بسم الله الرحمن الرحيم

Sudan University of Science and Technology

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Production of beverage from whey and Karkade (*Hibiscus sabdariffa*)

إنتاج مشروب من مصل(شرش) اللبن والكركدي

A Dissertation Submitted in Partial Fulfillment for the Requirements of B. Sc (Honor) Degree in Food Science and Technology

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الآية

بسم الله الرحمن الرحيم

قـال تـعـالـى:

﴿ إِنَّ اللَّهَ يَأْمُرُ بِالْعَدْلِ وَالإِحْسَانِ وَإِيتَاءِ ذِي الْقُرْبَى وَيَنْهَى عَنْ الْفَحْشَاءِ وَالْمُنكَرِ وَالْبَغْيِ يَعِظُكُمْ لَعَلَّكُمْ تَذَكَّرُونَ﴾

سورة النحل

الاية (90)

DEDICATION

To My:

Family,

Teachers and Friends.

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With all due humbleness and gratitude I render ultimate thanks and special praise to GOD (Almighty) who gave me health, power and patience to accomplish and conduct this work.

My deepest thanks and appreciation are genuinely expressed to my supervisor Prof. Ahmed Al-awad Al-faki for his continuous assistance, valuable advices, patience and encouragement through the course of this study.

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Abstract

The aim of this study was to make use of whey of white cheese as beverage and make it palatable. The beverage was formulated from different levels of whey and extract of Karkade (*Hibiscus sabdariffa*), karkade:whey (10:90, 15:85, 20:80, 0:100) exhort combined health benefits to consumer like neutraceutical compound, anthocyanin. Sugar were added to improve the taste of beverage, citric acid was used to hydrolysis lactose to obtain (glucose-glactuose) to increase the sweetness of beverage. Beverage was subjected to sensory evaluation and found that according to the overall acceptability sample B (15:85) was more acceptable than other treated samples. Extract of karkade 15% was optimum to prepare suitable beverage from whey, slightly increase in protein from 0.73% to 0.87%. Whereas there was observed increase in total solids from 4.7% to 16.3%, with decrease of moisture content from 88.16% to 77.3%. whey beverage Was packed in plastic bottles were storied at 4℃. Ideal pH for the whey beverage was found to be 3.8, through storage period for month there was a decrease at pH to 2.5. According to above results whey with karkade is recommended as the beverage for human consumption.

ملخص الدراسة

الهدف من هذه الدراسة هوتحقيق الإستفادة من مصل لبن الجبن الابيض كمشروب وجعله مستساغ، تم تحضير مشروب مصل اللبن بنسب مختلفة من مصل اللبن و مستخلص الكركدي(كركدي:مصل اللبن) (20:80,15:85,10:90,0:100, لحشد الفوائد الصحية للمستهلك مثل مركبات النيوتريسيتك والانثوسيانين. تمت اضافة السكر لتحسين طعم المشروب، وحامض الستريك أستخدم لتحلل اللاكتوز المائي للحصول على (الجلوكوز- الجلاكتوز) لزيادة حلاوة المشروب.تم إجراء تقييم حسي للمشروب،بناءآ على التقييم الكلي وجد ان العينة (ب) كانت أكثر قابلية من العينات المعاملة الاخري. ووجد أن نسبة 15% من مستخلص الكركدي كانت الأمثل لتحضير مشروب مناسب من مصل اللبن وبالتالي أدت الى زيادة طفيفة في نسبة البروتين حيث كانت 0.73%في ماء الشرش واصبحت 0.87%في مشروب مصل اللبن، في حين وجدت زيادة ملحوظة في نسبة الجوامد الصلبة الكلية حيث كانت 4.7% واصبحت 16.3%، مع انخفاض محتوى الرطوبة من88.16% الى 77.3%. تم تعبئة مشروب مصل اللبن في عبوات بلاستيكية وتم تخزينهاعند درجة حرارة 4°C. كان ال pH المثالي للمشروب 3.8. حيث انخفض ال pH خلال فترة التخزين لمدة شهر الى 2.5. بناءآ على النتائج أعلاه مصل اللبن مع الكركدي يوصى به كمشروب للاستهلاك الادمي.

CHAPTER ONE

1. INTRODUCTION

Whey is greenish-yellow aqueous portion of milk when coagulum is separated during manufacture of cheese, paneer, chhana, and casein etc., representing 80-90%volume of transformed milk (Moreno-indias, *et al.*, 2009). Use of whey in nature is limited although it is considered an important nutritional source of proteins and lactose. The biological oxygen demand (BOD) of whey is very high (40,000-50,000 mg/kg), constituting a major ecological burden if disposed off as a waste material (Dabur and Brahm Prakash, 2007).

Whey beverages have been recognized as a genuine thirst quencher, light, refreshing, healthful and nutritious. Whey based fruits beverages are more suitable for health as compared to other drinks. Whey and its biological components have proven its effects in treatments of cervical chronic diseases like cancer, cardiovascular etc. It is nutritionally rich hence, it can also be used in beverages, infant, geriatric and athletic food (Devraj 2005).

Besides that, addition of many other fruits like concentrates of apple, pear, peach, apricot and cherry has also been applied. The additions of berries which are known as a good source of iron and antioxidants have proved to be very useful. That is especially important in production of whey beverages with improved nutritional value (Miglioranza *et al*., 2003).

Hibiscus sabdiriffa var. sabdariffa, commonly known as hibiscus or roselle, grows in many tropical and sub-tropical countries and is one of highest volume specialty botanical products in international commerce.(FAO,Anne ,2004). Even though, the uses of different parts of

Roselle are many and varied both in food and in traditional medicine, all parts of Roselle including seeds, leaves, fruits and roots are used as a food in different parts of the world. Fleshy red calyxes of Roselle are commonly used for the production of soft drinks and tonic without alcohol like wine, juice, jam, jelly, syrup and also dried and brewed into tea and spice. These are rich in carotene, riboflavin, anthocyanins, ascorbic acid, niacin, calcium, iron and vitamin C. The

young leaves and tender stems of Roselle are consumed raw as green vegetable.(Mahadevan, *et al*.,2009).

The world's best roselle comes from the Sudan, but the quantity is low and poor processing hampers quality. Virtually all of Sudan's production is exported to Germany. (FAO,Anne ,2004). so that whey utilization in many industrial at India utilize in dairy industrial to meet the consumer requirement using whey as water to replacer without much change in the compostion . Also in Switzerland developed the one most successful beverage called (Rivella). But in sudan cannot utilize the whey in any industrial rether than it disposed at emptiness. So that must use to formulation the beverage, research has been taken up with the fallowing objectives.

General objective:

The main goal of this study is to develop stable whey kharkadia beverage from whey of white cheese.

Specific objectives:

1-To study the physicochemical and sensory characteristics of kharkadia whey beverage.

2-Tostudy the shelf stability of Karkade blended whey beverage.

3-To study the effect of roselle on nutritional value of whey.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. What is whey actually?

Whey is a by-product obtained from cheese manufacture Depending on the type of casein coagulation, whey can be sweet or acid Composition and properties of whey mainly depend on the technology of cheese manufacture and on the quality of milk used for cheese production (Tratnik, 1998).

2.1.1. Composition of the whey:

There is approximately 3.6% protein in liquid bovine milk. This protein fraction is composed of 20% whey and 80% casein. Whey is the liquid substance obtained by separating the coagulum from milk or cream in cheese making (Madureira, et al., 2007). In its raw liquid form, whey is composed of naturally occurring macronutrients-i.e., water (93%) protein (0.8%), fat (0.3%), lactose (4.8%) and minerals-referred to as ash (0.5 %). Whey is the collection of globular proteins isolated from the liquid fraction (micronutrients) that are biologically active- beta (β) - lactoglobulin, alpha (α)-lactalbumin, glycomacropeptide, bovine serum albumin, immunoglobulins, lactoferrine and lactoperoxidase.

Whey, by definition from 21 U.S. Code of Federal Regulations (CFR) §184.1979a, is the liquid substances obtained by separating the coagulum from the milk or cream in cheese making (Farrell, *et al*., 2004). The milk is often standardized before cheese making in order to optimize the protein (casein) to fat ratio.

This is accomplished by adding protein solids (i.e., condensed skim milk and non-fat dry milk NFDM) to the standardized cheese milk (SCM) in order to improve cheese (compositional) quality and production yields. Rennet (animal –derived) or chymosin preparation (fermentation-derived) 21 CFR § 184.1685 calcium chloride (21 CFR184.1193), and dairy cultures are added to the SCM. These nonorganic substances are allowed as ingredients in or on processed products labeled as organic (Kang, *et al*., 2010). The casein coagulates in the presence of rennet or (lactic) Acid to form the cheese curd. The pH of the cheese milk drops from 6.7 to 5.3 which causes the casein protein to coagulate and form a curd.

The curd traps most of the lactose, fat, and ash. The liquid whey protein that remains is further processed into a variety of commercial ingredients from dried whey (13 percent protein) to whey protein concentrates (25 to 89 percent protein) and whey protein isolates (greater than 90 percent protein).

One pound of cheese produces nine pounds of liquid whey protein. In high moisture fresh cheeses such as cottage cheese (where a portion of the original raw milk is returned to the cheese as cream dressing) the ratio may be as low as 6:1 (Burrington, 2012., Etzel, 2004., Brown, 2014., and Wastrel *et al*., 1999).

Cording to its average composition whey is approximately 93% water and contains about 50% of total solids present in the milk of which lactose is the main constituent Whey proteins constitute less than 1% of dry matter (Beucler *et al*., 2005). Minerals and milk fat are also present but in less amounts, however whey composition is very variable and significantly depends on the technology of whey production.

However, in processes of traditional cheese manufacture, regardless of casein coagulation process, almost all whey proteins cross into the whey due to their insensitivity to enzyme activity and acids (Tratnik, 1998). Thereat whey proteins are the constituents which put whey into spotlight on the diary products market.

Whey proteins include several thermosensitive fractions like ß-lactoglobulin, α-lactalbumin, bovine serum albumine, immunoglobulins and termostable fraction of proteose peptones. Due to the high content of essential aminoacids (notably lysine, cysteine and methionine) and cystin, whey proteins are one of nutritionally most valuable proteins.

