEVELOPMENT OF FUNCTIONAL BEVERAGE FROM CHEESE WHEY AND TEFF FLOUR

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Design and Development of Functional Beverage from Cheese Whey and Teff Flour

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ACRONYMS AND ABBREVIATIONS

AOAC:	Association of Official Analytical Chemists
ANOVA:	Analysis of Variance
APC:	Aerobic plate count
ASE:	Accelerated Solvent Extraction System
BCAAs:	Branched-chain amino acids
BOD:	Biological Oxygen Demand
BSA:	Bovine serum albumin
CFU:	Colony forming units
CMC:	Carboxymethylcellulose
cP :	Centipoise
CSA:	Central Statistics Agency
CW:	Cheese-whey
DE:	Degree of esterification
DM:	Degree of methylation
DRBCA:	Dichloran Rose Bengal Chloramphenicol Agar
Eq.	Equation
FAAS:	Flame Atomic Absorption Spectrophotometer
FAO:	Food and Agricultural Organization of the United Nations
GMP:	Glycomacropeptides
HG:	Homogalacturonan
HM:	High methoxy pectin

LM:	Low methoxy pectin
LSD:	Least significant differences
PCA:	Plate count agar
PER:	Protein Efficiency Ratio
PKU:	Phenylketonuria
USA:	United States of America
UF:	Ultra-filtration
WP:	Whey powder
WPC:	Whey protein concentrates
WPI:	Whey protein isolate

ABSTRACT

Cheese whey is one of the main by-products of the dairy industry. The nutritional component of the whey is overlooked; however, in many parts of the world and only little effort if not none has been made to utilize it into different forms of products. The current study aims at producing a functional beverage from Gouda cheese whey, teff and stabilizer. A D-optimal Experimental model of the Design-Expert®, version 7.0 (from Stat-Ease, Inc) was used in order to optimize whey (88-94%), teff flour (5-10%), and pectin (1-2%). The components were blended and cooked at a temperature of 65°C for 15 minutes with frequent stirring. The raw materials and the final products were characterized using standard test methods for proximate, microbiological, mineral, viscosity and sensory attributes. Vanilla and mango flavors, artificial food color and sweetener were used to increase the acceptability of the final product. Microbiological quality was performed for the selected consistent final products across a period of seven days at room and refrigeration temperatures. From the formulations, the nutritional values are found to be: moisture (78.3-89.24%), protein (7.49-11.99 g/100g), fat (0.23-1.71 g/100g), ash (2.78-5.63 g/100g), fiber (1.74-2.48 g/100g), carbohydrate (80.75-87.47 g/100g), energy (370.50- 385.76 Kcal); minerals: Fe (9.49-21.28 mg/100g), Ca (177.07- 399.41 mg/100g), Mg (77.14-167.99 mg/100g), Zn (1.03- 2.09 mg/100g) and Na (124.95-369.90 mg/100g). Two trials; Tr-2 with 93.3% whey, 5 % Teff, and 1.7 % stabilizer; and Tr-13, with 94% whey, 5 % Teff, and 1.0% stabilizer, were found to have good consistency. Microbiological results of the formulated products were; *aerobic bacterial count* (160-220 Cfu/g), *yeast and mold*, *s. aureus* and *Enterobacteriaceae* count (<10 Cfu/g).From the sensory evaluation vanilla flavour has the highest acceptability, plain ranked second and Mango flavour with the least preference. Trial 13 was found to have the highest overall acceptability than Trial 2 by consumer panelists. The viscosity of the products falls in the range of minimum 24.32 and a maximum of 99.99 cP. Products stored at room temperature showed good consistency over those stored at refrigeration temperature, which exhibited synereses up on storage. Based on the findings of this study, it is possible to produce a noble functional beverage from cheese whey using teff flour and pectin as a complementary blend. The final product has exhibited a slight off-flavour after the seventh day of storage and considered a challenge of the current study.

CHAPTER ONE

1. INTRODUCTION

1.1.Background

Whey is a liquid by-product of the dairy industries during the production of cheese by coagulating and separating of casein from milk Tsakali *et al.*,(2010).There are two types of whey; sweet and acid whey. Sweet whey is produced by means of rennet type enzyme and has a pH of about 5.6. Acid whey, on the other hand, is produced from dairy products that involve coagulum formation by acidification in a pH range of approximately 5.1 and less Boersma & Murarka, (1995). Whey is believed to contain about half of the milk solids, most of the lactose, about one fifth of the proteins, vitamins and minerals Divya, (2009).

According to Chavan *et.al.*, (2015)whey proteins are present in small quantity but have high protein efficiency ratio (3.6%), net protein utilization (95%), biological value (104%) and as compared to all other protein sources available whey is next to egg protein in terms of nutritive value. Whey proteins are sources of α-lactalbumin(α-La), β-lactoglobulin(β-Lg), bovine serum albumin, caseino-macropeptides, immunoglobulin, lactoferrin and lysozyme which are often associated with health benefits, such as enhanced immunity, anticancer properties, anti-adhesive effect against pathogenic properties, as well as antiviral, antimicrobial (iron binding properties) and antihypertensive properties.

Despite their nutritional provision, however, there is a food allergens concern related to the consumption of milk proteins. Hypersensitivity to cow milk proteins affects mostly but not exclusively infants, while it may persist through adult age and can be very sever in some instances. Milk protein allergy originates from protein components present in milk, causing reactions to either the protein fractions in emulsion (caseins) or in whey (milk albumin) Mc Williams & Collins, (2014).

Whey could be utilized in different forms. The common types are whey protein concentrates (WPC), whey protein isolates (WPI), whey powder (WP), lactose permeate, use as feed and fertilization. Apart from these products, whey could also be used as beverages of different forms. Whey fruit juice beverage , dairy type whey beverages (fermented and unfermented), whey based energy drinks, whey based thirst quenching carbonated beverages, dietetic beverages with hydrolyzed lactose, whey-cereal based beverages and sports drinks are the basic ones. The utilization of whey in different forms help to make use of its nutritional

component, generate additional income and address adverse environmental issues by the discharge of the same Marshall,(2004).

Teff (*Eragrostis tef*) is a cereal grain believed to have been originated and domesticated in Ethiopia and Eritrea about 5000 years ago. It is widely known among the people of the two nations for a traditional staple food, 'Injera' Baye, (2014).Apart from its main use as Injera, teff can be used in the making of various products such as gruel, kitta, traditional alcohols and can also be elaborated to produce brownies, baked goods, cookies and crackers among others Hopman, (2008). It is a common practice in Ethiopia to use *Atmit* as a traditional functional beverage for patients since it is easily digestible than solid foods and supplies nutrients. Atmit could be made from various cereals and ingredients and could be customized as needed. A teff based *Atmit* is among such preferences, which have also been used to make Juice at an industrial level with a brand name *Amandin, (*Figure 2-4), due to its nutritional content and gluten free nature. Teff has considerable nutritional profile of protein, carbohydrate, phytate and minerals. However, its phytate content impairs the absorption of iron and zinc Baye, (2014).

Functional food products are thought to provide health benefit in addition to their basic nutritional content. Such foods reduce the risk of certain diseases and other health conditions. Nowadays, the advances in scientific research support the idea that diet may fulfil nutritional needs and exert a beneficial role in some diseases Otles, (2012).

Hydrocolloids are long chain polymers with a property of forming viscous dispersion and gels in water. Their main functional application in foods include enhancing viscosity, thickening, gelling, emulsifying, stabilization and coating Burey*et.al.,* (2008).

The utilization of whey in the production of various food products is considered to design a functional beverage using teff flour and pectin as a stabilizer. The development of such a product is believed to enable the bioactive components of whey to be utilized effectively and increase the bioavailability of nutritional components of teff.

1.2. Statement of the problem

The dairy industry is advancing, both in number and capacity, currently in Ethiopia. Studies also show the demand for ready to eat dairy products such as cheese, yoghurt and butter are increasing, especially in urban areas FAO, (2011). As the production of such products increased, the dairy wastes will also increase. The current study shows possible ways of

alleviating environmental pollution through whey utilization, which results from its high BOD level and organic matter components.

Cheese whey is considered a waste in many dairy plants. However, it contains valuable nutritional profile. The current study helps the community to understand the nutritional values of whey.

One of the main reasons that dairy industries fail to utilize cheese whey is largely due to its high technological cost implication. This study directs the dairy industries to consider cost effective possibilities of whey utilization.

The majority of the population in Ethiopia suffers from quality and nutritious food supply. The study insights the alternative approaches of combating such nutritional problems in the community through the development of value added products from dairy wastes.

Although several researches were conducted on different ways of whey utilization worldwide, it is hardly possible to find similar studies in Ethiopia. Hence, this study will serve as a bench mark for further studies on industrial application of whey.

It is a common practice in Ethiopia to use Atmit as a traditional functional beverage for patients since it is easily digestible than solid foods and supplies nutrients. Atmit could be made from various cereals and ingredients and could be customized as needed. A Teff based '*Atmit*' is among such preferences, which have also been used to make Juice at an industrial level with a brand name *Amandin* due to its nutritional content and gluten free nature.

Teff has been used for thousands of years in Ethiopia Gebremariam, (2012). The only possible ways of its utilization is in the form of 'Injera', 'Kita' and other products mostly for house hold purpose. The current study shows industrial application of teff in the production of a functional beverage, products that have health benefits in addition to their nutritional supplement.

In reference to those suggestions, cheese whey could be blended with teff flour in order to produce a functional beverage with sound nutritional and energy value, thereby alleviating the environmental pollution due to whey disposal.

1.3. Significance of the study

- A nutritious and noble product will be designed and developed from whey and teff flour.
- The study will be used as bench mark for utilization of whey in functional food products.
- The study will show possibilities of cheese whey utilization by the dairy industries.
- Environmental pollution, by the discharge of whey, will be minimized through the utilization of whey.

1.4. Objective

1.4.1. General objective

The general objective of this research was to design and develop a functional beverage from cheese whey and teff flour for its energy and nutrient utilization.

1.4.2. Specific objective

- Evaluating the nutritional composition of raw materials (cheese whey and teff flour).
- Design the utilization of teff in beverage production.
- Evaluating the physicochemical and microbiological properties of whey-teff beverage.
- Evaluating the sensory attributes of whey-teff beverage.
- Examining characteristic parameters of functional beverages.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Overview of the dairy processing Industry

Dairy processing is practiced all over the world. Because of their sizes and variation of the types of products manufactured, however, it is hard to give general characteristics. The primary objective of dairy processing is to extend the saleable life of the products which is typically achieved by heat treatment to ensure that milk is safe for human consumption and has extended quality, and preparing a variety of dairy products in a semi-dehydrated or dehydrated form such as butter, hard cheese and milk powders to mention some. Nearly all the process steps, or unit operations, that are applied in food processing are applied in the dairy industry Peiter, (2006). (Figure 2-1) shows the main units operations and process steps in a typical dairy processing plant.

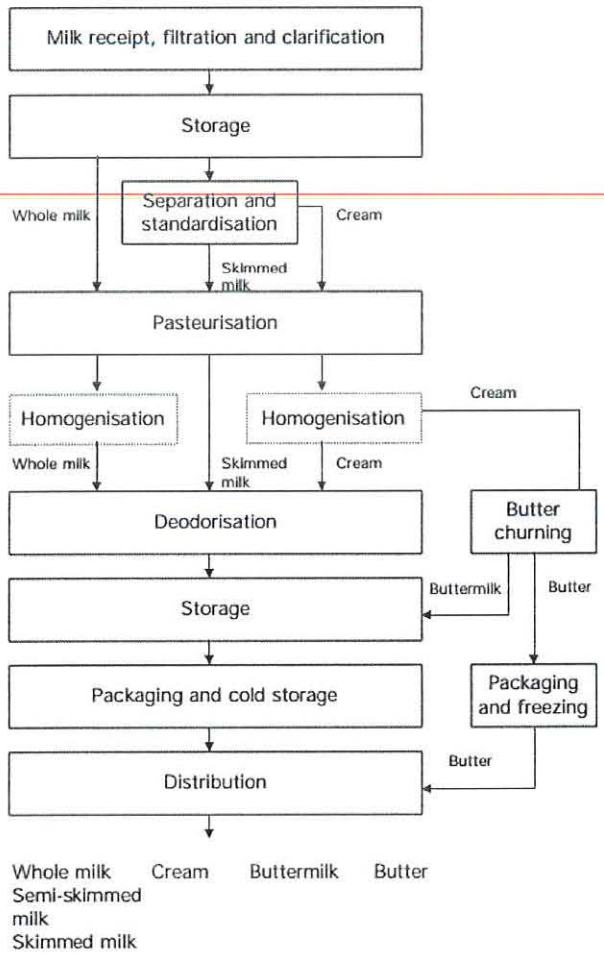

Figure 2.1. Flow diagram for processes occurring at a typical milk plant Peiter,(2006).

2.2. Whey production and utilization

2.2.1. Whey production

Whey is a by-product of cheese making or a casein production. About 96% and 6% of whey is produced from cheese making and casein production respectively. Based on the method applied for casein coagulation, there are two categories of whey, sweet and acid whey. Acid whey is produced by acid action and sweet whey by rennet type enzymatic action Guimarães *et al.*, (2010).

For every 100 litres of Milk used for cheese production, on average, 80-90 litres of whey is produced. However, the average amount of whey produced depends on the type of cheese product, whether it is soft, semi-hard or hard cheese Guimarães *et al.*, (2010). There are number of factors that determine the composition and sensory characteristics of whey. The type of whey, whether sweet or acid, the source of milk (cow, sheep, bovine milk), and the feed of the animal that produced the milk, the technique of cheese processing employed are among the major factors Divya, (2009).

The production of whey on a global scale is estimated from 180 to 190 Million tons/year; out of which only 50% is processed. This estimate is nine fold of the cheese production worldwide with an annual growth rate of 1-2 % Baldasso *et.al.* (2011).

Disposal of liquid whey poses a serious environmental pollution due to its high organic matter content largely due to its lactose, protein and fat content. The Biological Oxygen Demand (BOD) of whey ranges from 39,000 to 48,000 ppm, which is roughly 200 times more as threat the whey before disposal, which is found to be uneconomical Divya, (2009). A discharge of 40,000 litres of untreated milk whey results in an environmental pollution equivalent to that produced daily by a population of 250,000 people.

2.2.2. Whey utilization

The history of using cheese whey goes back to the ancient Greeks; Hippocrates, in 460 B.C recommended as a therapeutic beverage for human consumption for different health problems. In the middle Ages, whey was recommended by many doctors for varied diseases, and, by the mid-19th century, whey curing reached a high point with the establishment of over 400 whey houses in Western Europe Susli, (1956). As late as the 1940's in spas in Central Europe, dyspepsia, uremia, arthritis, gout, liver diseases, anaemia, and even tuberculosis were treated with the ingestion of up to 1500 g of whey per day Holsinger, (1995); Pien, (1943).

Whey contains more than half of the solids present in the original whole milk, including whey proteins (20% of the total protein) and most of the lactose, water-soluble vitamins and minerals. As a result, whey can be considered a valuable by-product with several applications in the food and pharmaceutical industries.

Approximately 50% of worldwide cheese-whey (CW) production is treated and transformed into various foods and feed products. About half of this amount is used directly in liquid form, 30% as powdered cheese-whey, 15% as lactose and its by-products and the rest as cheese whey- protein concentrates Spalatelu, (2012).

Whey could be used in many forms Mollea *et.al.,* (2013), (Figure 2-3).The common types of whey utilization are whey protein concentrates (WPC), whey protein isolates (WPI), whey powder (WP), lactose permeate, use as feed and fertilization. Apart from these products, whey could also be used as beverages of different forms. Whey fruit juice beverage , dairy type whey beverages (fermented and unfermented), whey based energy drinks, whey based thirst quenching carbonated beverages, dietetic beverages with hydrolyzed lactose, whey-cereal based beverages and sports drinks are the basic ones. The utilization of whey in different forms help to make use of its nutritional component, generate additional income and address adverse environmental issues by the discharge of the same Marshall,(2004).

