CHAPTER ONE

Introduction

The livestock population in Sudan was estimated to be about 29618000 cows, 39296000 sheep, 30649000 goats and 4715000 camels the total is 104278000 head according to federal ministry for animal resources fishery and range (F M A R FR, 2012).

The total milk production in the Sudan is estimated 4361000tonnes, where the target production of milk for consumption is 4426000.731 tones .The available production of milk for consumption is 2705000.68 tones, so the deficit is 1721000.051 tones, where the individual consumption for the year is 79.5liters, where the target amount of milk for individual consumption per year 120 liters (ministry for Animal Resources Fishery and Range, 2014).

Yogurt is produced by the lactic acid fermentation of milk with addition of a starter culture containing (Streptococcus thermo philus and Lacto bacillus delbrueckiisp. Bulgaricus). In some countries less traditional microorganisms, such as (Lactobacillus helve tic us and Lactobacillus delbrueckiissp. Lactis) are sometimes mixed with the starter culture (McKinley, 2005).

Yogurt is a popular food and is believed to be one of the oldest fermented foods, originating in the Middle East and Asia (Tamime and Robinson, 1999).Although fermented milk products such as yogurts were originally developed simply as a means of preserving the nutrients in milk, it was soon discovered that, by fermenting with different microorganisms, an opportunity existed to develop a wide range of products with different flavors, textures, consistencies and more recently, health attributes, so that the market now offers a vast array of yogurts to suit all meal occasions. Yogurts come in a variety of textures (e.g. liquid, set and stirred), fat contents (e.g. regular fat, low-fat and fat-free) and flavors (e.g. natural, fruit, cereal, chocolate), can be consumed as a snack or part of a meal, as a sweet or savory food, so according to this versatility, together with their acceptance as a healthy and nutritious food, hassled to their widespread popularity across all population subgroups (Mckinley2005).

physical characteristics are important for quality of yoghurt (Amatayakul italic., 2006). The stabilizers are used to improve the consistency, viscosity and reduce the synersis rate of the yoghurt (Lucey, 2002).Gelatin, starches, pectin, alginate, carrageenan, derivatives of methylcellulose, gum arabic, tragacanth, karaya, locust bean gum (LBG), guar and xanthan gums are the compounds used as stabilizers in yoghurt (Tamime and Robinson, 1985).

The stabilizers used for improve the consistency and dispersion properties of spray-dried yoghurt powder in water (Ramirez-Figueroa et al., 2002).Advantages of soluble fibers stabilizer has their beneficial effects for human health (Labell, 1990). consuming fiber in the diet decrease or prevented disease, such as hypertension, diabetes, hypercholesterolemia and gastrointestinal disorders (DelloStaffolo et al., 2004).

Objectives at the study:

- To study the possibility of using natural stabilizers of nutritional value in the manufacture of yoghurt.
- To examine the influence of stabilizers impact on viscosity and sensory characteristics for yoghurt.
- To increase the nutritional value of yogurt by using different stabilizers.
- To study the economical impacts of using different natural stabilizers in yoghurt manufacturing.

CHAPTER TWO

Literature Review

2.1 Definition of milk

Milk is the natural secretion of mammals to feed their offspring. It is defined as a dynamically balanced mixture of protein, fat, carbohydrates, salts, and water co-existing as emulsion colloidal suspension and solution (Hargrove and Alford, 1980).

2.1.1 Importance of milk and milk products in diet

stated that the fluid milk is a source for a whole range of dairy products consumed by mankind. Fluid milk is about 87% water and 13 % solids. The fat portion of the milk contains fat-soluble vitamins. The solids other than fat include proteins, carbohydrate, water-soluble vitamins, and minerals. Milk products contain high quality proteins. The whey proteins constitute about 18% of the protein content of the milk. Casein, a protein found only in milk, contains all of the essential amino acids and accounts for 82 % of the total proteins in milk. Milk also contains calcium, phosphorus, magnesium, and potassium. The calcium found in milk is readily absorbed by the body; Vitamin D plays a role in calcium absorption and utilization. Milk is also a significant source of riboflavin (vitamin B2), which helps promote healthy skin and eyes and also stated that the dairy products such as yogurts, cheeses and ice creams contain nutrients such as proteins, vitamins and minerals(Sharma, et al 2006).

Consumption of dairy products has been associated with decreased risk of osteoporosis, hypertension, colon cancer; obesity and insulin resistance syndrome, besides its main dietary source of calcium and vitamin D (Weaver, 2003).

2.1.2 Milk Consumption

Milk can be consumed fresh in the form of homogenized whole milk, fortified milk, flavored milk, low, fat milk and free fat milk (Webb *et.al.*, 1980).

Heat treatment applied to milk such as pasteurization and sterilization in order to enable its use for a period of time from some days (pasteurization) to some months [sterilization] (63-72°C).