Due to such aminoacid composition whey proteins have much higher biological value (but also other parameters that determine nutritional value) in comparison with casein or other proteins of animal origin, including egg proteins which have been considered for a long time as referent proteins.

Protein utilization in human organism is tightly related to cystine/methionine ratio which is about 10 times higher in whey proteins than in casein. Therefore, it is not surprising that thermally denatured lactalbumns are being almost totally (100%) absorbed in the digestive

system while this ratio is significantly lower (about 75%) regarding casein absorption.

Whey protein content is similar in sweet and acid whey. Nevertheless, it is important to mention free aminoacid content which varies a lot and is mostly dependant on the level of casein hydrolysis in cheese making process. Thereby, free aminoacid content is about 4 times higher in sweet whey and about 10 times higher in acid

Whey than in milk (Tratnik, 1998). Besides that, whey proteins have excellent functional properties like good solubility, good viscosity, good emulsifying and gelation abilities. Therefore whey protein concentrates are largely used in food industry. Due to the fact that whey proteins have much higher digestibility than casein, they are often used in production of infant formulas and to improve the nutritional value not only of dairy products but also of many other food products. Also, it is important to mention immunoglobulins and other glycoproteins (lactoferrine, transferrine) and enzymes (lysozime, lactoperoxidase) which are very notable factors of immunactive system of whey. They have antimicrobial properties and have the ability to reduce or even inhibit allergic reactions (Tratnik, 2003).

However, lactose is the main constituent (about 70%) of the whey dry matter and is a very important source of energy, but it has several roles. Some of beneficial effects of lactose are stimulation of peristaltic activities in the digestive tract, alleviation of calcium and phosphor absorption, establishment of lightly acid reaction in the gut which inhibits the growth and expansion of pathogens. Furthermore, lactose also assures the optimal amount of magnesium and improves digestion of milk fat and other nutrients in human organism and it does not participate in plaque formation. Heat treatments of whey cause transformation of certain amount of lactose into lactulose which is bifidobacteria growth promoter (Tratnik, 2003).

Water soluble vitamins present in the milk also pass into the whey, but their amounts are very variable and highly dependent on the storage conditions of whey. Thereby riboflavin, folic acid and cobalamine are present in significant amounts. The latter ones are bounded to whey proteins and mostly remain in whey after cheese manufacture. It is interesting that they can contain higher amounts of riboflavin than milk, due to the activity of some lactic acid bacteria used in cheese manufacture. Due to relatively high content of riboflavin, whey has a characteristic yellow-green color (Popović-Vranješ and Vujičić, 1997; Tratnik, 1998).

Mineral composition in whey dry matter is the variable (7-12%) and depends on the technological process of cheese production (Popović-Vranješ and Vujičić, 1997).

Whey contains almost all soluble salts and microelements present in the milk, but also salts added in the cheese manufacture process Thereby calcium and phosphates are partially remaining bounded in the case in contained in cheese, and their contents are much higher in acid whey because of much higher solubility in acid medium (Tratnik, 1998).

2.1.2. Whey proteins:

The major proteins in whey are β-lactoglobulin (β-lg), α-lactalbumin (α-La), bovine serum albumin (BSA), and immunoglobulin (Ig). Whey contains low concentration of other proteins such as lactoferrin, and enzymes such as lysozyme, lipase and xanthine oxidase. The most abundant whey proteins are β-lg which represents about 50% of the total whey proteins in bovine milk. β- lg plays a key role in any processing operation (Swaisgood, 1982). At the pH of the milk (pH 6.7) β-lg is a dimer (36.7 kDa) due to electrostatic interactions between amino acids Asp130 and Glu134 of one monomer with the corresponding lysyl residues of the other monomer. At pH values below 3.5 and above 7.5 the protein forms monomers. In the pH 3.5-5.2 an octamer is formed (147 kDa). Most of the tertiary structure of β-lg is maintained by a thiol group (residue 119 or 121) and two disulfide bonds (66-160 and 106-119 or 121) (Swaisgood, 1985).

Alpha-lactalbumin is the second most important whey protein and represents about 20% of the serum protein in bovine milk. This protein is a co-enzyme, which plays a role in the final 23 stage of lactose synthesis (Sienkiwicz and Riedel, 1990). A total of 123 amino acids are present in the primary structure of this protein which has a molecular weight of 14,000 Daltons and four disulfide bonds. Bound calcium stabilizes the molecule against irreversible thermal denaturation (Hiraoka and Sugai, 1984). Removal of the calcium leads to the reduction of transition temperature and thermal denaturation and aggregation (Kronman et al., 1981).

2.1.3. Interactions of whey proteins:

Whey proteins can interact with or without heating, with various non-dairy components including pectins, tannins and polyols (Rawel *et al*., 2001). Low methoxyl pectins interact with whey proteins yielding sedimentation. Devkota (1991) found that the presence of both whey protein and pectin in a 2:1 ratio resulted in the production of sediment in

a dispersion containing a mixture of whey and pectin Incompatibility was found for mixtures of native whey protein-nonionic hydrocolloids such as maltodextrins, dextrans and methylcellulose at pH 5 to7 (Syrbe *et al*., 1995).

However, ionic hydrocolloid-whey proteins solutions including high-methoxyl pectin, sodium carboxymethylcellulose or sodium alginate performed differently at pH 6 to7. Unless whey24 proteins were denatured, no polymer segregation occurs in these mixtures. If denatured, phase separation as well as gel formation occurs concurrently. Two types of gels are observed with both anionic and nonionic hydrocolloids (Syrbe *et al*., 1998).

Heat induced gelation of β- lactoglobulin-low-methoxyl pectin solutions is influenced by calcium ions on both sides of the pI (Ndi et al., 1996). At pH 3.5 self-association of low-methoxyl pectin in presence of high levels of calcium was promoted, which prevented formation of insoluble complexes with positively charged β-lactoglobulin. However, at H 6.5 repulsive forces were reduced between protein and polysaccharide, leading to more homogeneous gel structures

2.2. Whey beverage:

In recent two decades numerous patents containing recipes for production of whey beve0rages with addition of fruit concentrates have been registered. Thereby citrus flavoured drinks and drinks with addition of other tropical fruit aromas like mango, banana or papaya have been most frequently suggested, since they have proved to be very efficient in covering up the undesirable odour of cooked and salty-sour flavour of fresh whey (Duric, *et al.,* 2003).

Besides that, addition of many other fruits concentrates of apple, pear, peach, apricot and cherry has also been applied. The additions of berries which are known as a good source of iron and antioxidants have proved to be very useful. That is especially important in production of whey beverages with improved nutritional value. Brazilian group of scientists who have developed awhey drink flavored by addition of strawberry concentrate and fortified with ferrous bisglycinate.

They have proved that long-term consumption of this drink had an impact on reduction in the prevalence of anemia in children and

adolescents (Miglioranza *et al*., 2003). Rice bran has shown to be one of the best choices in this category of flavouring agents since, it has the proper balance of soluble and insoluble dietary fibers, there is almost no sediment formation during storage of rice bran fortified beverages and it does not contain allergy causing proteins. Addition of honey to such beverage instead of sugar or other sweeteners results in fortifying it with numerous other nutrients like vitamins, adding fruits like apples, pears and bananas) is the formation of sediment due to the high amount of fruit dry matter and interactions of proteins with components in fruit dry matter. The sediment arises with time and consequently such whey beverages minerals (Hammond, 1992).

However, the main problem occurring in all these recipes especially when do not perform well on the market. On the other side, if the amount of fruit dry matter is not high enough, the end product does not have good sensory properties like color, flavor and odor (Koffi *et al.*, 2005; Duric *et al*., 2003). 18 Citric acid and diverse sweeteners like fructose, succrose or lactose hydrolysates for adjusting odor and flavor. Nowadays some authors have suggested the addition of CO2 combined with fruit add-ins to overcome the undesirable flavor and odor of beverage (Sherwood and Jenkins, 2007).

2.3. Health importance of whey based beverage:

Whey beverages have been found to contain valuable amounts of calcium and riboflavin. Since, the whey beverages have nutritionally best protein and whey component which make whey beverages superior for consumption by the consumers who have limited intake of milk. It was found that when drinks were manufactured from the whey which had high content of calcium and flooded with additional whey protein than it was seen that there was lactose hydrolysis which became the centre of attraction and ultimately led to the minimization of the lactose intolerance (Jelen *et. al.,* 1987).

(Katsanas *et al.,* 2008) concluded that both the whey proteins and essential amino acids are known for the stimulation of the anabolism of muscle protein. Hence, it was found that whey protein intake helps in improving the skeletal muscle protein accrual by the mechanisms which are beyond the essential amino acid contents, therefore are considered to improve the anabolism of muscle in old individuals. It was observed that

the short term postprandial enriched water beverage though there was not much effect on food intake when assessed two hrs after consumption (Robin, *et al.,* 2013).

2.4. Whey-fruit beverages:

Some success has been achieved in developing beverages fortified with whey. The beverages produced are nutritious (acid whey contains about 120 mg Ca/100ml), refreshing but less acidic than fruit juices, and have a good potential for profit margins (Holsinger *et al*., 1974). RivellaR is one of the most successful beverages developed in Switzerland in 1952 (Anon, 1960). It is a sparkling, crystal clear infusion of herbs in deproteinized whey, promoted as therapeutic tonic. Other beverages that have received attention include BodrostR (Len'kov, 1969), an alcoholic beer-like beverage made in Russia from pasteurized, clarified whey with the addition of sugar and raisins. TaiR from Brazil is a soft drink fortified with a whey protein concentrate to contain 1.5% protein (Anon, 1973). Other whey beverages (from deproteinized whey) sold in Europe includes Big MR, FrusighurtR (Germany) and TaksiR (Holland) (Lang and Lang, 1969). Heat precipitation of the whey proteins by steam injection is commonly used during deproteinization (Sienkiwicz and Riedel, 1990).