The most successful way to recover cheese whey proteins is the production of whey protein concentrate (WPC), (Figure 2-2), owing to the technological applications of ultra-filtration system Cheryan, (1984); Marshal, (1988). Whey proteins are widely used as food ingredients due to their high nutritional composition and possess useful functional properties. The term whey protein concentrate (WPC) is being used for the dried whey having more than 25% protein; however, there is a wide variation in protein composition of resultant WPC, ranging from 25-90 % protein, which is the determining factor of its end use Huffman, (1996).

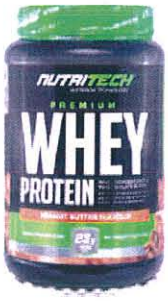

Figure 2-2. Commercial whey protein concentrate (www.bodybuilding.nodeloc.net)

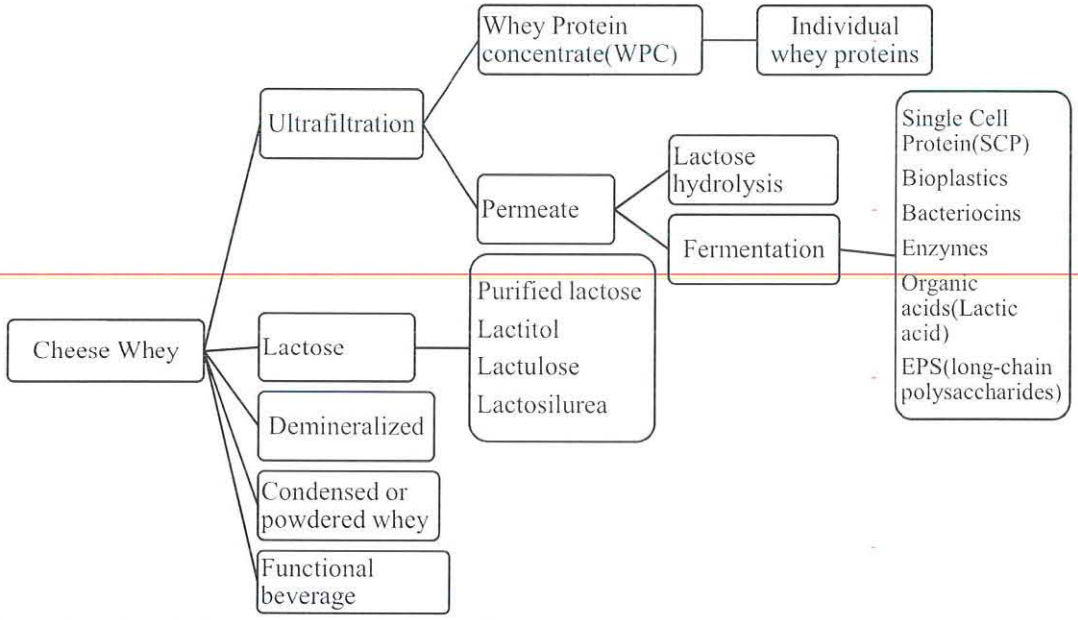

Figure 2-3. Possibilities of cheese whey utilization Mollea *et.al.*, (2013)

2.3. Nutritional composition of whey

There are research findings that indicated dairy constituents could be used as a functional food and its positive impact on human health is measurable Gill *et.al.*,(2000). Whey is among such products whose components have been proved to promote human health hence; it could be used to produce other products after the production of cheese Walzem *et.al.*, (2002).

Approximately 54% of the nutrients from milk are found in the fluid sweet whey of Cheddar cheese, while about 73% of the nutrients of the non-fat milk used for Cottage cheese show up in fluid acid whey Kosikowski, (1967).

Walzem*et.al.*, (2002) indicated, whey contains beta-lactoglobulin, alpha-lactalbumin, bovine serum albumin, lactoferrin, immunoglobulins, lactoperoxidase enzymes, glycomacropeptides, lactose and minerals. The amino acid profile of whey is greater when compared to different food protein sources such as soy, corn and wheat gluten. Moreover, those amino acids are easily absorbed and assimilated to the body relative to free amino acids in solution Kinsella & Whitehead, (1989).

The chemical composition of whey varies in relation to method used for its production (acid whey or sweet whey). Whey usually contains about 50% of milk constituents, such as lactose , whey proteins , minerals and some fat, (Table 2.1). The main differences are in the calcium, phosphate, lactic acid and lactate contents, which are higher in acid whey than in sweet whey Chiara *et.al.*,(2013).

It is established by nutritional experts that only 14.5 gram whey proteins in native form will satisfy the daily requirement of essential amino acids as compared to 17.4 grams Egg proteins; 12 grams whey proteins are equivalent to 20 grams of Casein proteins in terms of weight gain Shreyansh, (2013).

The nutritional composition of sweet and acid whey is discussed in the following table.

Table 2.1. Composition of sweet and acid whey (g/L) Jelen, (2011)

Type of Whey	Total solids	Lactose	Proteins	Calcium	Phosphates	Lactate	Chloride
Sweet	63.0-70.0	46.0-52.0	6.0-10.0	0.4-0.6	1.0-3.0	2.0	1.1
Acid	63.0-70.0	44.0-46.0	6.0-8.0	6.0-8.0	2.0-4.5	6.4	1.1

2.4. Bioactive components of whey

Bioactive compounds are chemical compounds derived from a plant, animal, or marine source, that exert the desired health/wellness benefit Mollea *et al.*,(2013).There are several proven health and clinical benefits one can get from the biological components of whey. Such benefits include; anti-oxidant activity by contributing cysteine rich protein that helps in the synthesis of glutathione, potent anti-oxidant; anti-viral, anti-fungal and anti-bacterial effects with the presence of lactoferrin; anti-cancer effect with the help of amino acids of the

precursors of glutathione; helps to manage obesity since calcium is thought to influence energy metabolism because intracellular calcium regulates adipocyte lipid metabolism and triglyceride storage and the amino acids in whey protein helps to synthesize proteins that assists in maintaining body mass index in individuals involving in exercise, to mention but a few Burke *et.al.,* (2001); Shah, (2000); Tomita, (2002); Walzem *et.al.,* (2002); Zamel, (2003).

2.4.1. Lactoferrin

Lactoferrin, an iron-binding glycoprotein, is a non-enzymatic antioxidant found in the whey fraction of milk as well as in colostrum. The lactoferrin component of whey consists of approximately 689 amino acid residues, while human lactoferrin consists of 691 residues. Whey lactoferrin is composed of a single polypeptide chain with two binding sites for ferric ions Pierce *et al.*,(1991).

2.4.2. ß-Lactoglobulin (ß-Lg)

One of the components of functional whey proteins is ß-lactoglobulin, which approximately accounts half of the total protein in bovine whey while human milk contains no ß – lactoglobulin Kinsella & Whitehead, (1989).In addition to being a source of essential and branched amino acids, a retinol-binding protein has been identified within the ß - lactoglobulin structure. This protein, a carrier of small hydrophobic molecules including retinoic acid, has the ability to modulate lymphatic responses Guimont, (1997).

2.4.3. α -Lactalbumin (α-La)

α–Lactalbumin is a protein component that is found both in human and bovine milk. It accounts for about 20-25 percent of whey proteins and contains a wide variety of amino acids, including a readily available supply of essential and branched chain amino acids Pierce *et al.*,(1991).

According to the studies made by Bounous, (1985), α–lactalbumin enhances antibody response to systematic antigen stimulation, both in its native and hydrolysed state. The same group revealed that α–lactalbumin has a direct effect on B-lymphocyte function, as well as suppressing T-cell dependent and independent responses.

2.4.4. Lactoperoxidase

Different types of enzymes could be listed in the composition of whey. Some of these enzymes include; hydrolases, transferases, lyases, proteases, and lipases. Lactoperoxidase,

however, is an important and the most abundant enzyme in the whey fraction of milk, the majority of which ends up in whey following the curding process. Lactoperoxidase accounts for 0.25-0.5 percent of total protein found in whey. It has the ability to catalyze certain molecules, including the reduction of hydrogen peroxide Bjorck, (1978).

This enzyme system catalyzes peroxidation of thiocyanate and some halides (such as iodine and bromium), which ultimately generates products that inhibit and/or kill a range of bacterial species. During the pasteurization process, Lactoperoxidase is not inactivated, suggesting its stability as a preservative Kussendrager & Van Hooijdonk, (2000).

2.4.5. Glycomacropeptides

Glycomacropeptides (GMP) is also referred to as casein macropeptide. GMP accounts about 10-15 percent of whey composition. It is high in branched chain amino acids and lacks the aromatic amino acids including phenylalanine, tryptophan, and tyrosine. It is one of the few naturally occurring proteins that lack phenylalanine, making it safe for individuals with phenylketonuria (PKU) Brody, (2000).

2.4.6. Bovine Serum Albumin

Bovine serum albumin (BSA) is a large protein that makes up approximately 10-15 percent of total whey protein. BSA is a source of essential amino acids, but there is very little available information regarding its potential therapeutic activity Marshal, (2004).

2.5. Whey protein-Pectin Interaction

The functional properties of whey proteins, which include; the water binding capacity, emulsification, foaming and gelation are related basically to their structural and other physicochemical properties. These features are influenced by many factors, among which is the interaction of whey proteins with pectin Cayot & Lorient, (1997); De Wit, (1998).

Pectin assumes various structural forms which influence and control functional properties of whey. According to Tolstoguzov, (1997), attraction and repulsion between unlike macromolecules are the two interactions responsible for complex formation and the immiscibility of biopolymers. The interaction between pectin and β- lactoglobulin (the main globular protein of whey), are mainly caused by hydrogen bonding between carboxyl groups of pectin and peptide linkage of protein. The compatibility of β- lactoglobulin with pectin in aqueous solution is greatly influenced by pH, ionic strength and the structural features of pectin Bédié *et al.*, (2008); Girard *et al.*, (2002); Wang & Qvist, (2000).

Whey protein and pectin are present in dairy and food products such as yoghurt and milk drinks. The control or manipulation of its macromolecular interactions is vital in the development of novel food processes and products as well as in the formulation of fabricated food products Tolstoguzov, (1997).

2.6. The role of Whey protein on Iron Absorption

According to Yip, (1994), iron deficiency, with or without anaemia, is one of the most significant nutritional problems all over the globe, affecting approximately 20% of the world population. Since iron deficiency anaemia mainly results from insufficient intake of iron, the strategy of iron fortification of food is practiced worldwide to prevent the case Clugston & Smith, (2002); Demment *et al.*, (2003). Despite such practices, the incorporation of iron into foods leads to a variety of problems such as its oxidation and precipitation which results in lower bioavailability Douglas *et al.*, (1981); Hurrell, (1997). It is, therefore, crucial to consider not only intake of iron but also its bioavailability to prevent iron deficiency.

According to Douglas *et al.*, (1981), heme iron is widely used in the food industry as an iron supplement. However, it is insoluble in neutral pH and their absorption and bioavailability have not yet been sufficiently considered. Lactoferrin and casein phosphopeptide are widely accepted to be functional proteins or peptides, which enhances iron absorption by improving its solubility in animal intestine, and are applied extensively as iron supplements and ingredients Jovaní *et al.*, (2003); Kawakami *et al.*, (1988); Uchida *et al.*, (2006); Yeung *et al.*,(2002). However, they are expensive food ingredients due to the high cost of preparation.

Allen, (2002) indicated, the presence of amino acids in the intestine increases iron absorption. Therefore, the use of suitable proteins in the diet may increase the absorption rate of dietary iron. Whey proteins produced as the principal by product of cheese or casein manufacturing are widely used as food ingredients such as whey protein concentrate (WPC) or whey protein isolate (WPI), because they have high nutritional value and functional properties such as gelling and emulsifying properties . In other study, Remondetto *et al.*, (2004) reported that the iron in whey protein hydrogels was superior in intracellular iron absorption in the Caco-2 system that was used to estimate intestinal absorption, because whey protein hydrogels released most of their iron during the intestinal phase of a simulated digestion.

Nakano *et al.*, (2007) conducted a study on Bioavailability of Iron-fortified Whey Protein Concentrate in Iron-deficient Rats. They examined the bioavailability of iron in Fe-WPC using Caco-2 monolayer combined with in vitro gastric and intestinal digestion. Caco-2 cells

were exposed to the filtrate obtained from the simulated gastro-intestinal dissolution, because Caco-2 cell monolayers behave similarly to human intestinal mucosa Puyfoulhoux *et al.*, (2001). The increase of ferritin in cells was evidence that iron has entered the cell because cells produce ferritin in response to increases in intracellular iron Glahn *et al.*, (1996). Therefore, ferritin formation in the cells was used as an indicator of iron bioavailability.

2.7. Stability of whey proteins during thermal processing

Whey proteins can be effectively utilized by humans and provide a considerable amount of essential amino acids for growth. However, they are easily denatured during thermal processing, that is, they undergo conformational changes due to unfolding of their initially folded molecule Qi *et al.*, (2015).

In general, whey protein aggregation involves the interaction of a free –SH group with the S–S bond of cystine-containing proteins such as β-Lg, κ-casein (κ-Csn), α-La, and BSA via –SH/S–S interchange reactions Considine *et al.*, (2007). These protein–protein interactions lead to irreversible aggregation of proteins into protein complexes of varying molecular size depending on the heating conditions and protein composition. Knowledge of the ways of inhibiting the formation of these protein complexes is needed in order to minimize the negative practical consequences that may arise.

2.8. Milk Protein Allergy

Adverse reactions to food intake have very diverse etiology and symptomatology. Regarding milk, its food allergy is presented as lactose intolerance, the sugar in milk, or allergy to milk protein. Despite having different symptoms, confusions among allergic conditions to dairy and its mediators are common. Milk protein allergy originates from protein components present in milk, causing reactions to either the protein fractions in emulsion (caseins) or in whey (milk albumin)Mc Williams& Collins, (2014).The allergic reaction produces severe cellular damage and it triggers physical, mental and emotional symptomatology that may vary in time, intensity and severity. Lactose intolerance is originated by total or partial absence of the enzyme that digests this disaccharide. Hypersensitivity to cow milk proteins affects mostly but not exclusively infants, while it may persist through adult age and can be very sever in certain cases. The agents responsible for all of these reactions are cow's milk proteins, such as casein, ß-lactoglobulin, α-lactalbumin, serum albumin, and immunoglobulins Rangel *et al.*,(2016).

2.9. Overview of Teff utilization

2.9.1. Teff consumption and health benefits

Teff (*Eragrostis tef*) is a cereal grain believed to have been originated and domesticated in Ethiopia and Eritrea about 5000 years ago. It is widely known among the people of the two nations for a traditional staple food, Injera Baye, (2014).

Ethiopians used Teff for making their staple food, Injera, for thousands of years. Teff has rich nutritional profile of protein, carbohydrates, fat and minerals. The amino acid composition of teff is well balanced with higher amount of lysine, a limiting amino acid which many cereals fail to contain. Other types of amino acids including isoleucine, leucine, valine, tyrosine, threonine, methionine, phenylalanine, arginine, alanine, and histidine are found in higher amount in teff compared to other cereals. Mineral composition of teff is also higher compared to other cereals and indicated in Table 2.2.According to Hopman, (2008), teff contains no gluten, an additional feature that makes it preferable especially by people with celiac disease, who happen to be gluten intolerant.

Table 2.2. Mineral composition of teff compared to other cereal grains (mg/100g) Baye,(2014).

Mineral	White Teff	Red Teff	Mixed Teff	Maize	Sorghum	Wheat	Rice
Iron	9.5- 37.7	11.6->150	11.5->150	3.6-4.8	3.5-4.1	3.7	1.5
Zinc	2.4-6.8	2.3-6.7	3.8-3.9	2.6-4.6	1.4-1.7	1.7	2.2
Calcium	17.124	18-178	78.8-147	16	5.0-5.8	15.2-39.5	23
Copper	2.5-5.3	1.1-3.6	16	1.3	0.41	0.23	0.16

According to Gebremariam, (2012), teff is used for making porridges, unleavened breads (kitta), gruels (atmit), and traditional alcoholic beverages, such as tella and arakie; in addition to its common use as injera, though to a lesser extent.