Research in the United States has been directed toward utilizing whole whey (to minimize costs) in the form of nutritious whey beverages, both carbonated and non-carbonated 25 (Holsinger *et al*., 1974). The citrus flavored beverages (particularly orange), have received the highest acceptability. The orange-flavored drink contained 33% untreated cottage cheese whey. Citric acid was added to pH 3.6 to overcome the buffering capacity of the whey (Nelson *et al*., 1971). No more than 33% of unmodified whey could be used without some objections being raised about the unpleasant insipid taste of whey. The same author noticed that the choice of the flavor was very important. Passion fruit juice at 2-3% has contributed considerably to the acceptability of whey beverages. Synthetic flavors including raspberry have been used successfully. When using fruits, it is recommended that natural fruits be well ripened. Various fruits including peach puree (20% level), strawberry (10%) and red raspberry (10%) have been used successfully at these levels. Hannigan (1978) developed what he called citrus milk. The beverage was obtained by mixing (85-92%) of 14 Brix orange juice (or 54-57% of 6-14 Brix

grapefruit juice), soluble protein (WPC) to obtain 1-6% protein (3-4% best). Soluble stabilizers in the range of 0.1-1% were added to improve texture, viscosity and to prevent sedimentation during storage. A number of sweeteners are added as well as various extracts (vanilla, citrus essences etc). The author recommends that the viscosity should be at least about 40 cps at 80 oF. LactofruitR, a whey drink developed in Switzerland was made by hydrolyzing deproteinizedwhey to 50% with lactase to increase sweetness and avoid lactose intolerance. The beverage contained 25g/L galactose, 4.5 g/L minerals, 2 g nitrogenous substances, B vitamins and vitamin C

Jelen (1992) reported that the lack of high marketability for whey beverages, when compared to other beverages, was due to poor flavor blends. Whey contains high amounts of lactose and salts which makes it a difficult material to utilize. Various flavor notes including 26 diacetyl, acid, saltiness, astringency, bitterness and sweetness have been found (McGugan *et al*., 1979).

2.5. Roselle:

Hibiscus sabdariffa commonly named as "red sorrel" or "Roselle" is a member of malvaceae family. It is a medicinal plant with a worldwide fame and has more than three hundred species which are distributed in tropical and subtropical regions around the world. Roselle can adapt to a variety of soil in a warmer and more humid climate. Roselle is rich in organic acids including citric, malic, tartaric, and allo-hydroxycitric acids (singh *et al*., 2017).

2.5.1. Description of *Hibiscus sabdariffa*:

Hibiscus has more than three hundred species distributed in tropical and subtropical regions around the world and are used as ornamental plants. Research on have shown that some species of Hibiscus possess certain medicinal properties of which *Hibiscus sabdariffa* is one (Yadong Qi, *et al*., 2005) Hibiscus *sabdariffa* is commonly named as "red sorrel" or "Roselle". Even though permeable soil is the best, Roselle can adapt to a variety of soil in a warmer and more humid climate (Duke JA,1938).

Hibiscus sabdariffa, a member of Malvaceae family, is a known medicinal plant with a worldwide fame (Abbas , *et al*.,2011) and the plant

can be found in almost all warm countries such as India, Saudi Arabia, Malaysia, Indonesia, Thailand, Philippines, Vietnam, Sudan, Egypt and Mexico (Rao ,1996). Roselle is mainly cultivated to be consumed and the main producers of Roselle blossoms are Egypt, Sudan, Mexico, Thailand and China. Other hibiscus varieties are planted for their fibers they produce (Naturland ,2002).

Among numerous verities of Hibiscus, *Hibiscus altissima* and *Hibiscus sabdariffa* are the commonest and better introduced. *Hibiscus altissima* is branchless plant with yellow flowers and red because of its high fiber content. The other distinct type *Hibiscus sabdariffa* or "Roselle" grows in a bush with many branches. The flowers of Roselle are axillaries or in terminal racemes, the petals are white with reddish center at the base of the stamina column and this species is widely used as food (Abu-Tarboush, *et al.*, 1997).

Roselle is an ideal crop for developing countries if market demand is favorable. It is drought tolerant, relatively easy to grow, not suitable for mechanized harvest, labor intensive to process, and can be grown as part of multi-cropping system. In addition to fodder and fiber, it is used for other purposes as well. In China the seeds are used for their oil and the plant is used for medicinal properties, and in West Africa the leaves and powdered seeds are a local foodstuff. It has many other local names including sorrel, l'oiselle (French), Jamaica (Spanish), bissap (Wolof/Senegal) and (dâBambara) among others. In North Africa and the Near East Roselle is called karkade (Arabic), the name also used in the pharmaceutical and food flavoring trade in Europe. Like many specialty botanical products, market information is not readily available for Roselle. Prices and production are not tracked like a conventional agricultural commodity and there are few, if any, published market (FAO, Anne, 2004).

2.5.2. Post-Production Operations (FAO, Anne,2004)

2.5.2.1. Pre-harvest operations:

Roselle is an annual bushy shrub that grows to approximately 2.4 meters in one growing season. While it is relatively easy to grow, it is more difficult to produce consistently high quality. This is a function of seed stock, local growing conditions, harvest time, and postharvest handling, in particular the drying process. Roselle is quite hardy and

grows in most well drained soils. It can tolerate poor soil, and is often grown as a supplemental rather than a primary crop. It requires 4-8 months with nighttime temperatures not below 21oC. In addition, it requires 13 hours of sunlight during the first 4-5 months of growth to prevent premature flowering. Roselle requires a monthly rainfall ranging from 5-10" (130-250 mm) in the first 3-4 months of growth. Dry periods can be withstood and are desirable in the last months of growth. Rain or high humidity during the harvest time and drying can downgrade the quality of the calyces and reduce the yield.

2 .5.2.2.planting:

Hibiscus sabdariffa is very sensitive to changes in the length of day This photoperiodic quality of blooming, when the days become shorter, require the planting time to be set according to the day length and not according to the rainfall requirements. It is a deep-rooted crop, therefore deep plowing is recommended in preparing the seedbed. To produce a large calyx 1,000-2,000 pounds of manure are added per acre (1-2 tonnes/ha). Seeds are planted at a rate of 6-8 pounds or less per acre (6-8 kg/ha) and approximately one inch (2.5 cm) deep. Seeds are usually planted in the spring at the beginning of the rainy season, 2-3 feet (0.60-1 m) between rows and 18-24 inches (45-60 cm) within the rows. The reduced planting rate produces a larger calyx. Sowing can be done by hand, or with a modern grain drill. A good alternative tool would be a corn planter small enough to accommodate the hibiscus seeds. Thinning is done by hand as well. There are over 100 cultivars or seed varieties of *Hibiscus sabdariffa*.

The major commercial and Mali In most other countries, small farmers carry out the bulk of production, but in the Sudan, nomadic goat herders are known to pick the product from semi-wild sources.

2.5.2.3. Growth:

Flowering of the hibiscus is induced as the days become shorter and light intensity reduces. Flowering begins in September or later depending on the country in question, and continues through October or later when the entire field is in bloom. Flowers begin to drop at the end of October or later. Flowers are diurnal and last only a day. The seedpods begin ripening towards the bottom and proceed to the top. In Sudan,

growers will sometimes allow the seed to completely ripen and the leaves drop prior to harvest.

2.5.2.4. Harvesting:

Hibiscus sabdariffa is harvested from late December onwards. The harvest is timed according to the ripeness of the seed. The fleshy calyces are harvested after the flower has dropped but before the seedpod has dried and opened. The more time the capsule remains on the plant after the seeds begin to ripen, the more susceptible the calyx is to sores, sun cracking, and general deterioration in quality. All harvesting is done by hand. Total yield is about half a ton per hectare Special care must be taken during harvesting operation to avoid contamination by extraneous material. At no time should the calyx come in contact with the ground or other dirt surfaces. Clean bags or containers should be used to transport from the field to the drying location. Different harvesting methods are in use today. In Mexico the entire plant is cut down and taken to a nearby location to be stripped of the calyces. In China only ripe calyces are harvested with clippers leaving the stalks and immature calyces to ripen in the field. The field is harvested approximately every ten days until the end of the growing season. The calyx is separated from the seedpod by hand, or by pushing a sharp edged metal tool through the fleshy tissue of the calyx separating it from the seedpod Thailand has perhaps the most sophisticated production system. Growers and collectors are more organized, and during the growing season collectors monitor the crop and estimate the yield for the whole country.

2.5.3. Primary Products (FAO, Anne, 2004)

2.5.3.1. Fresh hibiscus:

Roselle fruits are best prepared for use by washing, then making an incision around the tough base of the calyx below the bracts to free and remove it with the seed capsule attached. The calyces are then ready for immediate use. They may be merely chopped and added to Fruit salads. In Africa, they are frequently cooked as a side-dish eaten with pulverized peanuts. For stewing as sauce or filling for tarts or pies, they may be left intact, if tender, and cooked with sugar. The product will be almost indistinguishable from cranberry sauce in taste and appearance. For making a finer-textured sauce or juice, syrup, jam, marmalade, relish, chutney or jelly, the calyces may be first chopped in a wooden bowl or

passed through a meat grinder. Or the calyces, after cooking, may be pressed through a sieve.

2.5.4. Secondary and derived product (FAO, Anne, 2004):

2.5.4.1 Frozen Hibiscus and dried:

The calyces are either frozen or dried in the sun or artificially for out-of-season supply, marketing or export. In Mexico today, the dried calyces are packed for sale in imprinted, plastic bags. It is calculated that 11 lbs (5 kg) of fresh calyces dehydrate to 1 lb (0.45 kg) of dried roselle, which is equal to the fresh for most culinary purposes. However, dried calyces as sold for "tea" do not yield high color and flavor if merely steeped; they must be boiled. For retailing in Africa, dried roselle is pressed into solid cakes or balls. In Senegal, the dried calyces are squeezed into great balls weighing 175 lbs (80 kg) for shipment to Europe, where they are utilized to make extracts for flavoring liqueurs. In the United States, Food and Drug Administration regulations permit the use of the extracts in alcoholic beverages.

2.5.4.2. Juice and wine:

Juice made by cooking a quantity of calyces with 1/4 water in ratio to amount of calyces, is used for cold drinks and may be frozen or bottled if not for immediate needs. In sterilized, sealed bottles or jars, it keeps well providing no sugar has been added. In the West Indies and tropical America, Roselle is prized primarily for the cooling, lemonade-like beverage made from the calyces. In Egypt, Roselle "ado" is consumed cold in the summer, hot in winter. In Jamaica, a traditional Christmas drink is prepared by putting Roselle into an earthenware jug with a little grated ginger and sugar as desired, pouring boiling water over it and letting it stand overnight. The liquid is drained off and served with ice and often with a dash of rum. A similar spiced drink has long been made by natives of West Tropical Africa. The juice makes a very colorful wine.