It is a common practice in Ethiopia to use *Atmit* as a traditional functional beverage for patients since it is easily digestible than solid foods and supplies nutrients. *Atmit* could be made from various cereals and ingredients and could be customized as needed. A Teff based *Atmit* is among such preferences, which have also been used to make Juice at an industrial level with a brand name Amandin, (Figure 2-4), due to its nutritional content and gluten free nature. The production of a functional beverage of whey-teff flour will introduce a new approach of using the long standing home utilization of teff to an industrial level.

Figure 2-4. Teff Juice-Amandin (www.trendhunter.com)

2.10. Functional Foods

Functional foods are foods that are thought to provide health benefit in addition to their basic nutritional content. Such foods reduce the risk of certain diseases and other health conditions. The idea of health promoting foods has a long standing history as Hippocrates wrote 2400 years ago, "Let food be thy medicine and medicine be thy food", and Asian communities are well aware of the concept of functionality of foods and herbs. Nowadays, the advances in scientific research support the idea that diet may fulfill nutritional needs and exert a beneficial role in some diseases Otles, (2012).

Different types of commercially available functional products are produced and could be grouped as dairy-based beverages; including probiotics and minerals/ω-3 enriched drinks; vegetable and fruit beverages, and sports and energy drinks. Such food products include baby foods, baked goods and cereals, dairy foods, confectionery, ready meals, snacks, meat products, spreads, and beverages. In particular, beverages are by far the most active functional foods category because of convenience and possibility to meet consumer demands for container contents, size, shape, and appearance; ease of distribution and better storage for refrigerated and shelf-stable products; great opportunity to incorporate desirable nutrients and bioactive compounds Ofori, (2013).

2.11. The Dairy Sector and utilization of whey in Ethiopia

There was no objective evidence on whey utilization in Ethiopia so far. However, according to the information obtained from the dairy industries, whey is mostly discharged with other dairy effluents and very seldom sold for animal feed. According to the 2010 estimation survey conducted by the Central Statistics Agency, Ethiopia has the largest cattle population

in Africa which accounts 50.9 million heads among which 55% are dairy cows. However, the dairy sector in the country has not developed to the expected level. The annual growth rate in milk production of 1.2 percent falls behind the annual human population growth estimated at 3 percent GRM International,(2007).Felleke, (2003) indicated that, the traditional milk production system is dominated by indigenous breeds of low genetic potential for milk production and this accounts for 97% of the annual milk production in the country.

Over the last 10 years or so, milk production in the country has generally increased from about 1.5 billion litres in 2001 to about 2.2 billion litres in 2005 and around 2.9 billion litres in 2010. This increasing trend is mostly associated with an increase in the number of cows. However, the per capita milk consumption has declined from 26 kg per annum in 1980, to 22 kg in 1993, 19 kg in 2000 and 16 kg in 2009. This is likely to be attributed to the mismatch between the growth rate of milk production and human population Yilma *et al.*, (2011).

In 2011, a projection was done to increase the additional milk requirement to supply the growing consumer needs in Ethiopia. In the projection, the annual growth rate of 2.72 percent of the human population was used in the calculation based on the report of the 2007 population census figure of 82,101,998 for 2011, (Table 2-3). Milk available for consumption was estimated based on the report of Felleke & Geda, (2001), who indicated that 68 percent of the total annual milk production was to be consumed. The value recommended by FAO (62.5 kg/year/person) to be maintained for a balanced diet is considered as a target to be achieved Crawford, (1990).

Table 2-3. Projected demand for Milk in Ethiopia from 2011-2020

Year	Population in '000' based on the current growth rate (2.27%)	Milk production in million litres based on current growth rate (4.1%)	Milk available for consumption (68% of the produce) in million litres	Demand for milk, in million litres based on FAO recommendation (62.5kg)	Gap between projected milk available for consumption and demand based on FAO's recommendation in million litres
2011	82102	3 061	2 081	5 131	3 050
2012	84 335	3 186	2 166	5 271	3 105
2013	86 629	3 317	2 256	5 414	3 158
2014	88 985	3 453	2 348	5 562	3 214
2015	91 406	3 594	2 444	5 713	3 269
2016	93 892	3 742	2 545	5 868	3 323
2017	96 446	3 895	2 649	6 028	3 379
2018	99 069	4 055	2 757	6 192	3 435
2019	101 764	4 221	2 870	6 360	3 490
2020	104 532	4 394	2 988	6 533	3 545

Source: FAO, 2011

2.11.1. Consumption of Milk in Ethiopia

The consumption of dairy product in Ethiopia is 19 Kg per capita per year. This is one of the lowest levels in Sub-Saharan Africa, due to economic and cultural factors. FAO recommends 175 Kg. Combinations of cultural and economic factors are main reasons of the low consumption level. In recent years, the demand of milk has been rising due to urbanization, transformation of habits and population growth. Moreover, according to the survey made by the Central Statistics Agency, 13 % of the milk produced is used for the production of cheese Staal *et al.*, (2008).

2.12. Production of Gouda Cheese

Gouda cheese, originated in the vicinity of Gouda in the province of South Holland, is one of the most important Dutch type varieties of cheese produced in the world. It belongs to semi-hard to hard varieties of cheese with few or no eye holes. It is made from fresh cow's milk having fat such that the product contains at least 40% fat in the dry matter, starters consisting of *mesophilic lactococci* and *leuconostocs* that produce CO_2, clotted by rennet, pressed to obtain a close rind, are salted in brine after pressing and have no essential surface flora Gouda cheese is made from fresh unskimmed milk and was matured for 6-60 weeks Luyten *et al.*,(1991).

Gouda cheese contains high bioavailable calcium compared to acid- coagulated cheese such as cottage cheese. Bioavailability of the calcium from cheese is equivalent to that from milk. It has been reported that 22.9, 26.7 and 25.4% of total calcium was absorbed from cream cheese, whole milk and yoghurt, respectively. Adequate calcium intake during childhood and in teenage years is important in development of high bone mass which may prevent osteoporosis in the later years Khetra *et al.*, (2016). The overall process of Gouda cheese production is given in Figure 2-5.

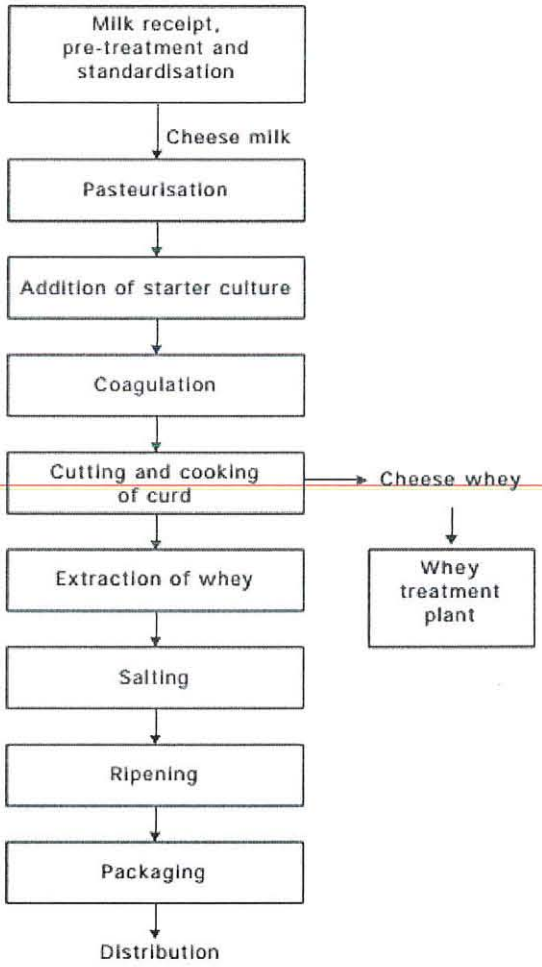

Figure 2-5. Flow diagram for cheese making Kanawjia, (2016).

2.12.1. Standardization, renneting and cutting

For the manufacture of good quality Gouda cheese, cow milk is considered to be the most suitable raw material. Manufacturing of Gouda cheese starts with acidification of the standardized milk. The milk is standardized so as to give 40-50% fat in cheese on dry matter. Starter is added at 0.7% of the milk. No ripening is done for this type of cheese.

Renneting is usually done at about 30°C at 0.022% of milk and allowed to set for about 20-30 minutes. After the curd is properly set, it is cut in cubes of some 8-15 mm size. Stirring, at first gently till acidity rises by 0.02% (to minimize loss of fines) and later more vigorously, is done with the knives used for cutting or with special stirrers Kanawjia, (2016).

2.12.2. Scalding

After cutting, part of the whey (about one third) is removed for more effective stirring and to promote synereses. It also facilitates partial removal of lactose, which aids in achieving a lower acidity. The temperature is also increased at the same time to aid synereses process but not too high to injure starter organisms. Usually, it is kept below 38°C. This process of heating curd in whey is called scalding. The temperature is increased usually by addition of hot water at about 60°C (about equal quantity of whey drained) which also helps in controlling water content and the pH of the cheese Fox *et.al.* (2017).

2.12.3. Draining and pressing

After the curd has lost enough moisture i.e. around 65% moisture is left in the curd and pH is around 6.5, stirring is stopped and the curd grains are allowed to sediment. Continuous mass of curd is then formed due to fusion of the curd grains. This curd can now be cut into blocks and taken out of the whey. Blocks may be pressed further to remove whey. This may also cause considerable loss of fines. Fat loss in whey can be recovered by passing it through cream separator and loss of fines can be recovered by the use of hydrocyclones. The blocks are then put into moulds and pressed Fox *et.al.* (2017).

2.12.4. Brining

Brine salting is generally done using about 18-20% brine in tank. The pH of brine is adjusted to 4.8-4.9 to prevent dissolution of cheese protein in the brine. Cheese blocks should be inverted few times daily. Time taken for brining will depend on size, viz., 0.45 kg - 20 h, 0.90 kg - 36 h, and 3.83 kg - 3 days Kanawjia,(2016).

2.12.5. Paraffining and storage

After brining, paraffining is done and the cheese blocks are kept for ripening. A maximum of 3-4 months are required for development of flavour and texture of Gouda cheese Kanawjia,(2016).

2.13. Food Hydrocolloids

Hydrocolloids are a diverse group of long chain polymer characterized by their property of forming viscous dispersion and/or gels in water. The affinity of water binding is increased by the presence of hydroxyl groups. Hydrocolloids have a wide functional application in food including; viscosity enhancing or thickening, gelling, emulsifying, stabilization and coating among others. For most applications, it is used at a concentration range of 0.15-3.1% Burey *et.al.* (2008).

Similar to their functional diversification, hydrocolloids are obtained from different sources such as plant hydrocolloids; Carboxymethylcellulose (CMC), Mannans and galactomannans, Xyloglucans, Arabinogalactan and Pectins; Seaweed hydrocolloids; alginates, Carrageenans and Agar; Microbial hydrocolloids; Xanthan gum, Pullulan and Gellan gum; and Animal hydrocolloids; Chitin and chitosan, and Gelatin Dickinson, (2003).

According to Burey *et al.,* (2008), a stabilizer is a single chemical component or mixture of components which can offer long term stability on an emulsion, possibly by a mechanism involving adsorption but not necessarily so. The primary applications of stabilizers such as pectin is modification of the rheological properties of aqueous systems by functional characteristics such as retardation of precipitation of dispersed solid particles, prevention of aggregation of dispersed particles and prevention of synereses of gelled systems to mention but a few Dickinson, (2003).

2.14. Function and chemistry of Pectin

For many years, Pectin has been used for its functionality in many foods. Ubiquitous inthe conserves and preserves industries, development of pectin has centered on its use toimpart texture in high sugar systems. Although Pectin has been studied widely, it remains difficult to characterize as a model system due to the heterogeneous nature of the polymer. Industrial use has mainly been focused on tailoring the polymer to specific needs Sharma *et al.,* (2006).

2.14.1. Structure

Pectin is a complex polysaccharide which contains 1.4-linked α-D-galactosyluronic residue, (Figure 2-6). So far, there are three types of pectic polysaccharides; homogalacturonan, rhanmogalacturonan-I and substituted galacturonan, that has been isolated from a plant cell wall. Homogalacturonan (HG) assumes a linear chain of 1.4-linked α-D-galactosyluronic residues, in which some of the carboxyl groups are methyl esterified. They have been isolated

from sunflower heads and apple pectin but were obtained by extraction treatments likely to cleave covalent bonds so they have been released from a heterogeneous pectic polysaccharide Dronnet *et al.*, (1996).

Preparations of pectin consist of sub-structural entities that depend on their source and extraction methodology. Commercial extraction causes extensive degradation of the neutral sugar-containing side chains.

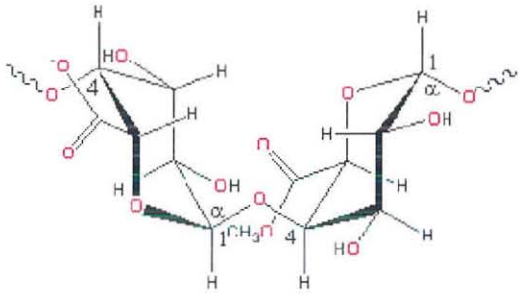

Figure 2-6. Structure of pectin (www.1.lsbu.ac.uk)

2.14.2. Functional Groups

Pectin also carries non-sugar substituent, essentially methanol, acetic acid, phenolicacids and occasionally amide groups. The esterification of galacturonic acid residues with methanol or acetic acid is a very important structural characteristic of pectic substances. Thedegree of methylation (DM) is defined as the percentage of carbonyl groups esterified with methanol. If more than 50% of the carboxyl groups are methylated the pectins are called high-methoxy pectins (HM), and less than that degree of methylation are called low methoxy(LM) pectin. This same principal applies to acetylation although the degree of acetylation(DAc) can be larger than 100% as galacturonosyl residues can be acetylated with more than one group per monosaccharide Ridley *et al.*, (2001).

2.15. Gelation of Pectin

2.15.1. Low methoxy pectin (LM)

LM pectins can gel in the presence of divalent cations, usually calcium. In these systems gelation is due to the formation of intermolecular junction zones between homogalacturonic smooth regions of different chains. The structure of such a junction zone is generally ascribed to the so called 'egg box' binding process. Initial strong association of two polymers into a dimer is followed by the formation of weak inter-dimer aggregation, mainly governed by

electrostatic interactions. The gel forming ability of LM pectins increases with decreasing degree of methylation. LM pectins with a block wise distribution of free carboxyl groups are very sensitive to low calcium levels. The presence of acetyl groups prevents gel formation with calcium ions but gives the pectin emulsion stabilizing properties Yoo *et al.*, (2006).

2.15.2. High methoxy pectin (HM)

HM pectins have the ability to form gels with sugar and acid, so-called low water activity gels or sugar-acid-pectin gels. Such a gel is considered a 2-dimensional network of pectin molecules in which the solvent (water) with the co-solutes sugar and acid are immobilized. This results in a system resisting deformation and showing a stress-strain relationship for small deformation. The build-up of the 3-D network is based on the formation of junction zones in which there are chain associations stabilized by hydrogen bonding between un-dissociated carboxyl and secondary alcohol groups and by hydrophobic interaction between methyl esters. The gelation mechanism of pectin is mainly governed by their degree of esterification (DE). For the low methoxy-pectins, denoted LMP (DE<50%), gelation results from specific non-covalent ionic interactions between blocks of galacturonic acid residues of the pectin backbone and with divalent ions such as calcium. The affinity of pectin chains towards calcium is known to increase with decreasing degree of esterification or ionic strength, and with increasing polymer concentration. Besides the influence of the charge density of the polygalacturonate chain, the distribution pattern of free and esterified carboxyl groups has an important effect on the strength of calcium binding Tsoga *et al.*, (2004).

2.16. Concluding remarks

Whey has a proven nutritional as well as health benefits to human. In most of the cases, however, it is discharged as a by-product. In my opinion, draining such valuable product into the sewage lines is a crime committed against a large number of people who suffers from food shortage and malnutrition in general and its utilization is worthconsidering.

Whey is one of the prominent dairy wastes introduced to the environment. Due to its high Biological and Chemical Oxygen Demands, it creates inhabitable ecological niche for the community. As the dairy sector in Ethiopia is growing owing to rapid urbanization and population growth, the demand for dairy products is expected to escalate; which in turn contributes to the dairy effluent.