2.5.4.3. Sauce or syrup:

Roselle sauce or syrup may be added to puddings, cake frosting, gelatins and salad dressings, also poured over gingerbread, pancakes, waffles or ice cream. It is not necessary to add pectin to make a firm jelly. In fact, the calyces possess 3.19% pectin and, in Pakistan, Roselle has been recommended as a source of pectin for the fruit-preserving industry.

2.5.4.4. Seeds:

The seeds are somewhat bitter but have been ground to a meal for human food in Africa and have also been roasted as a substitute for coffee. The residue remaining after extraction of oil by parching, soaking in water containing ashes for 3 or 4 days, and then pounding the seeds, or by crushing and boiling them, is eaten in soup or blended with bean meal in patties. It is high in protein.

2.5.5. Composition of *Hibiscus sabdariffa*:

Roselle is mainly cultivated for its calyx, which is of three types: green, red and dark red. The red calyxes are the most used are characterized by their concentration anthocyanin. Delphinidin 3-Sambubioside and Cyanidin3-Sambubioside are the major anthocyanin. Roselle is also rich in organic acids, minerals, amino acids, carotene, vitamin C and total sugar in its calyx, leaves and seeds at variable levels depending on the variety and geographical area (Mady, *et al*.,2009). According to(Manita–mishr. 1999) a number of compounds have also been isolated and characterized from Roselle including flavonoids, anthocyanidins, triterpernoids, steroids and alkaloids. Nutrient contents of different part of *Hibiscus sabdariffa* per 100 gram are clearly stated in table 3.

2.5.6. Nutritional and medical importance of *Hibiscus sabradiffa:*

Roselle, the safe medicinal plant having various medically important compounds called phytochemicalsis well known for delicacy and also for its nutritional and medicinal properties (Arvind, *et al*., 2011)

The application of the plant in managing different medical problems including cancer, inflammatory diseases, and different cardiovascular problems has been well investigated by different scholars in different settings (Okereke , *et al*.,2015).

Table (2.1): Nutritional value of *Hibiscus sabradiffa:*

Nutrients	Calyxes	Seeds	Leaves
Protein(g)	2	28.9	3.5
Carbohydrates(g)	10.2	25.5	8.7
Fat(g)	0.1	21.4	0.3
Vitamin A(I.E)	-	-	1000
Thiamine(mg)	0.05	0.1	0.2
Riboflavin (mg)	0.07	0.15	0.4
Niacin(mg)	0.06	1.5	1.4
Vitamin c (mg)	17	9	2.3
Calcium (mg)	150	350	240
Iron (mg)	3	9	5

Source: Naturland, 2002

2.5.7. Anti helmentic and anti microbial effects:

Roselle is known for its antibacterial, antifungal and anti-parasitic actions. Oil extracted from seeds of Roselle has been shown to have an *in vitro* inhibitory effect on *Bacillus anthracis* and *Staphylococcus albus* (Gangrade, *et al*., 1979). Aqueous and ethanol extracts were also found to be effective against *Schistosoma mansoni* and other microorganisms (ELkamali,*et al*.,2006) demonstrated the antibacterial effect of hibiscus extract on *Streptococcus mutans*, a bacterium from oral cavity. In a similar study, antibacterial potential of hibiscus was also observed on *Campylobacter* species (Yin, *et al*., 2008) An ethanol extract of the dried

leaves of Roselle reduce aflatoxin formation and have *in vitro* inhibitory effect against some fungi (EL-Shayeb , *et al*.,1984).

2.5.8. Anti-oxidant effect:

Protective property of a compound to inhibit the oxidative mechanisms by scavenging reactive oxygen and free radicals is known as antioxidative activity. It protects lining organelles from premature cell damage and reduces ageing. A large number of *invitro* and *invivo* studies have shown that Roselle calyxes contain potent antioxidant. According to (Augustine, 2011) both the whole aqueous and anthocyanin-rich extracts of Roselle are effective antioxidant. Studies have also highlighted that poly-phenolic acid, flavonoids and anthocyanins which are found in Roselle are potent antioxidants (Crawford, *et al*.,1998).

2.6. Role of ingredients in the preparation of Karkade whey beverage:

2.6.1. Sugar:

The preparation beverage from cheese whey as found to be better at 12 % sugar and 0.4% citric acid (Jayaprakasha *et al*., 1986). (Mandal *et al.,* 1997) indicated that the best quality of sterilized chhana whey beverage can prepared by the addition of 10% sugar 1.5% citric acid, 3% whey. Krishnaiah *et al*.,(1989) formulated whey beverages by blending 10% sugar on 0.2% citric acid. (Singh *et al*., 1999) observed enhanced overall acceptability guava whey beverage at 8% sugar with 1:3 ratio of guava extract to whey. (Khamrui and Rajorhia, 1998) reported that the ready to serve drinks usually contains up to 14% sugar. Khamrui *et al.,* 2001) developed a technology for manufacture of ready to serve whey based beverage mix. They found that it containing 40% Kinnow juice, 53% whey and 7% sugar was found to be highly acceptable lemon juice and 0.06% sorbic acid to the deproteinised chhana.

2.6.2. Hydrolysed Lactose:

An attractive way of improving the attributes of whey, as a beverage base is to hydrolyze its lactose to sweeter syrup consisting of glucose and galactose, as both of them are sweeter than lactose. Many research workers tried the lactose hydrolysed whey. Hydrolysis of lactose in milk and milk products to glucose and galactose would solve the problem of milk intolerant people and in whey it would avoid

environmental pollution and for an interesting possibility of by-product utilization (Mahmoud, 2011).

Branger (1999) prepared the Cottage cheese whey and grapefruit juice blended beverage for lactose hydrolysis. They used the enzyme lactase from *Aspergillus* with a pH optimum of 4.5 and hydrolysed at 55°C for 30 min. After completion of 30 min hydrolysis, the enzyme was inactivated by placing the samples in boiling water bath for 3 min. After processing, sensory test showed lactose hydrolysis increased sweetness and decreased cheesiness in blends with more than 50% whey.

Singh (2009) optimized the lactose hydrolysis in whey for production of instant energy drink. Lactose hydrolyzation results in production of glucose and galactose - monosaccharides with much higher sweetness, better solubility and better absorption ability than lactose. In that way, sweetness of whey is being enhanced by production of natural sweetening agents enables the use of such whey for production of low-energy beverages.

2.6.3. Citric acid:

The US food and drug administration and FAO expert committee both have classified citric acid as multipurpose and generally recognized as safe (GRAS) food substance (Dziezak, 1990 and Berry, 2001). It performs multi functional activities in beverages. It imparts the sour taste; it helps in maintain pH, higher water solubility, maintain viscosity and helps in enhancement and modification of flavors (Berry, 2001). 11 The other attributes of citric acid responsible for its wide spread uses in food and beverage industries include good palatability, readily assimilable nature, easy bio degradability, absence of health hazards, pleasant acidic taste, higher water solubility, emulsifying effects, bactericidal action, ability to deliver a burst of tortness, ability to enhance existing flavor, anti oxidative quality and chelating as well as buffering capacity (Dziezak, 1990; and Berry, 2001). It is most commonly used in juice based beverages it has a light citrous like character that makes it compitable with many, besides providing product stability (Giese, 1995). Addition of 0.15% citric acid resulted in the best quality sterilized chhana whey beverage (Mandal *et al.,* 1997). Citric acid is for the most widely used in the beverages. Its light and fruity character blends well with most fruit

flavors, in soft drink and citric acid has been used at concentration of 1.28 g/l (Brygmesteren, 1963).

2.6.4. Color and Flavor:

Color and flavor are used in beverages to improve the color and appearance, flavor acceptability. This also indirectly helps to improve the appealing or marketability of the beverage by improving appearance and flavor, also aids in masking the undesirable flavor (Suresha, 2004). Jayaprakasha *et al*. ,1986) and Suresha and Jayaprakasha ,2004) observed that the optimum levels of flavor for whey drinks were 0.4 ml per liter, 0.3 ml per liter and0.35 ml per liter for pinaple,mango and orange flavored whey beverages respectively. Beverage were prepared from cow milk whey and flavored with extract of Roselle (5, 10, and 15) % physicochemical and microbiological quality rheological properties and shelf life was determined.

2.7. Storage stability:

Cheese whey concentrated to about 50 per cent total solids was enzymatically hydrolyzed (65-70% lactose hydrolysis) and the resultant whey had a water activity (aW) 210.9. It was shown that pH adjustment to 5.0 together with the addition of 0.2 per cent potassium sorbet or 0.2 per cent calcium propionate (0.1% of each) resulted in a whey that may be stored without refrigeration for at least 3 months. The microbial stability of the product was challenged with inoculation of *Staphylococcus aureus, Osmophilic* yeasts and various moulds (Leiras *et al*., 1991).

Ultrafiltered whey permeates after pasteurization was used in the preparation of whey based fruit beverages. Whey permeates were blended with 5 types of fruit juices such as cherry, apple, grape, strawberry, and orange. Each was added to whey permeate at 20.0 to 40.0 per cent and sweetened with 7.0 to10.0 per cent sucrose and acidified with0.2 or 0.4 per cent sorbic acid. A total of 75 samples of whey beverage subjected to sensory evaluation. The study revealed that all the beverages had good sensory properties, characterized by satisfactory clarity, good color and pleasant typical odor and flavor (Vojonovic *et al*., 1993).

It was observed that higher sensory scores were awarded for hydrolysed fruit based beverage compared to unhydrolysed fruit based

beverage. Sensory evalution revealed that cheesiness and saltiness increased, while sourness, astringency, sweetness and grape fruit flavor decreased as the percentage of whey increased (Branger, 1999).

2.8. Who can consume whey based beverages?

Whey based beverages target a large scale of consumers - from old people to little children Because of its health benefits, it was used to treat some illnesses, such as tuberculosis and skin and digestive tract diseases, since the time of Ancient Greece. In the 18th century there were specialized institutions built for curing illnesses with whey which designated the start of detailed studies of nutritional and therapeutic properties of whey. These so called „whey cures were usual in countries like Switzerland, Germany and Austria at that time.

Whey was also successfully applied for treatments of diarrhea, bile illness, skin problems, scales in the urinary tract and some intoxications (Popović-Vranješ and Vujičić, 1997).

Due to high amount of proteins with high nutritional value these beverages are ideal source of energy and nutrients for athletes. Whey proteins are a rich source of branched chain amino acids (BCAA) like isoleucine, leucine and valine. BCAAs unlike other essential aminoacids are metabolized directly into the muscle tissue and are first amino acids used during periods of exercise and resistance trainings (Sherwood, *et al*., 2007).

Whey protein fractions include also lactoferrin - an iron-binding protein, glycomacropeptide (GMP) which derives after cheesemaking using rennet and is naturally free of phenylalanine and alpha-lactalbumin which is a calcium-binding protein. That way, due to presence of lactoferrin whey beverages can be used as functional food intended to improve iron absorption from food and/or help to keep pathogens from attaching to the intestinal walls. That is very important for nutrition of little children and babies. Furthermore, these beverages may improve absorption of calcium what is very important for older population which is often suffering from osteoporosis. Besides that, a drink with addition of GMP isolate would be a very good source of energy and micronutrients for those suffering from phenylketonuria (Miller, 2005).

They are also being used as meal replacement for people suffering from overweight problems, older population and athletes or as a healthy alternative to fast food. Food market studies have shown that fermented and/or fruit whey beverages are mainly consumed by health conscious women, children and working people who consume those beverages for breakfast or as a snack (Miller, 2005, Huth, *et al*. 2006).

2.8.1. Whey Proteins-Nutraceuticals Application:

Jul- Aug.2013 Whey proteins are the wonder proteins for Nutritional, Clinical, Dietetic, Sports, Infant, Protein foods etc. because of:

- The best possible essential amino acids profile
- The best protein efficiency ratio
- The best biological value
- Easy digestibility and assimibility
- Natural taste in native form
- completely and easily soluble

2.8.2. Sports Foods:

The importance of Whey protein supplementation is critical to any individual looking forward to build and maintain muscle mass. Proteins have been called as the **"Building Blocks of Life"** and without proper Whey protein supplementation the task of building and maintaining muscle mass are next to impossible. The reasoning for this is that Whey protein has superior BV. Every athlete is aware of the importance of protein supplementation. If you are on any strength-training program, building muscles will meet or defeat optimum performance. Whey proteins have been enriched by nature with Branched Chain Amino Acids (BCAA), namely- L-Isoleucine, L-Leucine, L-Valine. These BCAA must be present in the muscle cell to promote protein synthesis. These BCAA helps increase the bio-availability of high complex carbohydrate intake and are absorbed by muscle cells for anabolic muscle building activity. The current theory is that during prolonged exercise, the BCAA"s are released from skeletal muscle; the carbon part is used as fuel and the nitrogen part is used to make the amino acid alanine which then goes to the liver where it is turned into glucose for energy. So for athletes who

want to protect their existing mass, the idea is to take a BCAA source before and after breakdown exercise. The issues being investigated are whether BCAAs reduce muscle breakdown and act as an energy source during this period. While maintaining exercise performance and delaying exertion, BCAAs are very important for muscle growth. The use of BCAAs in sports nutrition is especially interesting in making exercise feel easier. Whey proteins also help in speedy repairing of injured and torn muscle during practice and performance **Jul- Aug. (2013).**

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Materials:

3.1.1. Whole milk:

Fresh cow milk was collected from Sudan University of Science and Technology, College of Agricultural Studies, Department of Animal Production for preparation of whey.

3.1.2. Karkade(***Hibiscus sabdariffa***):

Fresh kharkade was bought from local market.

3.1.3. Sugar:

Good quality sugar was bought from local market.

3.1.4. Citric Acid:

Got It from Laboratory of Food Science and Technology, Department of College of Agricultural Studies of Sudan University of Science and Technology, used at concentration of 1.28 g/L.

3.2 Methods:

3.2.1. Experimental Procedure for preparation of whey :

The cow milk was used to produce white cheese (Rinner. 1983) for preparation of whey. The milk was heated at 85℃ for 5 minutes then cooled to 45℃, add Rinner enzyme and 1% culture then incubated for 2 hours. After that separated the curd from whey by filtering with a Muslin cloth to get the fresh whey.

3.2.2. Optimization of kharkade(***Hibiscus sabdariffa***):

Extract of karkade was made by placing a quantity of calyces with1:4 water in ratio to amount of calyces at 30 minutes, The PH of the extract was 2 PH and the T.S.S was 3.4 %

3.2.3. Preparation of whey beverage:

The whey was cooled at 4℃ for 24 hours, then the whey was thoroughly mixed and filtered by Vacuum Suction through A Porcelain Buchner Funnel with filtered glass disc and filter paper in order to remove the majority of fat(Rizzolo and Cortellino.2017). The product had pH at 5,6 and T.S.S at 5%. the pH of whey was adjusted by citric acid at concentration of 1.28 g /L until reached pH of 4.0 then divided the whey in to four parts : A, B,C and D.

Different amounts of sugar was added(10% ,15% ,20%),(A,B,C, respectively and D(control)) then heated at 45 ℃ for 30 minutes, after that blended with karkade (*Hibiscus sabdariffa*) at different levels of karkade to whey A (10:90) B (15:85) C (20:80) and one control D (100 %whey). After that filtered and bottled then subjected to physicochemical and sensory studies to adjust the optimal level of sugar and karkade in beverage. The final beverage had pH at5,4-3,8-4-5,6 forA,B,C,D, respectively and T.S.S. 13% , 17%,15and5% for A, B, C and D, respectively according to the flow diagram:

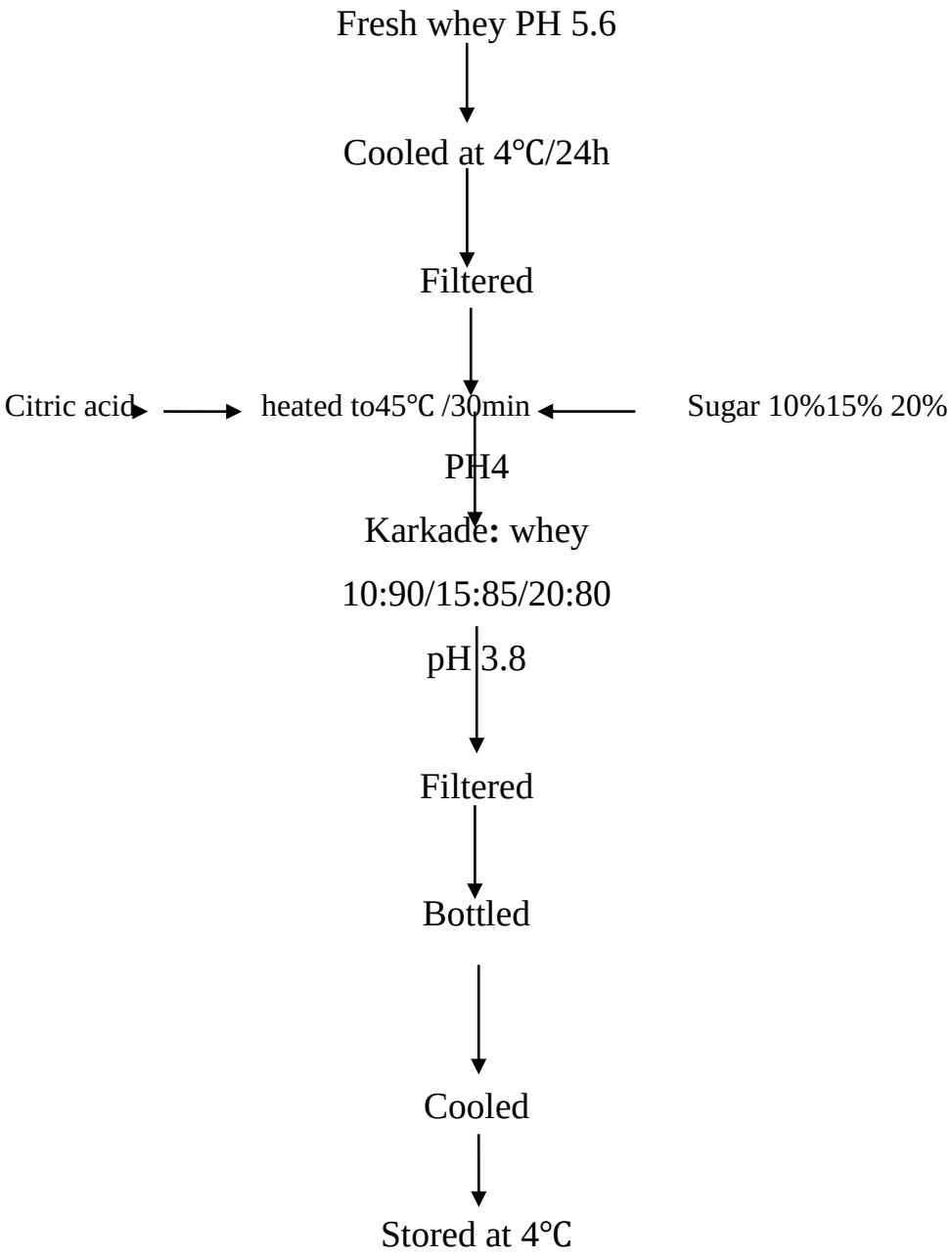

Figure 1: diagram of production Karkade blended whey beverage

3.3. Chemical analysis:

3.3.1. Fat content:

Fat content was determined by Gerber method according to Bradley *et al*. (1992). 10ml of sulphuric acid 10±0.2 ml were measured into Gerber butyrometer, and then the sample was withdrawn using a pipette (11.07) that allowed draining slowly to prevent a violent reaction with the acid. Then one ml of amyl alcohol was added, the lock stopper was inserted securely and the butyrometer was shaken until the curd was completely digested. The butyrometers were placed in a water bath at 60-63℃ for at least five minutes. The straight line at the bottom of the fat column was gently pushed upward to the nearest whole percent graduation mark. The fat content was then recorded as the difference between the lower and upper readings.