Whey beverage fortified with teff is such one remedy in exploiting the advantages of the golden waste for human use nutritionally, convert it to money for the industries and addressing the environmental pollution concern that often frowned by the experts of the field.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1.Location of the Study

The experiment was conducted in Addis Ababa, Ethiopia, which lies at an altitude of 2,500 meters and is located at 9.03°N 38.74°E. / 9.03; 38.74. Raw materials and finished products were processed and prepared at Bless Agri Food Laboratory Services P.L.C and Addis Ababa University, Centre for Food Science and Nutrition.

3.2. Sample collection and Sample preparation

3.2.1. Sample Collection

Gouda cheese whey was collected from Lame Dairy Processing P.L.C, Addis Ababa and stored at 4°C until the laboratory test is carried out. Teff sample (DZ-Cr-387 Quncho variety) was collected from Debre Zeit Agricultural Research Center, Bishoftu, Ethiopia.

3.2.2. Sample Preparation

Teff flour:

Teff sample (DZ-Cr-387 Quncho variety) was milled using High speed all-purpose laboratory grinder (Model: JK-APG-100, Shanghai China) Jingke Scientific Instruments Co. LTD) to produce whole flour.

3.3.Determination of particle size

The particle size of teff flour was determined according to the Ethiopian Standard ES 3880:2015, which is in the range of 54-90 % using a 250μm sieve size. Hence, the particle size of the teff flour was calculated using the following procedure:

100g teff flour was weighed (W_1).The sieve was placed on the receiving container so that it fits properly. The flour was sieved until only larger particles are retained. The mass of the flour passed through the sieve was weighed (W_2). The percentage of particles was calculated using Eq. 1.

$$\text{Particle Size (\%)}: \frac{W2}{W1} * 100 \quad \text{.....Eq.1}$$

Where:

W_1= Sample weight before sieving

W_2 = Sample weight after sieving

Accordingly, the percent particle size of the flour that passes through the 250μm was:

W_1= 100.0136g

W_2= 99.2754

$$\% \text{ Particle size (pass)} = \frac{W2}{W1} * 100$$

$$= \frac{99.2754}{100.0136} * 100$$

$$= 99.26 \pm 0.11\%$$

The flour was then analysed for its microbiological quality, proximate composition and mineral content.

Cheese whey:

After aseptic collection, the whey was strained with cheese cloth and analysed for its microbiological quality, proximate composition, mineral content and pH values at Bless Agri-Food Laboratory Services PLC.

Maltodextrin:

Corn Maltodextrin (MAL-Cr) was procured from Nutriset, France, and stored at room temperature. Maltodextrin is a modified starch used in food for improving texture, mouth feel and consistency of products.

Pectin:

Pectin (PECT-AC13, Producer: HERBS TREITH & FOX) was procured from Warren Chemical Specialities (PTY) LTD, South Africa and stored at room temperature. Pectin was chosen for this research work because it is an important polysaccharide used in a number of foods as a gelling agent, thickener, texturiser, emulsifier and stabiliser.

Flavours:

Artificial flavouring agents; Vanilla (Foster Clarks Products LTD) and Mango (Guanzgzhou Xinlei Flavor Co., LTD) was procured from a supermarket to enhance the flavour of the final product.

Artificial sweetener:

Artificial sweetener (aspartame) tablets of 18mg; (Lo-Kal Gold, Cipla LTD, India) was procured from a local pharmacy and used to enhance the sweetness of the final product.

Food Color:

Artificial color, Yellow Jaune (Foster Clarks Products LTD), was procured from a supermarket and used to enhance the color of the final product.

3.4. Preliminary product development

3.4.1. Maltodextrin as a stabilizer

Prior to the actual design and optimization of the product, preliminary laboratory test was conducted at Bless Agri Food Laboratory Services P.L.C. The preliminary test was carried out based on the principle of teff juice formulation using maltodextrin as a stabilizer. According to the label, *Amandin* is made from water, teff (10%), sunflower oil, corn maltodextrin and sea salt.

Cheese whey was produced in the laboratory from pasteurized milk. The milk was boiled and vinegar added in order to separate the curd after which the whey was collected. Cooking temperature of 55°C and a holding time of 30 minutes were proposed based on trials made in the lab. The product was not consistent at the proposed cooking temperature and holding time. It exhibits synereses upon storage, (Figure 3-1).

Figure 3-1. Preliminary trail test using maltodextrin

3.4.2. Pectin as a stabilizer

After the preliminary product development failed using Maltodextrin as a stabilizer, another trial was made in the lab using pectin, (Figure 3-2). Moreover, the previous cooking temperature of 55°C was adjusted to 65°C and the holding time lowered to 15 minutes instead of 30 minutes. Pectin was replaced for maltodextrin to be used as a stabilizer after which the problem of synereses was overcome.

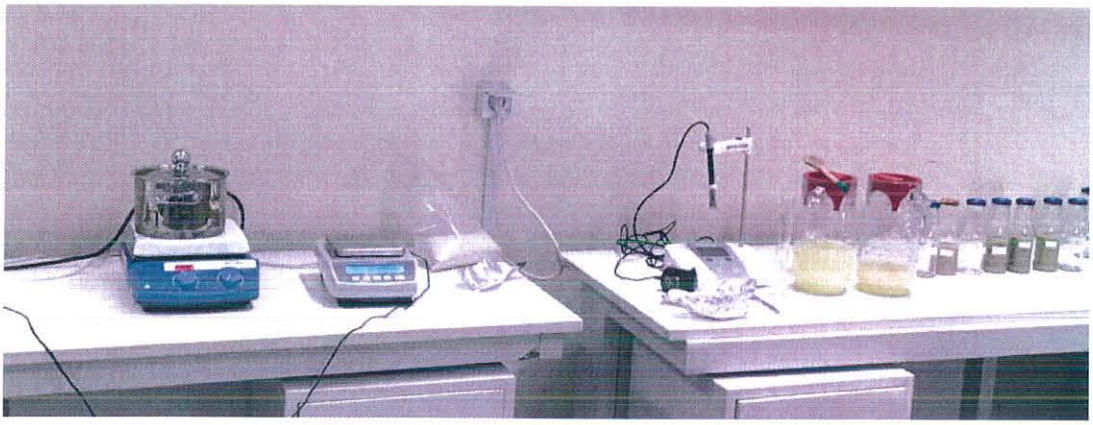

Figure 3-2. Preliminary trial test using pectin

Actual runs

The general process flow diagram during the production of whey-teff product is stated in Figure 3-3.

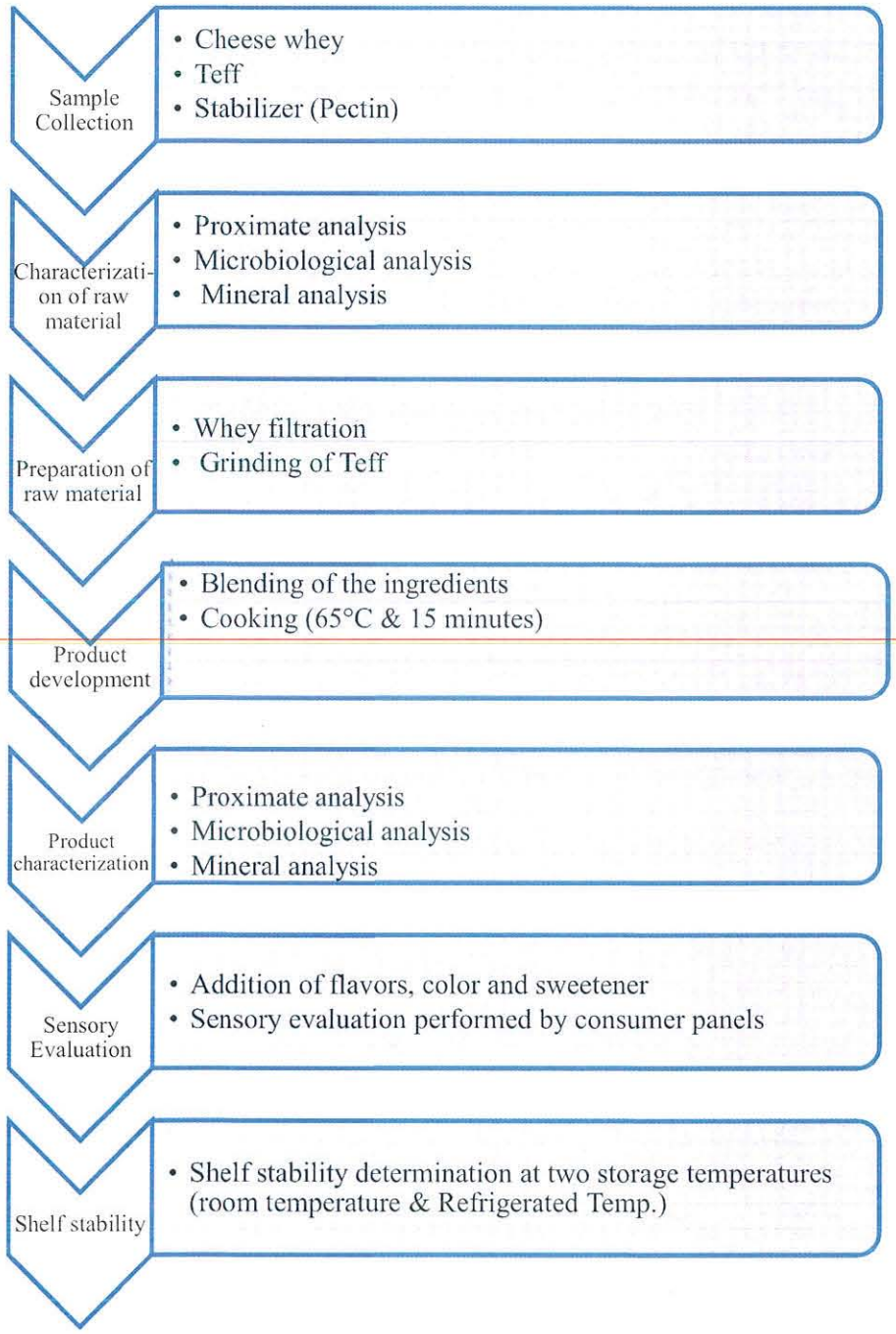

Figure 3-3. Process flow diagram of whey-teff beverage production

3.5. Formulation of Experimental Design

Three mixture components; whey, teff flour and pectin were designed in the proportion presented herein. Whey was proposed in the range of 88-94%, teff flour in 5- 10%, and stabilizer in the range of 1-2%, (Table 3-1). Maximum values of 10 % and 2 % for teff and stabilizer respectively was set on the basis of the composition of similar whey and cereal based beverages. The maximum and lower levels of the three variables are chosen on the basis of preliminary laboratory experiment conducted at Bless Agri Food Laboratory Services P.L.C.

A cooking temperature of 65 °C and a holding time of 15 minutes was chosen due to the fact that, most whey protein components are denatured at a temperature ≥ 70 °C and starches for teff component gelatinizes at a temperature of ≥ 74°C Ubwa *et al.*,(2012).

Table 3-1. Real values of coded variables

Variable	Coded value			Real Value (%)		
	Lower level	Middle Level	Higher Level	Lower level	Middle Level	Higher Level
Whey	0	0.5	1	88	91	94
Teff	0	0.5	1	5	7.5	10
Stabilizer	0	0.5	1	1	1.5	2

3.6. Design for the development of functional beverage

D-optimal Experimental design was selected in order to design and optimize the mixture components using Design- Expert ®, version 7.0 (from Stat-Ease, Inc). This design is suitable for building quadratic models with the selected mixture components.

A D-optimal design with three factors; cheese whey, teff flour and stabilizer (pectin), each with 3 levels were specified. Accordingly, a total of 16 run (6 model points and 5 lack of fit) was performed at different blend ratios, (Table 3-2) and (Table 3-3). Beverage optimization was performed by analysing the responses; sensory attributes; proximate analysis and mineral contents.

Table 3-2. Experimental Design of three factors (Cheese whey, Teff flour and stabilizer)

Standard Run	**Component 1**A: Whey	**Component 2** B:Teff	**Component3** C:Stabilizer	Sensory responses					Proximate test						Mineral content				
				Taste	Color	Flavour	Consistency/viscosity	Acceptability	Carbohydrate	Crude Protein	Crude Fat	Crude Ash	Dietary Fiber	Moisture	Fe	Ca	Zn	Mg	Na
	%	%	%																
1	0.5	0.5	0.5																
2	0.5	0	0.5																
3	0	1	0																
4	0.5	0.5	0																
5	1	1	1																
6	0.5	0	0.5																
7	0.167	0.169	0.664																
8	0.666	0.164	0.17																
9	0.168	0.664	0.168																
10	0	0.832	0.168																
11	0.168	0	0.832																
12	1	1	1																
13	1	0	0																
14	0	0	1																
15	0.5	0.5	0																
16	1	0	0																

Table 3-3. Actual values for the designed experimental factors

Run	Component 1	Component 2	Component 3	Taste	Flavour	Consistency	Acceptability	Proximate Test						Mineral Content				
	A: Whey	B:Teff flour	C:Stbilizer					Carbohydrate	Crude Protein	Crude Fat	Crude Ash	Crude Fiber	Moisture	Fe	Ca	Zn	Mg	Na
	%	%	%															
1	91.095	7.377	1.529															
2	93.285	5	1.715															
3	90.369	8.181	1.451															
4	89.865	8.135	2															
5	88	10	2															
6	93.285	5	1.715															
7	91.88	6.633	1.487															
8	92.963	6.037	1															
9	88.992	10	1.008															
10	89.838	9.162	1															
11	88.992	10	1.008															
12	88	10	2															
13	94	5	1															
14	88.933	9.067	2															
15	89.865	8.135	2															
16	94	5	1															

3.7. Laboratory Analysis

3.7.1. Proximate analysis of the designed formulation

The proximate analyses of the designed proportions were analysed by standard AOAC and ISO methods.

3.7.1.1.Moisture Content

In order to measure moisture content of the samples, AOAC Official Method 925.10 was employed. A clean crucible was dried at 92°C in a drying oven for 1 hr. The crucible was transferred to the desiccator and cooled for 30 min. The empty crucible was weighed (M_1) to the nearest mg. The heating, cooling and weighing were repeated until the difference between two successive weighing was less than 3-5 mg. For liquid samples, 15g sample was weighed accurately and evaporated first on a water bath. For solid and semisolid samples, 7.5g sample was weighed accurately. Then the sample was transferred to pre-weighed crucibles and weighed (M_2). The crucibles were placed in the oven and dried at 92 °C for 6 hrs. The crucibles were removed from the oven and cooled in the desiccator for 30 minutes and re-weighed. They were further complete dried for 1 hr at 92 °C, and cooled again in the desiccator. After drying was completed, final weighed weight measurements were taken (M_3). The heating, cooling and weighing were repeated until a constant weight had been achieved. The moisture content of each sample was calculated from the equation:

$$\text{Moisture (\% W/W)} = \frac{(M2 - M3)}{M2 - M1} \times 100 \quad \text{.....Eq.2}$$

Where:

M_1 = Weight of the dried crucible,

M_2 = Mass of the dried crucible and the sample before drying,

M_3 = Mass of the dried crucible and the sample after drying.

3.7.1.2.Crude Protein

The protein content of the samples was calculated using AOAC Official Method 976.05.A 0.5 g dried sample was weighed and transferred into the digestion tube. Then 6 ml of the acid mixture (85% orthophospsoric acid and 98% sulphuric acid) and 3.5 ml of hydrogen peroxide solution were added in to the digestion flask step by step. The tubes were shaken until the violate reaction disappeared. About 3g of the catalyst mixture containing 0.5 g of copper sulphate and 100 g of potassium sulphate was added in to the digestion tube. The solution was then digested at 370 °C for 1hr and 30 min by Gerhardt digester (TTM, Germany) until a clear solution was obtained. Then 30 ml of water was added and shaken to avoid precipitation of sulfate in the solution.