3.3.2. Protein content:

Nitrogen content determination were made in fat free meals by kjeldal technique following the AOAC(2000). About 2 gm of sample was weighted accurately into kjeldal flask, 4 gm of catalyst mixture and 35 ml of concentrated sulphuric acid were added, the flask was placed in the digestion equipment for 24 hours. The digested sample was then placed in the distillation apparatus 20ml of 40%NaOH were added and the ammonia evolved was received in 8ml of 2% boric acid solution. The trapped ammonia was titrated against 0.02N/HCL using universal indicator (Methyl red+ bromocresol green)

$$N\ \% = \frac{\text{Volume of HCL} \times 0.02\text{N/HCL} \times 14}{\text{Weight of sample}} \times 100$$

Protien%=N×6.38

3.3.3. Moisture content:

Moisture was determined according to the AOAC (2000). Two grams of sample were dried in an oven at 103℃ for three hours, the test repeated three times then the average was taken.

$$\text{Moisture } \% = \frac{\text{Weight of loss (gm)}}{\text{Weight of sample}} \times 100$$

3.3.4. Lactose content:

Lactose content determined according to the AOAC,(2000). 10ml of fehling's solution A were added into a conical flask followed by fihling's solution B, 25ml of the sample were added into the conical flask containing solutions A and B then the conical flask was heated until boiled then three drops of methylene blue indicator were added. Then lactose solution was added to the boiling solution drop wise at intervals of ten seconds until the blue color of the methylene blue indicator disappeared. Then the volume of lactose used at the end point of the reaction was recorded.

Lactose%= ((x-y)*0.05)/2.5

X= volume of lactose of blank sample

Y= volume of lactose of sample

3.3.5. Total solids:

Total solids content was determined by forced draft oven method according to Bradley et al. (1992). Aluminum dishes were dried for five hours at 100℃ and stored in a clean desecrator until used. Quickly and accurately 3±0.5gm of sample were weighted into per weighed dish on an analytical balance. The dishes with the cover under each were placed on the metal shelf in the forced draft oven for 16 hours, removed from the oven and cooled in the dessicator for at least 30 minutes or until they reached room temperature and then weighed. The total solids content was then calculated as follows:

$$\text{Total solids\%} = \frac{\text{Weight after drying}}{\text{Weight of sample}} \times 100$$

3.3.6. Ash content:

Ash content was determined according to the AOAC (2000) 2g of sample was ignited at 550℃ in a muffle furnace for 3hours.

$$\text{Ash content\%} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

3.3.7. pH :

pH of the sample was determined by using a digital pH meter. At25℃.

3.4. Sensory evaluation:

Sensory evaluation of whey beverage was done by eleven panelists of five Hedonic ratings. Hedonic rating is used to measure the consumer acceptability of food products. Samples were served to the panelist at one session. The acceptability of the product was rated based on a scale of points ranging from “like extremely” to “dislike extremely” according to the following instruction:

Pleas taste these samples and check how much do you like or dislike each one. Use the appropriate scale to show your attitude by checking at the point that best describes your feeling about the sample. Please give a reason for the attitude. Remember you are the only one who can tell what you like. An honest expression of your personal feeling will help us:

Response	**Points**
Excellent	5
Very good	4
Good	3
Acceptable	2
Unacceptable	1

3.5. Storage studies

Both control and experimental samples were stored at 4℃ for one month. The samples quality was analyzed with respect to PH meter, every 7 day for one month.

3.6. Statistical analysis:

Average scores secured by experimental samples were compared with those of the control samples and the results were analyzed for one way (Analysis of variance) ANOVA by using MINITAB-10 software.

CHPTER FOUR

4. RESULTS AND DISCUSSION

4.1. Chemical and physicochemical characteristics of whey beverage:

Table (4.1) showed the approximate analysis of tested whey and whey beverage samples. Fat content was 0.3 and 0.46 sample of control and whey beverage, respectively. These results mean that increase in Fat content in cases added karkade to whey (15:85).whereas on statistical analysis was no significant difference compared to sample B (15:85). The karkade blended whey beverage at (15:85) was awarded maximum total solids scored (16.3%) camper to control (0:100) were score (4.7%). Whereas the moisture content of the control were 88.16% and it was considered a high percent compared with sample B (15:85) were 77.3 also on statistical analysis was significant difference. Also observed that protein% at control (0:100) had 0.73% protein which considered a low percent compared to sample B (15:85) were 0.87%. The Ash content of control was 0.4% but at sample B (15:85) were 0.63% whereas COH % of karkade blended whey beverage at (15:85) were 15.2 % and considered a high compared to the control (0:100) was 4.2 % . And the pH of control (0:100) was 5.6 pH and for the sample B (15:85) was 3.8 pH.

4.2. Effect of various levels of karkade on the chemical quality of whey beverage

Effect of various levels of karkade on the chemical quality whey beverage is presented in Table-(4.1). The total solids content of whey beverage was increased with karkade levels. The total solids % were (4.7), (13), (16.3) and (22) were present with the increase in (karkade : whey) level at 0:100, 10:90, 15:85and 20:80 respectively. The increase in the total solids content as affected by karkade level was found to be statistically ($P \leq 0.05$) significant. The moisture content decreased with increase in karkade level due to increase in %TS. This variation in the TS and moisture similar trend was also observed by Jelen *et al* (1987) and Bhavnagar *et al.* (2010).content could be due to the variation in the levels of karkade and sugar.

Table(4.1): proximate analysis of whey beverage:

Karkade :whey v/v%	Moisture %	Protein %	Fat %	CO H %	Ash %	Total Solids%	H
0:100(control)	88.16[a] ±2.77	0.73[a] [b]±0.12	0.3[a] [b]±0	4.2[b] ±0.208	0.4[a] [b]±0.1	4.7 [b]±0.6	.6[a]
15:85(B)	77.3[b] ±2.413	0.87[a] ±0.06	0.4 6[a]±0.2	15.2 [a]±0.9	0.6 3[a]±0.2	16. 3[a]±0.6	.8[b]

All the values are average of 3 trials

Values are means ± SD.

Means having different superscripts in each column are significantly different ($p \leq 0.05$).

SD= Standard deviation.

4. 3. Sensory evaluation of whey beverage:

The scores pertaining to colour and flavour, consistency and overall acceptability of product as adjudged by a panel of judges during sensory evaluation of control and experimental samples of karkade blended whey beverage are presented in Table-4.2.and figure1.

4.3.1. Color:

The karkade blended whey beverage at 15:85 level was awarded maximum color score of 4.18 compared to control and other treated samples whereas, control secured the lowest color score of 2.3. The color scores for karkade blended whey beverage at 0:100(control), 10:90, 15:85and 20:80 levels were, 2.3, 2.8, 4.18 and 3.18 respectively. On Statistical analysis there was a significant effect ($P \leq 0.05$) on color scores of karkade blended whey beverage compared to control

4. 3.2. Flavor

The flavor scores of karkade blended whey beverage at 0:100(control), 10:90, 15:85, 20:80 levels were 2.1, 3.3, and 4.09 and3.7 respectively. The karkade blended whey beverage at 15:85 level was awarded maximum flavor score (4.09) compared to control and other treated samples. Whereas, control secured the lowest flavor score of 2.1. On Statistical analysis there was no significant effect on flavor scores of karkade blended whey beverage compared to control.

4. 3.3. Taste:

The Karkade blended whey beverage at 0:100(control), 10:90, 15:85, 20:80 were 2.1, 3.2, 4 and3.5, respectively. The karkade blended whey beverage at15:85 was awarded maximum taste scored (4.09) compared to control and other treated samples.

4. 3.4. Consistency

The consistency scores of karkade blended whey beverage at 0:100(control), 10:90, 15:85 and 20:80 levels were 2.9, 2.9, 4 and3.5 respectively.

Table(4.2.): Effect of various levels of karkade on the sensory characteristics of whey Beverage:

Karkade:whey %v/v	color	Flavour	Taste	Consisteny	Overall Acceptability
10:90	2.8 ^{a}	3.3 ^{a}	3.2 ^{a}	2.9 ^{a}	3.0 ^{a}
15:85	4.18 ^{b}	4.1 ^{ab}	4.1 ^{b}	4.0 ^{b}	3.9 ^{b}
20:80	3.18^{bd}	3.7 ^{ac}	3.5 ^{a}	3.5 ^{cb}	3.5 ^{ab}
0:100	2.2 ^{a}	2.1 ^{d}	2.1^{d}	2.9 ^{a}	2.5 ^{ac}
Std	1.001	1.092	0.96	0.93	0.836

Means with the same superscript in a column indicates no significant difference at P≤0.05