The nitrogen content was calculated from the equation:

$$\% \text{ Nitrogen} = \frac{14 \text{ x } (V - B)\, N}{W} \quad \text{.....Eq.3}$$

The protein content was calculated from the equation:

Crude protein (%) = total nitrogen (%) x Factor specific for different products

Where: V = volume of sulfuric acid consumed

B = volume of sulfuric acid consumed blank

N = normality of the acid (0.1N sulfuric acid)

14 = eq. wt of nitrogen

W = Weight of the sample (g)

3.7.1.3.Determination of Crude Fat

Accelerated Solvent Extraction method was employed in order to determine the fat content of the raw materials as well as the formulated products, (Thermo Scientific™).

The aggregation of sample particles may prevent efficient extraction. Hence, dispersing the sample with an inert sand material (Thermo Scientific™ Dionex™ ASE™ Prep DE (diatomaceous earth) (P/N 062819) assisted in the extraction process.

For teff samples, 4 grams of the sample was added to 1 gram of Dionex ASE Prep DE and for cheese whey and whey-teff beverage, 4 grams of sample was added to 2 grams of Dionex ASE Prep DE. The sample and the drying or dispersing agent was mixed thoroughly in a small beaker, and then added to the extraction cell.

Fat is extracted from the samples using petroleum ether in an Accelerated Solvent Extraction System (ASE). The solvent from the extracted fat was evaporated in an oven92°C for 1 hr.and the difference in weight was used to calculate the fat content in the sample.

The fat content of the samples is calculated by considering the differences in mass of Empty solvent collection vial (W_1), Weight of Sample (W_2) and Weight of Solvent collection vial after extraction (W_3).

Weight of fat W = W3- W1

Fat g/ 100g fresh sample (%) $= \frac{W}{W2} * 100$**.....Eq.4**

Where: W = weight of fat

W_1 = weight of empty extraction flask

W_2 = weight of the sample

W_3 = weight of fat + flask after extraction and drying

3.7.1.4. Crude Fiber

The Dietary Fiber content of the teff flour, and the designed product was analysed using analytical method described in ISO: 6865.2000. The test portion of about 1 g (W_1) was weighed and pre-treated with 30 ml Petroleum ether to each crucible and filtered with vacuum. 150 ml volume of Sulfuric acid was added to each sample and boiled for about 30 minutes. The mixture was filtered through a crucible using vacuum and washed several times with distilled water. The residue was transferred to a beaker and 150 ml Potassium hydroxide was added and boiled for about 30 minutes. The mixture was washed with 30 ml acetone and dried. The crucibles were put in an oven set to a temperature of 103±2°C and for 4 hours. The crucibles were then placed in a desiccator to cool and weighed to the nearest 0.1 mg (W_2). The crucibles were placed in a muffle furnace and incinerated to for 2 hours at a temperature of 550±20°C. Finally, the crucibles were put in a desiccator and allowed to cool. The final weight (W_3) was taken after removing to the nearest mg.

Calculation

Percent Crude fibre (% CF):

% CF (g/100g) $= \frac{W2-W3}{W1} * 100$**.....Eq.5**

Where;

W_1 = weight of the sample (g),

W_2 = weight crucible and residue after drying (g), and

W_3 = weight crucible and residue after incineration (g).

3.7.1.5.Total Ash

In order to measure the total ash content of the samples, AOAC 942.05.2000 method was employed. Porcelain Crucibles were cleaned and dried in an oven at 105°C for 30 min. The crucibles were cooled in a desiccator for 30 min and weighed to the nearest mg (W_1). About 3.75 g of the sample was weighed in to each crucible (W_2). Then the samples were charred at low temperature on a hot plate under a fume-hood and slowly increased the temperature until smoking ceased and the samples became thoroughly charred. The crucibles were then placed in a furnace at about 550°C for 1 hr. The crucibles were then removed from the furnace and were cooled. A 5 drop of deionized water was then added to each of the crucible to moisten the ash and evaporated the water on hot plate for 15 min and placed in the furnace at 550 °C for 30 min. Crucibles were again removed from the furnace, allowed to cool and 5 drops of nitric acid were added to each. The crucibles once again were placed inside the furnace until they became free from carbon and the residue appears grayish white. Then they were removed from the furnace and placed in desiccators for 60 min. Finally the mass of each crucible was weighed as (W_3).

The total ash was calculated from the equation:

$$\% \text{ Ash } = \frac{W3-W1}{W2} * 100 \quad \text{.....Eq.6}$$

Where: W_1 = Weight of crucible

W_2 = Weight of ash + crucible

W_3 = Weight of fresh sample

3.7.1.6. Carbohydrate

The total carbohydrate content was determined by difference i.e, subtracting the sum of the percentages of moisture, crude protein, crude fat, crude fiber and Ash content from100.

3.6.1.7. Energy values

Energy values were calculated using factors of 4, 9 and 4 for each gram of protein, fat and carbohydrate, respectively Wait *et.al.*, (1986).

3.7.2. Mineral analysis

Analysis of minerals; Fe, Ca, Zn, Mg and Na was determined according to Official Method of Analysis (AOAC) 999.10 by microwave digestion and Flame Atomic Absorption Spectrophotometer (FAAS).Products are digested with HNO_3 and H_2O_2under pressure in a closed vessel heated by microwaves. Solution is diluted with H_2O.

3.7.3. Microbiological Quality analysis

3.7.3.1.Total aerobic bacterial count

The aerobic plate count (APC) is intended to indicate the level of microorganism in a product. In order to determine the total aerobic bacterial count analytical method given in ISO 4833 was employed.10 g sample was weighed and diluted with 90ml of Phosphate Buffer. Serial dilutions were made from the first suspension in 1:9 sample-diluent ratios. 1ml of sample was transferred from each suspension into duplicate petri dishes after which about 15 ml of sterile molten Plate Count Agar (PCA) medium was added. The plates were let to solidify on an even surface and incubated (inverted) in an incubator set to a temperature of 30°C±1 for 72±2 hrs. Colonies were count after the incubation period according to the procedure described in ISO 7218.

3.7.3.2.Yeast and Mold count

Yeast and Mold count was determined according to the standard method given in Official Method of Analysis ISO 7954.The colony count estimates the number of viable aerobic mould and yeast per g or ml of the product. A 10 gram/ ml portion of the food homogenate was diluted with 90 ml of phosphate buffer. Serial dilutions were made in 1:9 samples to diluent ratio. About 15-20 ml of Dichloran Rose Bengal Chloramphenicol Agar (DRBCA) was poured into sterile petri dishes and solidified. A 0.1 ml portion of sample homogenate was spread on the solidified agar surface and incubated at a temperature of 25°C for 3-5 days. Typical colonies of Yeast and Mold were counted and results expressed according to the method described in ISO 7218.

3.7.3.3.Enumeration of Enterobacteriaceae

Enumeration of Enterobacteriaceae is determined according to the method described in ISO 21528-2. Enumeration is carried out by counting colonies in a solid medium after incubation at 37 °C, without pre-enrichment, for the enumeration of Enterobacteriaceae.

10 grams and ml of solid and liquid samples respectively were weighed and diluted with 90 ml phosphate buffer to make first dilution (10^{-1}). Petri dishes are inoculated with 1 ml of the test sample if the initial product and or suspension.15 ml of Violet Red Bile Glucose Agar was poured into sterile petri dishes and let to solidify. A 10 ml of the same medium was added as an overlay and solidified. Further Petri dishes are inoculated, under the same conditions, using decimal dilutions of the test sample or of the initial suspension. The plates were incubate aerobically at 37 °C for 20 h ±2 h. Characteristic colonies were enumerated and confirmed by means of tests for fermentation of glucose and presence of oxidase.

3.7.3.4.Staphylococcus aureus count

Coagulate positive staphylococcus strains was determined according to the analytical method given in ISO 6688-1.10 gram and ml samples were prepared with subsequent dilution, plated out on Baird parker agar and incubated at 35 °C for 20 h ±2 h. Typical colonieswere selected and biochemically confirmed using Brain Heart Infusion Broth (BHI)and Coagulase Tests.

3.7.4. Shelf stability, pH and Viscosity determination

The storage ability of the product was determined within a period of seven days at two different storage temperatures, 4°C and room temperature (ambient temperature). Microbiological quality parameters; *total aerobic bacterial count*, *yeast and mold count*, and *Enterobacteriaceae* count was determined. The pH of the cheese whey and final product (whey-teff beverage) was determined using bench top pH meter (Metler-bled AG FEP-20-Germany) with standard buffer solution of pH values 4.0 and 7.0 used for calibration.

The viscosity of the final product was determined using a DV-E VISCOMETER (Model- MS-12BB, Germany). The measurement was done using spindle numbers 63 and 64 for different formulations at 100 rpm (rotation per minute), at room temperature across a period of seven days.

3.7.5. Sensory Evaluation

A complete sensory plan, according to the method described in ES ISO 11136: 2016, was adopted to evaluate the taste, color, flavour and overall acceptability of the final products.

Hence, 30 consumer panellists were recruited from BLESS Agri Food Laboratory Services PLC. Criterion was set to choose individuals who have experience in consumption of the traditional atmit produced in household. The age group of the panellists was 23-43. Female participants were 13 and 17 of the panellists were male. The panellists have awareness of sensory evaluation procedures and meaning of the descriptive terms used.

Panellists were instructed to evaluate color first and then to evaluate taste, flavour and overall acceptability. A nine-point Hedonic scale with 1=Dislike extremely, 5=neither like nor dislike, 9=Like extremely was used for all attributes measured. A 50 gram sample was randomly coded and presented in a transparent food grade plastic cup. Three samples were given at a time for the panelists, at room temperature, (~22.3°C), totaste, swallow and rinse their mouth in between to minimize the effects of uncontrollable sources of variation or error and to eliminate bias Watts *et al.*, (1989).

To block possible effects of judges, the following model was used.

$Yij = \mu + Ti + Bj + Eij$.....**Eq.7**

Where;

- Yij- is the response
- Ti- is the treatment
- Bj- is the block effects
- Eij.- is random error

3.8. **Modelling**

The experimental data for each response variable were fitted to the quadratic model as $Y= \beta+X_1+X_2+X_3+X_1^2+X_2^2+X_3^2+X_1X_2+X_1X_3+X_2X_3$, where, Y=responses; β=constant; X_1, X_2, X3=linear regression; X_1^2, X_2^2, X_3^2=quadratic regression X_1X_2, X_1X_3, X_2X_3 =interaction regression; X1, X2, X_3= independent variables.

3.9. **Data analysis**

Data (Microbiological test results, Proximate test, Mineral tests and sensory values) obtained from the experiment was analysed using Duncan's Multiple Range test (IBM SPSS version 20.0) and in order to determine level of significance within means. A p-value below 0.05 was considered as significant. Both numerical optimization and graphical optimization technique were employed using the Design Expert® version7.0 software (State Ease Inc.) with the three components.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1. Proximate composition

Proximate composition of raw materials, whey & teff flour, and the final product were determined according to the proposed test procedures. Hence, the results of Moisture, Crude Protein, Crude Fat, Crude Ash, Total Carbohydrate, Crude fiber are depicted in Table 4-1.

Table 4-1. Proximate composition of cheese whey and teff flour (g/100g DM except moisture)

Product	Moisture (%)	Protein	Fat	Ash	Fiber	Carbohydrate
Cheese whey	95.20±0.75	10.91±0.81	3.59±0.52	11.20±0.46	NA	76.44±0.98
Teff flour	8.84±0.15	8.87±0.11	2.31±0.04	2.58±0.08	3.21±0.11	74.19±0.44*
*Values are given as Mean ± SD of triplicate run. NA- the test was not applicable for the sample						

The results are expressed as mean of triplicate trials and standard deviation in dry basis except for Moisture content, which is in wet basis. The mean values for protein of 10 g/100g; fat of 3.99 g/100g, and ash content of 10.67 g/100g of whey is close to the values found by (Gupta, 2000); which were protein (9 g/100g), fat (5.2 g/100g) and ash (6 g/100g) respectively. The fiber content of whey was below the value range to be determined by the employed method of analysis, which was close to zero.

The proximate composition of teff flour of protein (8.84g/100g), fat (2.3 g/100g), ash (2.58) and fiber content of (3.21g/100g) are close enough to the values determined by Baye, (2014); Bultosa, (2007); which were 11g/100g of protein, 2.5 g/100g of fat, 3.0 g/100g of fiber and 2.8 g/100g of ash respectively.

Table 4-2. Microbiological quality of cheese whey and teff flour (CFU/ml & CFU/g respectively)

Product	APC	Yeast and Mold	S.aureus	Enterobacteriaceae
Cheese whey	$3.8*10^4 \pm 2.10^4$	<10	<10	<10
Teff flour	$9.\ 8.*10^2 \pm 1.3*10^2$	<10	<10	<10
*Values are given as Mean ± SD of triplicate run.				

The microbiological quality of the raw materials indicated, no pathogenic (*S.aureus*) and spoilage (*yeast and mold*) and indicator microbes (*Enterobacteriaceae*) were detected. Moreover, the total bacterial load is very low which would further be reducing during processing of the final product, (Table 4-2).

Table 4-3. Proximate composition of formulated products (g/100g DM except moisture)

Trial	Moisture (%)	Crude Protein	Crude Fat	Crude Ash	Crude Fiber	Carbohydrate	Energy(Kcal)
R-1	86.32 ± 0.1^{a}	$8.84\pm0.54^{a,d,f}$	$0.59\pm0.01^{a,g}$	5.63 ± 0.06^{a}	$2.48\pm.54^{a}$	$82.47\pm0.97^{a,l,m,p}$	370.50 ± 2.39^{a}
R-2	88.73 ± 0.05^{b}	$9.17\pm0.03^{a,b,i}$	$0.94\pm0.05^{b,i}$	$4.72\pm0.03^{b,e}$	1.75 ± 0.17^{b}	$83.42\pm0.22^{a,b,d,j,k}$	378.82 ± 0.50^{b}
R-3	$85.85\pm0.23^{c,}$	$8.85\pm0.08^{a,d,f}$	1.67 ± 0.02^{c}	4.05 ± 0.06^{c}	$2.27\pm0.05^{a,b}$	$83.15\pm0.08^{a,c,d,p}$	$383.06\pm0.17^{c,f,g}$
R-4	85.62 ± 0.01^{c}	$8.92\pm0.02^{a,c,}$	$0.57\pm0.03^{a,g}$	$4.62\pm0.05^{b,j}$	$2.33\pm0.07^{a,b}$	$83.55\pm0.11^{d,g,h}$	375.04 ± 0.19^{d}
R-5	82.01 ± 0.01^{d}	8.40 ± 0.15^{d}	$0.50\pm0.01^{a,d}$	3.12 ± 0.02^{d}	$2.25\pm0.06^{a,b}$	85.73 ± 0.09^{e}	$381.03\pm0.29^{b,c}$
R-6	88.89 ± 0.02^{b}	11.99 ± 0.13^{e}	$0.38\pm0.03^{d,f}$	4.86 ± 0.02^{e}	$2.01\pm0.17^{a,b}$	80.75 ± 0.34^{f}	374.42 ± 0.59^{d}
R-7	85.75 ± 0.03^{c}	$9.29\pm0.11^{b,c,f,i}$	$0.49\pm0.03^{a,d}$	$3.83\pm0.06^{f,k}$	$1.88\pm0.09^{a,b}$	84.51 ± 0.03^{g}	379.65 ± 0.36^{b}
R-8	87.02 ± 0.02^{e}	10.07 ± 0.08^{g}	0.23 ± 0.02^{e}	4.49 ± 0.03^{g}	1.87 ± 0.13^{b}	$83.34\pm0.19^{a,h,j,k}$	375.73 ± 0.36^{d}
R-9	79.20 ± 0.10^{f}	7.49 ± 0.03^{h}	$0.29\pm0.07^{e,f}$	2.86 ± 0.06^{h}	$1.89\pm0.21^{a,b}$	87.47 ± 0.31^{i}	$382.46\pm0.52^{c,f,g}$
R-10	$82.90\pm0.02^{g,j}$	9.49 ± 0.02^{i}	0.67 ± 0.06^{g}	3.34 ± 0.03^{i}	$2.21\pm0.08^{a,b}$	$84.30\pm0.02^{g,j,n}$	$381.14\pm0.54^{b,c,f}$
R-11	82.86 ± 0.01^{g}	$9.39\pm0.04^{i,j}$	$0.69\pm0.01^{g,h}$	3.42 ± 0.07^{i}	$2.25\pm0.12^{a,b}$	$84.25\pm0.12^{g,k,n}$	$380.81\pm0.29^{b,c}$
R-12	78.83 ± 0.04^{h}	7.72 ± 0.17^{h}	0.86 ± 0.04^{b}	2.78 ± 0.09^{h}	1.86 ± 0.17^{b}	86.78 ± 0.27^{i}	385.76 ± 0.43^{e}
R-13	89.24 ± 0.09^{i}	$10.45\pm0.10^{g,l}$	1.58 ± 0.05^{c}	4.28 ± 0.06^{j}	$1.85\pm0.11^{a,b}$	$81.84\pm0.29^{l,o,q}$	383.37 ± 0.45^{f}
R-14	83.14 ± 0.06^{j}	$9.52\pm0.04^{i,k}$	1.05 ± 0.03^{i}	3.93 ± 0.08^{c}	$2.13\pm0.13^{a,b}$	$83.38\pm0.10^{b,c,h,m,n}$	$380.99\pm0.22^{b,g,h,i}$
R-15	$86.08\pm0.07^{a,k}$	11.84 ± 0.13^{e}	1.18 ± 0.06^{j}	$3.70\pm0.06^{f,k}$	$2.18\pm0.10^{a,b}$	$81.09\pm0.16^{f,o}$	$382.38\pm0.35^{c,f,h}$
R-16	88.96 ± 0.02^{b}	10.71 ± 0.06^{l}	1.27 ± 0.06^{j}	4.08 ± 0.04^{c}	1.74 ± 0.24^{b}	$82.20\pm0.29^{p,q}$	$383.04\pm0.63^{c,f,i}$
*Values are given as Mean ± SD of triplicate run. Values in the same column and not sharing the same superscript are significantly different at $p<0.05$.							