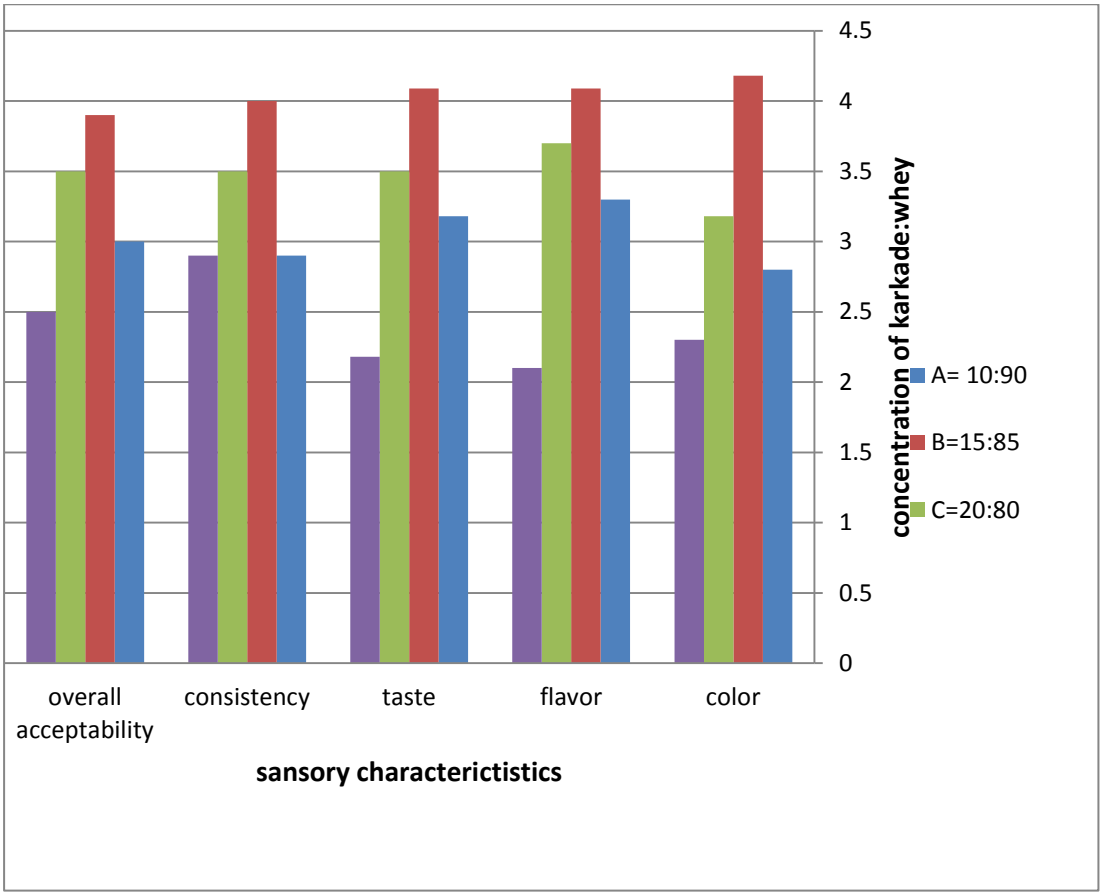

Figure2: Sensory characteristics of various level of karkade blended whey Beverage.

. The karkade blended whey beverage at 15:85 level was awarded maximum consistency score compared to control and other treated samples. Whereas, control secured the lowest consistency score of 2.9. However statistical analysis revealed a significant effect on consistency scores of all the samples of karkade blended whey beverages and control.

4. 3.5. Overall acceptability

The karkade blended whey beverage at 15:85 level awarded maximum overall acceptability score (3.9) compared to control and other treated samples. Whereas, control secured the lowest overall acceptability score of 2.5. The overall acceptability scores for karkade blended whey beverage at 0:100(control), 10:90, 15:85 and 20:80 were 2.5, 3, 3.9 and3.5 respectively. On Statistical analysis this was a significant effect ($P \leq 0.05$) on overall acceptability scores of karkade blended whey beverage samples and control. The product prepared at 15:85 level was awarded with maximum overall acceptability score of 3.9 Hence it was selected for further studies.

4.4. Effect of different levels of karkade on the sensory characteristics of blended whey beverages:

Effects of various levels of karkade on the sensory characteristics of blended whey beverage are presented in Table-(4.2). The colour score of beverage at15:85 (karkade:whey) level was awarded maximum score of 4.18 and it was found to be optimum. It was significantly higher when compared to the colour scores of control (2.3) and other treated samples.

The intensity of colour increased as the level of karkade blending increased. Whereas, at higher levels the intensity became very high and product colour appeared dark red, hence beverage prepared at 20:80 level was awarded significantly lower colour score of 3.18. Similarly, beverage prepared at 15:85 level was awarded maximum flavour score of 4.09 when compared to control (2.1) and other treated samples. Whereas, product prepared at 20:80 level was awarded significantly lower flavour score of 3.7. This might be due to blending of karkade extract at this level was found to be strong taste and smell.

So that blending of karkade with whey at 15: 85 levels imparted optimum natural colour and flavour to the product. So that slightly disappeared in nuture flavor of whey and earns new flavor from karkade

and flavor of strawberry. This trend was similar in the findings of Ritu *et al* (2007)

They reported that at higher levels of incorporation of guava pulp with whey lead to decrease in the taste sour of whey guava beverage. Similar trend was also reported by Bhavsagar *et al.* (2010), and Divya and Archana (2009).

The consistency scores of control (2.9) and beverage prepared at 10:90, 15:85 and 20:80 levels were 2.9,4, and 3..5 respectively. There was significant effect on consistency scores of control and other treated samples. Bhavsagar *et al* (2010) observed no significant difference in the consistency scores in pine apple pulp incorporated whey beverage.

The maximum overall acceptability score of 3.9 was awarded to the beverage prepared at 15:85 levels. There was a significant difference between the overall acceptability scores of the beverage prepared at 15:85 level when compared to control and other treated samples. Higher levels of incorporation of karkade (20:80) lead to strong karkade flavour with sour and slightly bitter taste. Hence, judges awarded lower sensory scores. At lower levels of karkade addition scores are the significantly reduced the intensity of karkade flavor and increased flavor of strawberry. Bhavsagar *et al* (2010) and Naik *et al* (2009) have reported same trend that at higher level of incorporation of pineapple and watermelon respectively lead to reduction in the overall acceptability scores of the beverage.

4.5. Effect of various level of sugar on the quality of karkade blended whey Beverage:

Effect of various levels of sugar on the quality of karkade blended whey beverage is presented in Table-(4.3). The color, taste and consistency scores for control and other treated samples was found to be significant. The colour scores of beverage prepared by adding sugar at 10,15and 20% were 2.5, 4.18, 3.18 and 2.3 respectively. There was no significant ($P \leq 0.05$) effect of sugar on colour scores of all the samples. The flavour scores of beverage prepared at 15% sugar level was awarded

maximum score of(4.3) and it was significantly higher when compared to the flavor scores of samples prepared at 15,% and 20% sugar levels. Whereas, product prepared at 0% sugar level was awarded significantly lower flavour score of (2.9).

This might be due to sour taste that predominated at 0% level of sugar in karkade blended whey beverage. Many authors were also added sugar at higher levels between 14-18% to overcome the acid taste of whey (Khamrui and Rajorhia 1998), Ritu *et al* (2007) and Jayaprakasha *et al*., 1986). The consistency scores of karkade blended whey beverage at all the sugar levels were found to be significant. Consistency scores of karkade blended whey beverage prepared by using sugar at 10, 15,20 and 0% were 2.9,3.9,3.5,and2.2 respectively. Whereas, at 0% level beverage secured the lowest consistency score of 2.2 On Statistical analysis there was a significant effect on consistency scores of karkade blended whey beverage.

Similar trend was also reported by Singh *et al.* (1999) The maximum overall acceptability score of 3.9 was awarded to be beverage prepared at 15% level. There was a significant difference between the overall acceptability score of the beverage prepared at 15% sugar level when compared to control and other treated samples. At higher levels of sugar addition (20%) leads to very sweetish flavour with more sweet taste. Hence, judges awarded less flavour scores for the beverage. Similarly, at lower sugar levels significantly ($P \leq 0.05$) lower flavor scores were awarded due to less sweetness. Similar trend was also observed by Reddy (1987) incorporated 9 to 14% sugar, while Khamrui and Rajorhia (1998) incorporated 6 to 8% sugar in whey beverage and also same sugar percentage was used by Kesarkar (2002).

Table(4.3.) Effect of various levels of sugar on the sensory characteristics of karkade whey Beverage:

Sugar levels%	Color	Flavor	Taste	consistency	Overall Acceptability
10	3.18[a]	3.3[a]	2.9[a]	2.9[a]	2.5[a]
15	4.2[ab]	4.3[b]	4.3[b]	3.9[b]	4.2[b]
20	3.5[ab]	3.7[ab]	3.5[c]	3.5[ab]	3.5[b]
Control	2.0[d]	2.9 [d]	1.9[d]	2.2[ac]	2.3[a]
Std	1.214	1.022	0.8	0.89	0.8

Means have the same superscript in a column indicates no significant difference at P≤0.05.

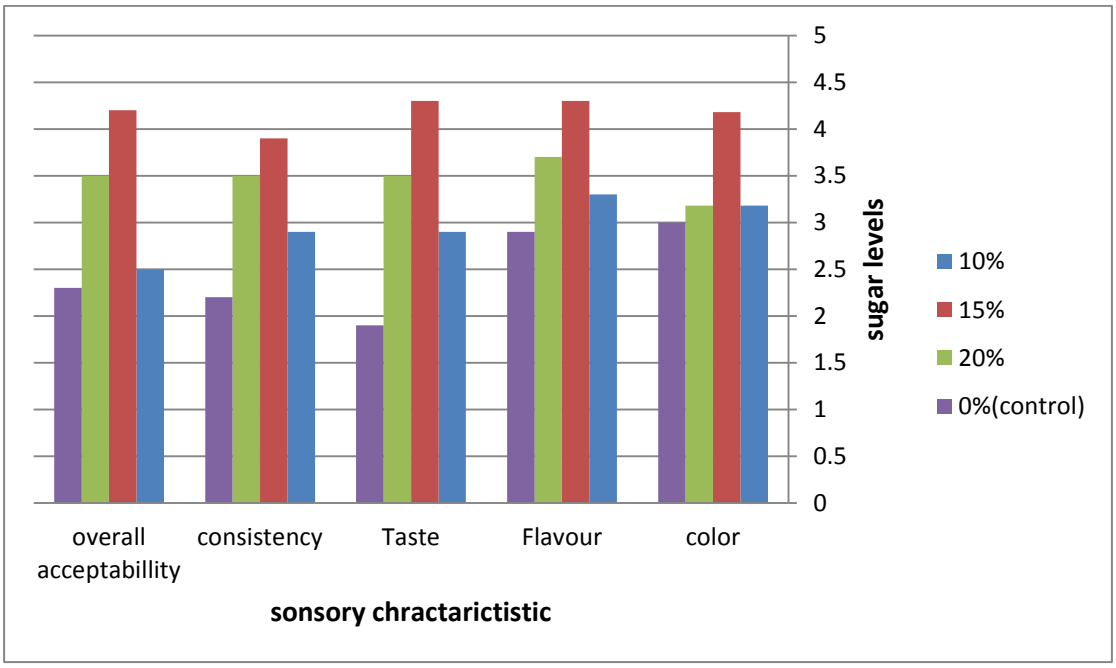

Figure3: Sensory characteristics of various levels of sugar on the quality of karkade blended whey Beverage.

4.6.Effect of pH on whey beverage during storage at 4℃:

The pH scores of karkade blended whey beverage at 10:90, 15:85, 20:80 and 0:100 (control) were 4.1, 3.4, 2.9 and 4.95 respectively. The control was awarded maximum scores (4.95) compared to other treated samples on statistical analysis there was significant effect on pH scores of karkade blended whey beverage. However the control and sample A(10:90) showed no significant effect on pH compared to samples (A, B). So that decrease in pH depended on increase in karkade level.

In control sample we found that the range of decrease was from read one to read four were (1.5) and that considered as a damage after 21 days compared to other treated samples , were the range of decrease was 0.5, addition to that the control seemed to had aromatic and acidic taste, but samples B and C had damage after 28 days and there was separated substance and alcoholic aroma. The optimum shelf life of the product was approximately 20 days , after that it would be acceptable until reached 28 days. It was shown that pH adjustment to 5.0 together with the addition of 0.2 per cent potassium sorbet or 0.2 per cent calcium propionate (0.1% of each) resulted in a whey that may be stored without refrigeration for at least 3 months. The microbial stability of the product was challenged with inoculation of *Staphylococcus aureus, Osmophilic* yeasts and various moulds (Leiras *et al*., 1991).

Table (4.4):The results of pH readings of beverage during storage at 4℃.

Beverage	R1(PH)	R2(PH)	R3(PH)	R4(PH)	Average
A	4.6	4.5	4.2	3.0	4.08[a]
B	3.8	3.8	3.5	2.5	3.4[ab]
C	3.2	3.2	3.0	4.0	2.9[b]
D(control)	5.6	5.6	4.6	4.0	4.95[a]

Means with the same superscript in a column indicates no significant difference at P≤0.05.

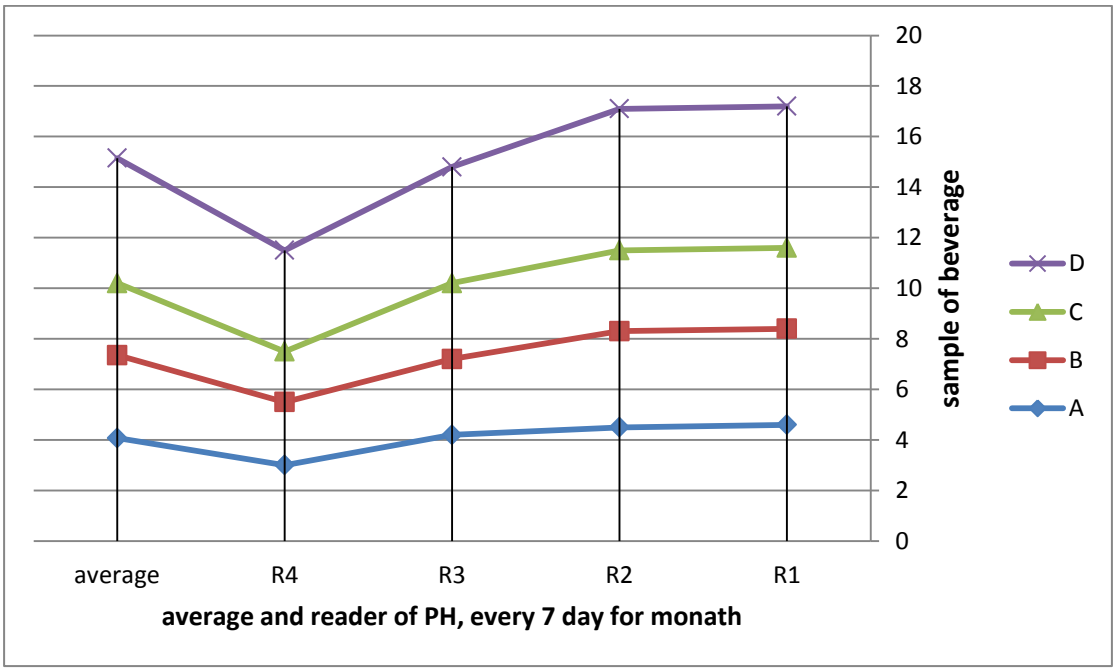

Figure4: change in pH of beverage during storage at 4℃.

CHAPTER FIFE

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion:

This study showed promising results that karkade can be incorporate into whey (15:85) to produce whey beverage, provide both nutrient inherent in whey as well as in karkade and also provides various therapeutic health benefits the developed karkade blended whey beverage not only help in by product utilization but also gives product with natural color and flavor.

5.2. Recommendation:

1. Fresh whey processing has the most economical technological solution, that by develop of beverage with addition of fruit concentration or flavors to produce a drink with acceptable sensory properties.
2. Recommended industry of dairy to introduce the whey as beverage in market because the market of functional beverages is growing around the world.
3. Whey with Karkade is recommended as beverage for human consumption because Roselle is safe medicinal plant having various medically important compound called phyto chemicalsis.
4. Extract of karkade at 15% to produce suitable whey beverage acceptable for consumer.
5. The product (karkade blended whey beverage) has high biological value so that it is suitable for humans whom have cracks in their feet.
6. More research must be done on this product especially on the shelf life and heat treatment.

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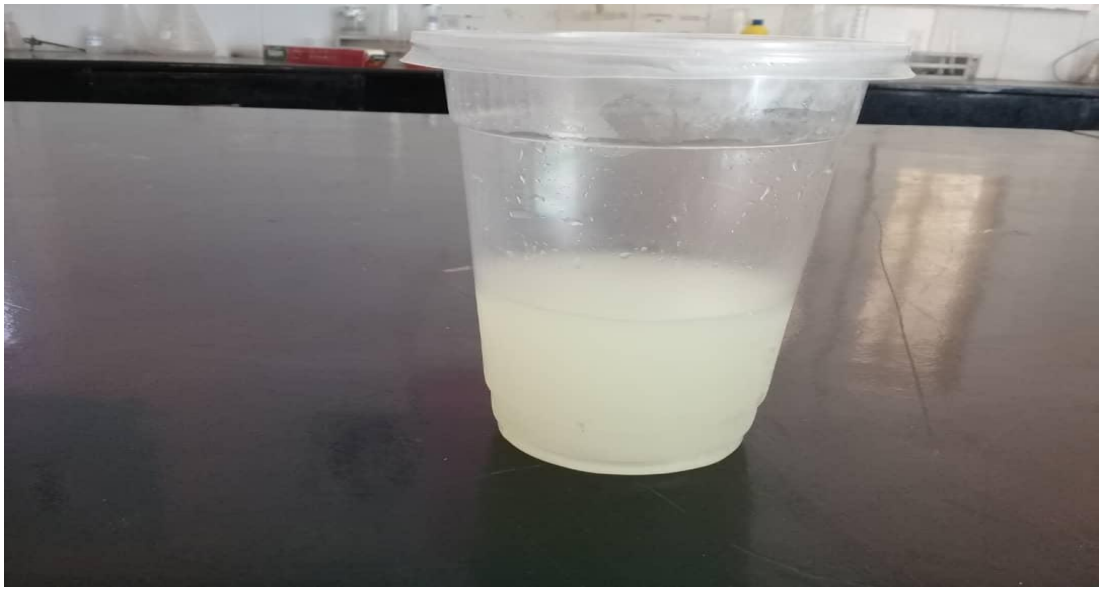
APPENDIXCES

Appendix 1: **White cheese during separation of whey**



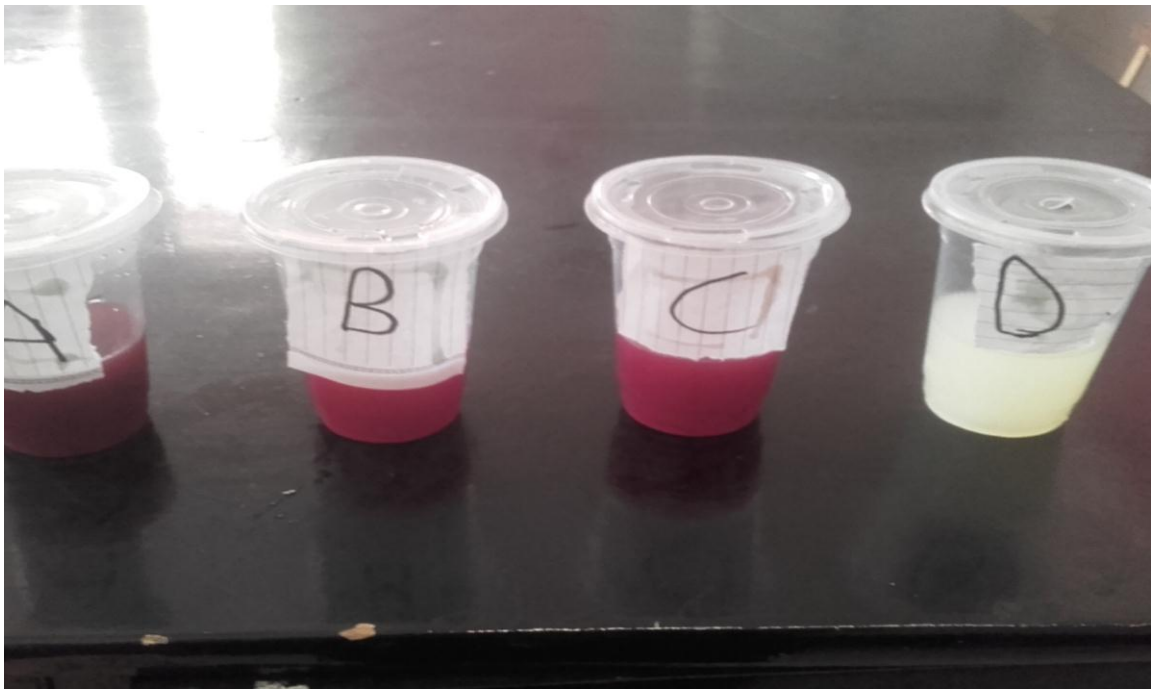
APPENDIX 2: fresh whey

Appendix 3: **Filter by pressure**

Appendix 4: water bath

Appendix :5 pHmeter

Appendix :6 Referactometer



Appendix 7: sample fo baverage

A=10:90(karkade:whey)

B=15:85(Karkade:whey)

C=20:80(Karkade:whey)

D=0:100(Karkade:whey) cotrol