The mean moisture content of the formulated products ranged from 78.83-89.24 %. The mean total ash, protein and fat content of the final products is in the range of 2.78- 5.63 g/100g; 7.49-11.99 g/100g, and 0.23-1.67 g/100g respectively, which is accounted from the higher composition of whey, (Table 4-3).

On the contrary, the mean crude fiber content of the final products ranges from 1.7- 2.48 g/100g which is mostly accounted from teff flour. The contribution of whey and pectin for fiber content is very insignificant.

The mean total carbohydrate of the formulated products ranges from 80.75-87.47 g/100g which slightly was contributed more from teff composition. This indicated, the formulated products with high teff composition will have more carbohydrate content, as represented in contour surface plot, (Figure 4-1), and 3D plot, (Figure 4-2), respectively.

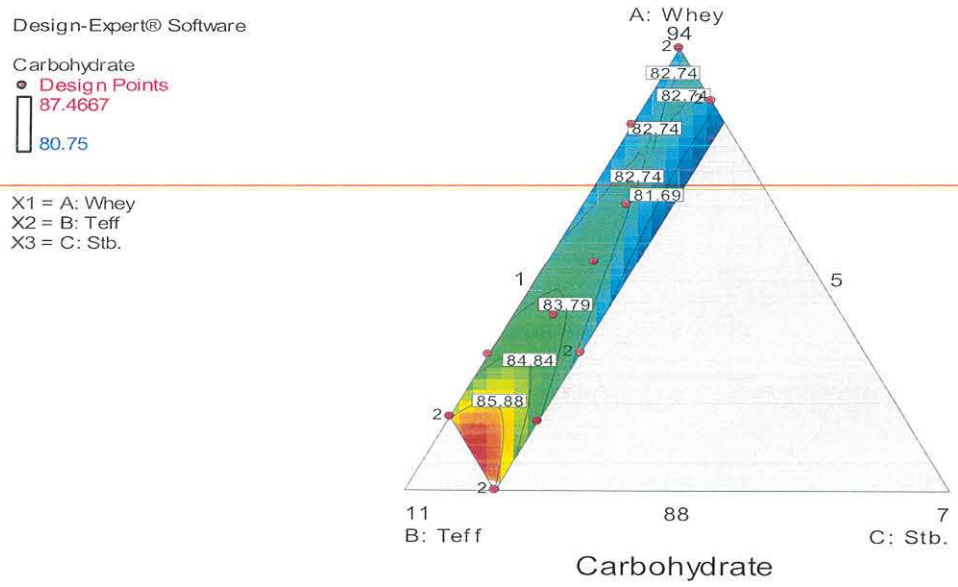

Figure 4-1. Contour plots for Carbohydrate

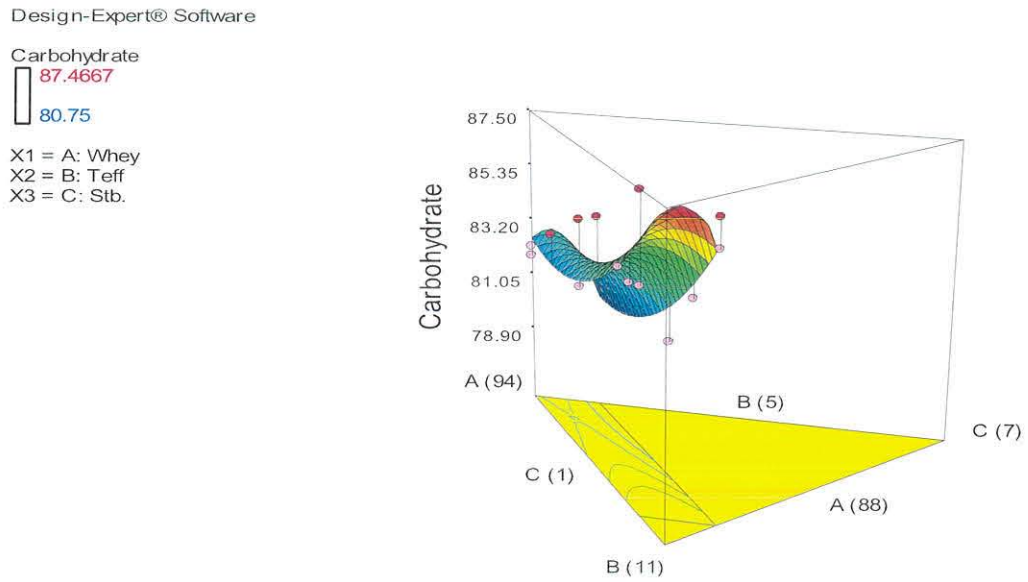

Figure 4-2.3D plots for Carbohydrate

The mean total protein content of the formulated products is in the range of7.49-11.99 g/100g. The higher the percentage composition of whey, the higher to amount of protein of the formulated products, as indicated in the contour, (Figure 4-3), and 3D surface plot, (Figure 4-4) given below.

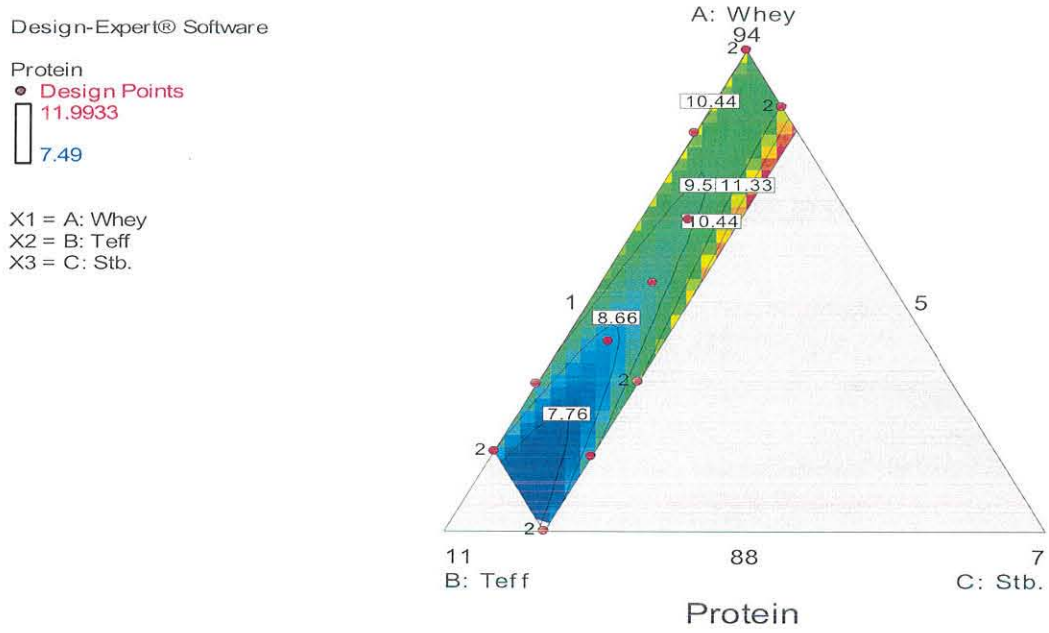

Figure 4-3.Contour surface plots for protein

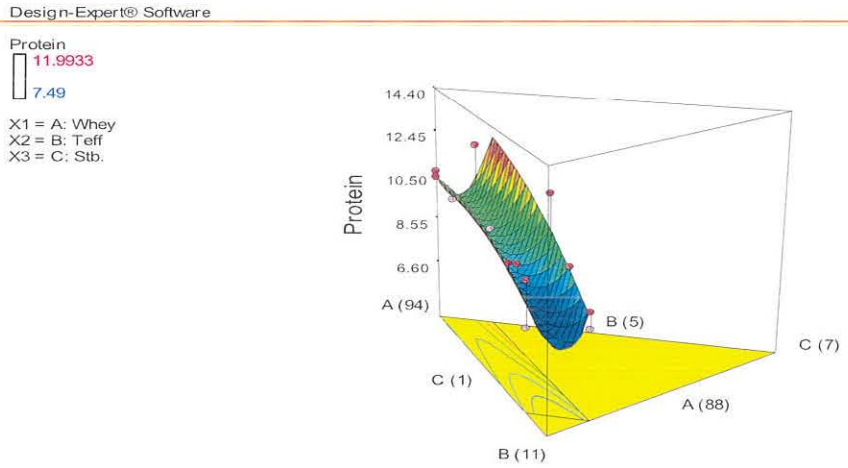

Figure 4-4. 3D plot for protein

The mean total fat composition of the formulated products ranges from 0.23-1.67 g/100g. As represented in the contour surface plot, (Figure 4-5), and 3D surface plot, (Figure 4-6), the values does not show inclination to any of the two major nutritional composition, teff and whey, rather the values aggregate towards the middle point. It is concluded that, the product contains a very small amount of fat which is considered to have positive health benefits, such as wait management, if the product is consumed.

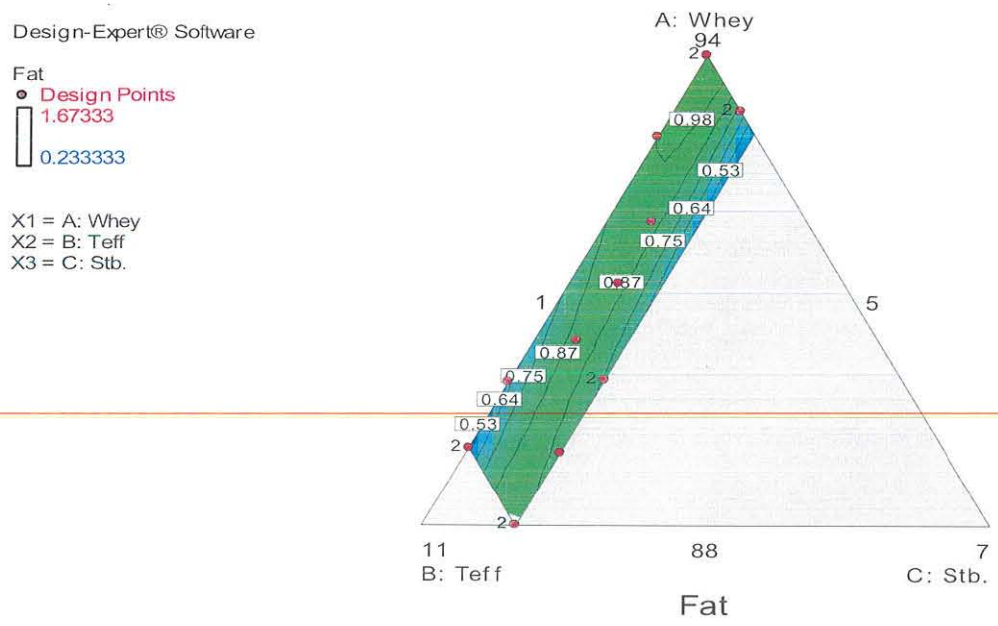

Figure 4-5.Contour surface plots for Fat

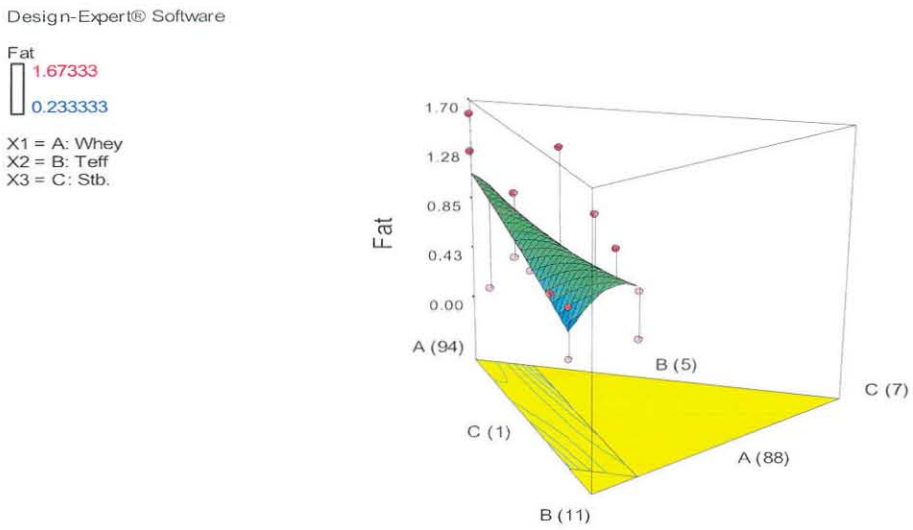

Figure 4-6. 3D plot for Fat

4.2. Mineral composition of ingredients and formulated product

Mineral composition of Fe, Ca, Zn, Mg and Na were determined for cheese whey and teff flour and the formulated products are presented in (Table 4.4)and (Table 4.5)respectively.

Total iron content (24.44 mg/100g), Calcium content (61.74 mg/100g), Zinc content (1.76 mg/100g) and Magnesium (189.0 mg/100g) content of Teff flour is close to the values of (9.5-377mg/100g) for iron, (17-124mg/100g) Calcium and (2.4-6.8mg/100g) zinc respectively, from previous work by Baye, (2014); Bultosa, (2007).

Table 4-4. Mineral content of raw materials (mg/100g DM)

Product	**Fe**	**Ca**	**Zn**	**Mg**	**Na**
Cheese whey	0.19±0.00	20.07±0.27	<0.0010	20.50±0.44	113.02±1.23
Teff flour	24.44±0.49	61.74±0.25	1.76±0.11	189.0.07±0.95	75.90±0.37
*Values are given as Mean ± SD of triplicate run.					

Table 4-5. Mineral composition of formulated product (mg/100g DM)

Trial	Fe	Ca	Zn	Mg	Na
R-1	12.16 ± 0.01^{a}	295.19 ± 0.02^{a}	$1.66\pm0.02^{a,d}$	$144.73\pm0.05^{a,b}$	280.35 ± 0.08^{a}
R-2	10.64 ± 0.02^{b}	323.90 ± 0.02^{b}	1.36 ± 0.04^{b}	$138.51\pm0.05^{a,b,c}$	292.39 ± 0.05^{b}
R-3	13.73 ± 0.01^{c}	282.52 ± 0.04^{c}	$1.75\pm0.06^{a,g}$	152.53 ± 0.01^{a}	369.90 ± 0.11^{c}
R-4	21.28 ± 0.03^{d}	249.48 ± 0.63^{d}	1.49 ± 0.02^{c}	$136.09\pm0.03^{a,b,c}$	248.46 ± 0.04^{d}
R-5	18.78 ± 0.12^{e}	241.03 ± 0.01^{e}	$1.65\pm0.01^{d,f,h}$	167.99 ± 0.19^{a}	204.44 ± 0.01^{e}
R-6	9.49 ± 0.12^{f}	261.52 ± 0.02^{f}	2.09 ± 0.02^{e}	$114.44\pm0.02^{a,b,c}$	232.97 ± 0.23^{f}
R-7	10.06 ± 0.04^{g}	268.42 ± 0.06^{g}	$1.55\pm0.05^{c,f,i}$	$117.11\pm0.77^{a,b,c}$	213.74 ± 0.60^{g}
R-8	10.57 ± 0.10^{b}	291.06 ± 0.01^{h}	1.82 ± 0.04^{g}	$121.37\pm0.06^{a,,b,c}$	234.79 ± 0.29^{h}
R-9	13.68 ± 0.03^{c}	181.75 ± 0.02^{i}	1.49 ± 0.02^{c}	$96.74\pm0.33^{a,b,c}$	124.95 ± 0.06^{i}
R-10	11.87 ± 0.06^{h}	238.38 ± 0.41^{j}	$1.70\pm0.01^{a,h,j}$	$115.7\pm0.14^{b,c,d}$	176.50 ± 0.05^{j}
R-11	12.47 ± 0.04^{i}	275.49 ± 0.06^{k}	$1.65\pm0.04^{d,i,j}$	$124.73\pm0.06^{a,b,c}$	204.59 ± 0.07^{e}
R-12	9.57 ± 0.02^{f}	200.38 ± 0.06^{l}	1.47 ± 0.04^{c}	103.51 ± 0.03^{c}	157.38 ± 0.06^{k}
R-13	13.03 ± 0.06^{j}	399.41 ± 0.03^{m}	1.35 ± 0.01^{b}	$142.26\pm0.02^{a,d,e}$	321.29 ± 0.02^{l}
R-14	12.07 ± 0.02^{a}	177.07 ± 0.02^{n}	1.03 ± 0.01^{k}	$77.14\pm0.01^{b,c,e}$	132.55 ± 0.01^{m}
R-15	13.49 ± 0.03^{k}	296.78 ± 0.18^{o}	1.54 ± 0.01^{c}	$131.10\pm0.11^{a,b,c}$	243.36 ± 0.07^{n}
R-16	11.50 ± 0.05^{l}	336.83 ± 0.05^{p}	1.47 ± 0.02^{c}	$132.29\pm0.06^{a,b,c}$	321.40 ± 0.05^{l}

*Values are given as Mean ± SD of triplicate run.

Values in the same column and not sharing the same superscript are significantly different at $p< 0.05$.

The total Iron content of the formulated products ranges from 9.48-21.28 mg/100g. The composition of Calcium, Zinc, Magnesium and Sodium of the formulated products ranges from 177.07-399.41 mg/100g; 1.03-2.09 mg/100g; 77.14-167.99 mg/100g and 124.95-369.9 mg/100g respectively.

The composition of Calcium tends to increase as the percentage of whey component increases as shown by the contour surface plot for Ca, (Figure 4-7), and 3D surface plot, (Figure 4-8).

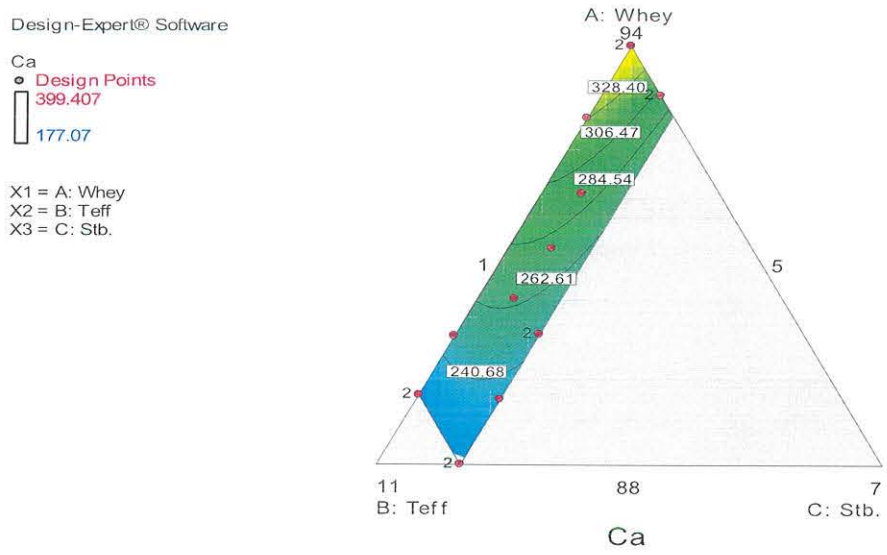

Figure 4-7. Contour plot for Ca

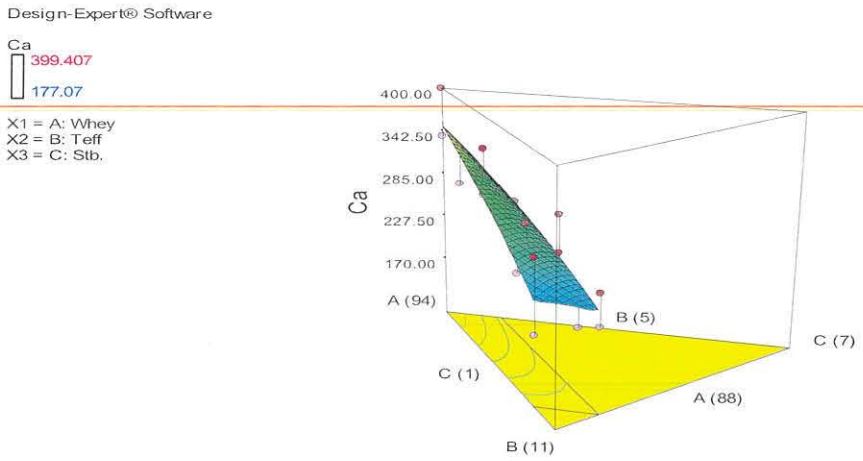

Figure 4-8. 3D plot for Ca

4.3.Sensory Evaluation

Sensory evaluation was performed for two treatments; Tr-2 and Tr-13, which showed good consistency across the storage days. Artificial flavours; Vanilla (1ml), Mango (0.5ml) and plain flavour; artificial sweetener of 144 mg (8 tablets) was added to each product, except a food color (yellow, 150µl) was added only to a product with mango flavour. A total of 6 separate products, three for each treatment, were evaluated by 30 consumer panelists as presented in(Figure 4-9) below.

Trial-2 **Tria-13**

Figure 4-9. Product presentation for sensory evaluation

Table 4-6. Sensory evaluation of the two selected treatments (n=30)

Brand	Code	Color	Taste	Flavor	Acceptability
T21	T2-Vanilla	6.8±1.24[a]	6.83±1.21[a]	7±1.20[a]	7±0.96[a]
T22	T2-Mango	6.7±1.91[a]	6.23±1.55[b]	6.23±1.77[b]	6.2±1.70[b]
T23	T2-Plain	6.43±1.38[b]	6.23±1.72[b]	6.2±1.49[b]	6.5±1.43[b]
T131	T13-Vanilla	6.9±0.92[a]	7.2±0.75[a]	7.1±0.78[a]	7.1±0.71[a]
T132	T13-Mango	7.1±1.14[a]	6.5±1.79[b]	6.23±1.94[b]	6.4±.57[b]
T133	T13-Plain	6.4±1.49[b]	6.4±1.45[b]	6.23±1.87[b]	6.33±1.45[b]

Values are given as Mean ± SD.

Values in the same column and not sharing the same superscript are significantly different at $p < 0.05$.

From the sensory evaluation carried out (Table 4-6),the mean value for color attribute is 6.64 & 6.80, for Trial-2 and Trial-13 respectively. There is significant difference, at $p<0.05$, between color preference of plain flavored product both with mango and vanilla flavored products of the two treatments. Taste has mean value of 6.43 (Trial-2) and 6.70 (Trial-13). Significant difference in taste, was exhibited between vanilla flavored products both with mango and plain flavored products of the two treatments at $p<0.05$.

Flavour has mean value of 6.48 and 6.52 for Trial-2 and Trial-13 respectively. Significant difference in flavor preference was observed between vanilla flavored products, with mango and plain flavored products of both treatments. The overall mean acceptability of the two treatments were 6.56 (Trial-2) and 6.61 (Trial-13). Products with vanilla flavors of the two treatments were preferred the most with mean value of 7.05; plain flavored products ranked second with mean value of 6.42 and mango flavored products had the least overall acceptability with mean value of 6.30.

4.4.Shelf stability, pH and Viscosity determination

The shelf stability of the selected consistent formulated products was determined by comparing their microbiological quality by storing at ambient (~22.3 °C) and refrigerator (4°C) temperatures for seven days.

The pH value of cheese whey and the final products were evaluated. As a result, whey had a mean pH value of 4.64 at 22.3 °C. The pH value of whey 4.64 was slightly lower than the expected pH for sweet whey, which is in the range of 5.8-6.6 proposed by Bund,(2005). This might result from the technological applications employed by the dairy industries to separate casein from whole milk and type of cheese produced; a practice that highly impacts the pH values of whey produced Fox *et.al,* (2017).

However, the mean pH value of the final product was 5.5 at the same temperature. The pH value of the final product, therefore, indicates a slightly acidic medium which creates unconducive environment for bacterial growth, which was witnessed by the lower count of microbial analysis. From the microbial results obtained, it can be inferred that both trials are free from *yeast and mold, S.aureus and Enterobacteriaceae count*. However, they have accounted lower counts of aerobic bacterial count, which slightly increases across the days.

The products stored at refrigerator temperatures have exhibited synereses on the third day. Hence, the test was halted and only early day counts were considered, which showed similar bacterial count with products stored at room temperature, (Table 4-7).

Table 4-7. Microbiological quality of selected trials across the days (CFU/g)

Product code	Test parameter	Storage Temperature	Test Date						
			Day-1	Day-2	Day-3	Day-4	Day-5	Day-6	Day-7
Tr-2	Aerobic bacterial count	Room	180	160	160	180	210	210	220
		Refrigerator	180	160	NA	NA	NA	NA	NA
	Yeast and Mold count	Room	<10	<10	<10	<10	<10	<10	<10
		Refrigerator	<10	<10	NA	NA	NA	NA	NA
	S.aureus	Room	<10	<10	<10	<10	<10	<10	<10
		Refrigerator	<10	<10	NA	NA	NA	NA	NA
	Enterobacteriaceae	Room	<10	<10	<10	<10	<10	<10	<10
		Refrigerator	<10	<10	NA	NA	NA	NA	NA
Tr-13	Aerobic bacterial count	Refrigerator	<10	<10	NA	NA	NA	NA	NA
		Room	180	160	160	180	210	220	220
Tr-13	Yeast and Mold count	Room	<10	<10	<10	<10	<10	<10	<10
		Refrigerator	<10	<10	NA	NA	NA	NA	NA
	S.aureus	Room	<10	<10	<10	<10	<10	<10	<10
		Refrigerator	<10	<10	NA	NA	NA	NA	NA
	Enterobacteriaceae	Room	<10	<10	<10	<10	<10	<10	<10
		Refrigerator	<10	<10	NA	NA	NA	NA	NA

<10Cfu/g implies no typical colony growth is observed.
NA implies the test was not performed on the specified dates.

4.5. Viscosity of the formulated product

The viscosity of the formulated product was measured across the seven days of the proposed storage period, which is stated in(Table 4.8).The viscosity of the formulated products ranges from 24.32 and 99.99cP. From the viscosity measurement, it was witnessed that most of the formulated products tend to gelatinize upon storage which could have resulted from the interaction of pectin with whey protein; whey protein-protein complexes and starch gelatinization Tester & Morrison, (1990). Therefore, only few trails were able to show good consistency and remained viscous up to the proposed days of storage.

There was no reference measurement found for similar product to bench mark the viscosity of the current formulated products. The minimum and maximum viscosity measurements, 24.32 and 99.99 cP., respectively at 100 rpm., on the basis of the actual viscosity measures and product consistency were taken for granted. Accordingly, trials within this range were evaluated to select the best consistent product using Design Expert software.

Table 4.8. Measurement of viscosity (cP)

Trial	Spindle No.	Viscosity measurement (cP)
R-1	64	92.05±3.14
R-2	63	61.00±2.12
R-3	64	97.85±2.0
R-4	64	94.95±3.0
R-5	64	99.99±0.2
R-6	63	71.53±0.23
R-7	63	86.34±0.21
R-8	63	73.25±7.7
R-9	64	99.99±0.1
R-10	64	97.89±0.1
R-11	64	99.99±0.2
R-12	64	99.99±0.3
R-13	63	24.32±2.2
R-14	64	98.76±1.4
R-15	64	88.44±3.2
R-16	64	35.86±1.4
Values are given as Mean ± SD of triplicate measurement.		

Among the formulated trials, Trial numbers Tr-2 and Tr-13 exhibited a consistent flow across the period of storage. The viscosities of the two trials are discussed in (Table 4-9).Optimization for viscosity of the formulated products within the minimum and maximum range resulted in four possible solution each with desirability value of 1. As a result, the best viscous products are obtained when whey is in the range of 93.13 -94 %; Teff flour in the range of 5.0-5.87 % and stabilizer at its lowest percentage, which is 1%.

Table 4-9. Viscosity of selected trials (cP)

Trial code	**Viscosity (cP) at 100rpm**
Trial-2	61.00±2.12
Trial-13	24.32±2.2

As can be seen from the contour surface plot, (Figure 4-10), and 3D plot, (Figure 4-11), respectively, the viscosity of the formulated products increases as the ration of teff decreases and whey composition increases. The more consistent product formulations, hence, were achieved with minimum teff and higher whey compositions.

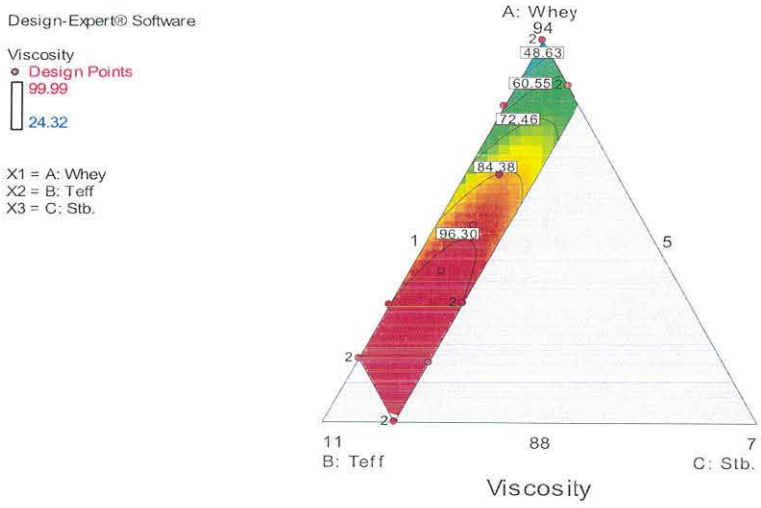

Figure 4-10. Contour plot for viscosity of formulate products

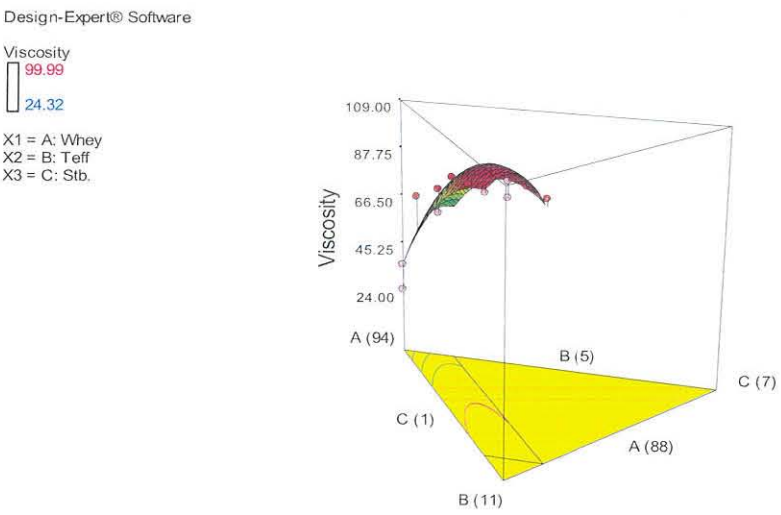

Figure 4-11. 3D plot for viscosity of formulated products

4.6. Nutrition Fact of the final product

The final product has a total volume of 282 g. Based on a 2000 calorie diet, designed for adults and children over 4 years of age. Nutritional provision of ≥ 20% is considered high or excellent, 10-19% good and <5% low based on a 2000 calorie diet Whitney & Ellie, (2008). The designed product (Trial-13) can provide 8.3%, 2%, 1% and 4% of the recommended daily allowance of carbohydrate, protein, fat and fiber, respectively. Moreover, the mineral composition of the formulated product accounts Ca (21.1%), Iron (22.2%), Zn (1%), Mg (12.3%), and Na (4.1%) of the recommended daily values.

Based on the above assumption, the product is an excellent source of Energy, and Iron; good source of Carbohydrate, Calcium and Magnesium; and provides low amounts of fat, protein and fiber. The low percentage composition of fat, however, could be considered good from healthy nutritional diet point of view. In general, the product could be consumed by all age groups excluding infants, and can provide a considerable amount of macro and micro nutrients.

Nutrition Facts/ የምግብ ይዘት		
Serving size	**1 1/2 cup (282g)**	
Total Calorie 130%		**Daily Value (%)*** **እለታዊ ስሌት(%)***
Total fat /የቅባት መጠን	0.5g(ግ)	1%
Carbohydrate/ ካርቦሃይድሬት	25g(ግ)	8.3%
Dietary Fibre/ፋይበር	1 g(ግ)	4%
Protein/ፕሮቲን	3.2g(ግ)	2%
Calcium/ ካልሲየም	121mg (ሚ.ግ)	12.1%
Iron/ብረት	4mg(ሚ.ግ)	22.2%
Zinc/ ዚንክ	0.4 mg(ሚ.ግ)	1%
Magnesium/ ማግኒዝየም	43.2mg(ሚ.ግ)	12.3%
Sodium/ ሶድየም	98mg (ሚ.ግ)	4.1%

*Percent daily values are based on a 2000 calorie diet
Ingredients: Cheese whey, Teff flour, Pectin, artificial sweetener and vanilla
ግብአት፡አጓት፣ጤፍ፣ፔክቲን፣ሰው ሰራሽ ማጣፈጫ፣ቫኒላ

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATION

5.1. CONCLUSION

The principal objective of this research was to design and develop a product with sound consistency and optimized nutritional composition from liquid whey, teff flour and pectin as a stabilizer.

With the help of Design Expert software, it was found out all sixteen trials were of considerable proximate and mineral composition. The optimum protein, fat and ash composition was achieved when higher percentage of whey was added to the formulations. However, the fiber content of teff flour contributes to the advantage of obtaining higher carbohydrate values when teff is at higher composition than whey.

The overall mineral composition of the final product is prominent. The tendency of the majority of the mineral composition does not show significant inclination towards any of the two main contributors, Whey and teff flour. However, calcium composition at large and sodium slightly tend to increase when whey is at higher percentage of the formulations.

Although pectin has considerable nutritional advantage, in addition to being used as a stabilizer, in this research, however, its nutritional contribution to the formulated product is insignificant since it was added in very low amount. Despite the nutritional composition of the formulated products, the viscosity of the product nature is the determining factor. To this end, among the sixteen trials made, only two trials were found to have best consistency.

Shelf stability of the product has showed that it stays consistently over a period of seven days at room temperature. However, at refrigeration temperature, the product exhibited synereses at early days of storage. Microbiological quality of the products during the storage period showed no significant increase and pathogenic microbes were not detected owing to the product's lower pH value. However, the product developed an off-odor that resulted from various flavour components contained by whey.

In order to increase the acceptability of the formulated product, artificial flavour and sweetener has played a vital role. Product with vanilla flavour has gained more

acceptability over the formulations with mango and plain flavour. Food color (yellow) was added to a product with mango flavour which was not preferred by most of the consumer panelists.

As the major outcome of this research; two consistent products with considerable nutritional value, of protein 9.17-10.45 g/100g; fat 0.94-1.58; carbohydrate 81.84-83.42 g/100g and energy value of 378.82-380.99 Kcal was obtained in addition to their apparent mineral composition. The final product was packed in a glass bottle with 282 gram and a serving can provide good deal of nutrients.

5.2. RECOMMENDATIONS

- The current research and previous studies depict the advantages of using whey in the formulations of valuable nutritious food from dairy by-product which could have otherwise been discarded. Hence, the formulated products are of good nutritional composition.
- The current research could also be an insight for the dairy industries to see the possibilities of utilizing whey in the formulations of various functional products. Therefore, these industries need to consider it as an opportunity for creating a side business and generating additional income.
- Whey could also be used for encapsulation of other vital minerals and vitamins. Hence, formulations of other functional products both for the food industries and pharmaceutical applications shall be considered.
- Commercializing such functional products could benefit consumers of all age group, owing to its considerable nutritional, sensory attributes and overall acceptability. However, further studies shall be made to improve its sensory, flavor and shelf life.
- The use of liquid cheese whey resulted in the development of an off-flavor due to various volatile aromatic compounds contained by whey. This would have been avoided if it was made in to powder, if not for its cost implication.
- The viscosity of the products needs further modification with the use of different formulations and modifications of the ingredients.

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ANNEXES

Annex-1: Ballot for Sensory Evaluation of Whey-Teff beverage product (English Version)

BALLOT FOR SENSORY EVALUATION OF TEFF-WHEY BEVERAGE PRODUCT

(9 scale hedonic test)

Name: _____ Sex: _____Age: _____Code: _____

Date: _____ Judge No._____

Instruction:

1. Please taste and evaluate each sample in the given order, from left to right, according to the scale provide and write the corresponding score that best reflects your feelings about the sample. Choose the descriptor which, in your opinion, is the most applicable to the characteristics being evaluated. Please rinse your mouth with water while testing each sample.

   Thank you in advance, for voluntarily participating in this sensory evaluation.

Score	Color	Taste	Flavor	Overall acceptability
Like extremely(9)				
Like very much(8)				
Like moderately(7)				
Like slightly (6)				
Neither like nor dislike(5)				
Dislike slightly(4)				
Dislike moderately(3)				
Dislike very much(2)				
Dislike extremely(1)				

Annex-2: Ballot for Sensory Evaluation of Whey-Teff beverage product (Amharic Version)

ከአጓት እና ከጤፍ የተሰራ የምግብ ናሙና ጣዕም እና ተቀባይነት መገምገሚያ ቅፅ

ስም፤_____ እድሜ፤_____ የናሙና መለያ ቁ፤ _____

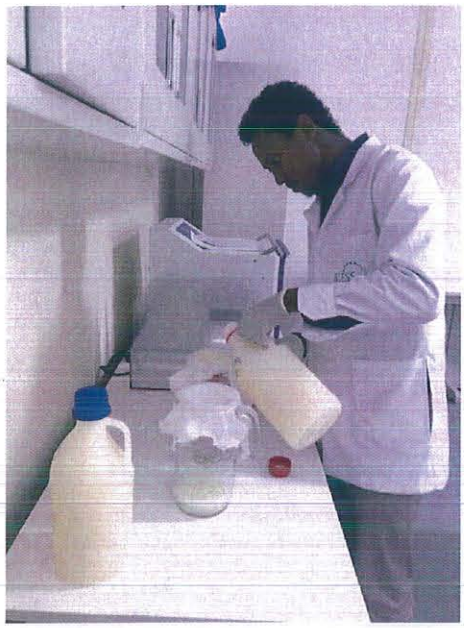
ቀን፤_____ የገምጋሚው ተ.ቁ፤ _____

መመሪያ፤

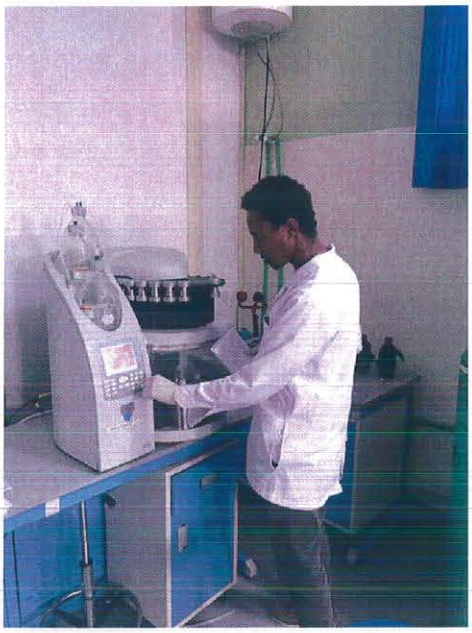
እባክዎ የተዘጋጁትን የምግብ ናሙናዎች በተቀመጠው ቅደም ተከተል መሰረት ከግራ ወደ ቀኝ ይቅመሷቸው። የምግብ ናሙናዎቹን በተጠቀሰው መስፈርት መሰረት ገምግመው በትክክል ገላጭ ይሆናል የሚሉትን የምግብ ባህሪ ይግለጹ። አንዱን የምግብ ናሙና ከቀመሱ በኃላ አፍዎን በውሃ ተጉመጥምጠው የሚቀጥለውን የምግብ ናሙና ይቅመሱ። በዚህ የምግብ ናሙና ቅምሻ ላይ ፍቃደኛ ሆነው በመሳተፍዎ በቅድሚያ እናመሰግናለን።

የመለኪያ መስፈርት	ቀለም	ጣዕም	ቃና	ተቀባይነት
እጅግ በጣም ወድጄዋለሁ(9)				
በጣም ወድጄዋለሁ(8)				
በመጠኑ ወድጄዋለሁ(7)				
በትንሹ ወድጄዋለሁ(6)				
አልወደድኩትም አልጠላሁትም(5)				
በትንሹ ጠልቼዋለሁ(4)				
በመጠኑ ጠልቼዋለሁ(3)				
በጣም ጠልቼዋለሁ(2)				
እጅግ በጣም ጠልቼዋለሁ(1)				

Annex 3: Pictorial representation of some research activities (BLESS Agri-food laboratory Services PLC)

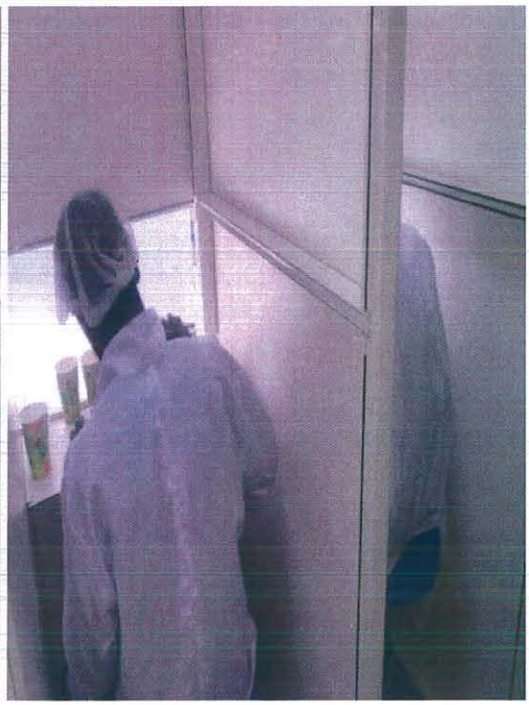


Straining of whey with muslin cloth



Fat analysis with ASE system

Sensory booth



Sensory analysis

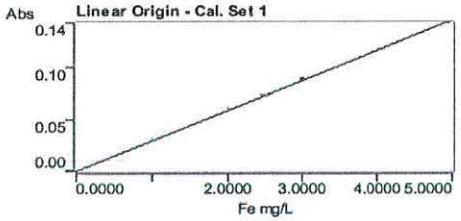
Annex 4: Calibration curve for mineral analysis

SpectraAA Report.

Methods Fe
Computer name BLESS-0-HP
Serial Number: MY14330003

Method: Fe (Flame)

Sample ID	Conc mg/L	Mean Abs
CAL ZERO	0.0000	-0.0005
STANDARD 1	1.0000	0.0314
STANDARD 2	2.0000e	0.0602e
STANDARD 3	3.0000	0.0871
STANDARD 4	4.0000	0.1152
STANDARD 5	5.0000	0.1417

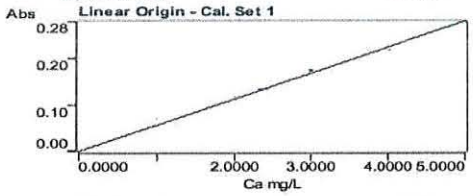


CCV 2.5 ppm Fe	2.4892	0.0716
MBLK	-0.0089m	-0.0003

Methods Ca
Computer name BLESS-0-HP
Serial Number: MY14330003

Method: Ca (Flame)

Sample ID	Conc mg/L	Mean Abs
CAL ZERO	0.0000	0.0000
STANDARD 1	1.0000	0.0681
STANDARD 2	2.0000	0.1151
STANDARD 3	3.0000	0.1727
STANDARD 4	4.0000	0.2166
STANDARD 5	5.0000	0.2794

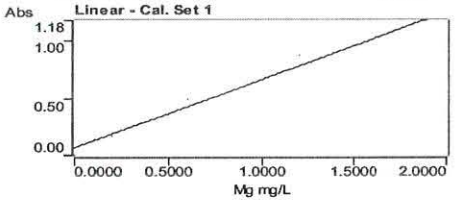


CCV 2.5ppm Ca	2.5612	0.1328
MBK	0.0093	0.0005

Methods Mg
Computer name BLESS-0-HP
Serial Number: MY14330003

Method: Mg (Flame)

Sample ID	Conc mg/L	Mean Abs
CAL ZERO	0.0000	0.0015
STANDARD 1	0.2000	0.1696
STANDARD 2	0.4000	0.3074
STANDARD 3	0.6000	0.4896e
STANDARD 4	1.2000	0.8670
STANDARD 5	2.0000	1.1799

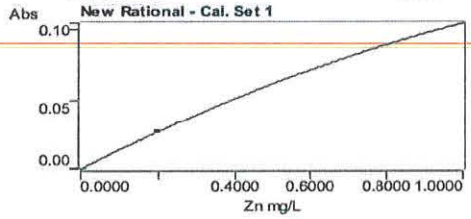


MBLK	<0.0500	0.0050
QC (0.5 ppm Mg)	0.5086	0.3696

Methods Zn
Computer name BLESS-0-HP
Serial Number:

Method: Zn (Flame)

Sample ID	Conc mg/L	Mean Abs
CAL ZERO	0.0000	-0.0003
STANDARD 1	0.2000	0.0271
STANDARD 2	0.4000	0.0498
STANDARD 3	0.6000	0.0704
STANDARD 4	0.8000	0.0882
STANDARD 5	1.0000	0.1046

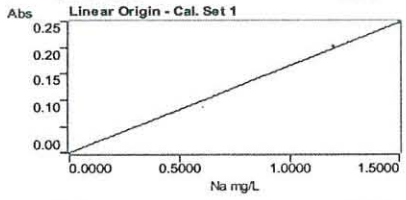


MBLK 5	0.0030	0.0004
CCV (0.5ppm Zn)	0.4973	0.0607

Methods Na
Computer name BLESS-0-HP
Serial Number: MY14330003

Method: Na (Flame)

Sample ID	Conc mg/L	Mean Abs
CAL ZERO	0.0000	0.0111e
STANDARD 1	-----	-----e
STANDARD 2	0.6000	0.0859e
STANDARD 3	0.9000	0.1472e
STANDARD 4	1.2000	0.2002e
STANDARD 5	1.5000	0.2496e



MBLK	-0.2849	-0.0468
CCV(0.8ppm Na)	0.7400	0.1216