2-2 Fermented milk

Fermentation, as defined by Gale (1948), is the process leading to the anaerobic breakdown of carbohydrates Kroger et. al(1989) Concluded that the fermented milk products are known for their taste, nutritive value and therapeutic properties. Fermented milk are products prepared from milks, whole, partially or fully skimmed, concentrated or milk substituted from partially of fully skimmed dried milk, either homogenized or non-homogenized, pasteurized or sterilized and fermented by means of specific microorganisms.

(Arab Organization for Agricultural Development, 1983) reported that therobe is the major fermented milk product in the Sudan. It is mainly produced from surplus milk of the rainy season by nomadic tribes. About 80% of the rainy season milk is turned into Robe by the household in this season, where they estimated the milk turned into dairy products in Sudan is 65% of the total annual production.

Dirar (1993) gave an estimate of 50 – 60%. Robe makes about 90% of all fermented milk products. The aim behind the souring of milk into Robe is not to obtain fermented milk for consumption, but the souring here is only an expedient to facilitate the extraction of butter from it.

Therefore it is considered as a by-product of butter production. Pastoralists commonly waste Robe away by spillage on the ground or give it to dogs or young animals and wild birds. Nonetheless they daily consume part of it in one form or another. Robe fermentation is not then a way of preserving the essential part of the milk, and that is butter, as far as the nomads are concerned. Every few days the accumulated butter is boiled to give ghee or butter oil, called Samin. The un-boiled butter or furssah finds no other use except that part of it is fed to babies. All other fermented dairy products of the Sudan are fermented to be consumed without removing the butter in advance (Dirar, 1993) .

Consumption of fermented-milk products is associated with several types of human health benefits (Wollowski et al. 2001). Recently, the role of fermented milks containing lactic acid bacteria (LAB), such as Lactobacillus ,Bifid bacterium and Streptococcus thermo philus, has been studied (Saikali et al. 2004).

A wide range of other health benefits, including improved lactose digestion, diarrhea prevention, immune system modulation and serum cholesterol reduction, have been ascribed to fermented milk consumption.

2-3Types of yoghurt:

Abdelkarim (2010) reported that the differentiation of yoghurt into divers types according to legal standards, technique of production, flavor and post incubation processing have been suggested, depending on method of production, the industries recognize two main types of yoghurt that are set and stirred, where this classification is based on the system of manufacturing and physical structure of the coagulum.

Based on flavor there are three different types of product namely natural or plain yoghurt which is the traditional type with sharp acidic taste, and yoghurt with fruit made by addition of fruit and sweetening materials to the natural yoghurt, flavored yoghurt which the fruit component is replaced with synthetic and coloring compounds, (Mohammed, 2011).

incubation processing, yoghurt can be differentiated into pasteurized yoghurt which is processed by conventional method of manufacture, in addition to this procedure the yoghurt undergoes heat treatment, and to extend its storage life, while frozen yoghurt which is prepared in a conventional mode, but is then deep frozen to-2o C , other classification is concentrated and dried yoghurt which contains total solids of about 24% and 90-94% respectively, besides the type of yoghurt in use is yoghurt cheese and acidophilus yoghurt (Abdelkarim, 2010).

2.4 Yogurt Processing

Although there are no standardized procedures for making a drinkable yogurt product, most processors agree on a general process, includes pre-treatment of the milk, heat treatment, homogenization, cooling, started culture addition and subsequent fermentation, and packaging (Tamime and Robinson, 1999).

2.4.1 Pre-treatment

The pre-treatment step involves adjusting the milk before processing. In set or stirred yogurt production and addition of milk solids to achieve desired viscosity. However, for a drinkable yogurt, milk without fortification is normally used (Tamime and Robinson, 1999). Pre-treatment also includes the standardization of the fat content in the milk. Standardization of the fat is an important step, not only for adherence to legal standards, but also for the overall effect on the flavor and texture attributes of the finished product, particularly relating to the viscosity and mouth feels.

According to (Tamime and Robinson 1999), milk can be standardized in a variety of ways. Part of the fat content may be removed from the milk, full cream milk may be mixed with skimmed milk; cream can be added to full fat or skimmed milk, or any combination of these using standardizing centrifuges.

Pre-treatment of the milk also includes the addition of any stabilizers, separation, a typical quality defect in drinkable forms of yogurt, may be prevented by the inclusion of various stabilizers such as gelatin (Tamime and Robinson, 1999).

2.4.2 Heat Treatment

Heating of milk is an important processing variable for the preparation of yoghurt since it greatly influences the physical properties and microstructure of yoghurt. Also heat treatment of milk is also used to destroy unwanted microorganisms, which provides less competition for the starter culture. Yoghurt starter culture is sensitive to oxygen so heat treatment helps to remove dissolved oxygen assisting starter growth, (Lee and Lucey, 2010).

2.4.3 Homogenization

Homogenization breaks the large fat globules into smaller ones, stable emulsion out of an oil-in-water mixture (Early, 1998; Spreer, 1998).

This is typically accomplished by directing the standardized milk through small valves at high pressure (Tamime and Robinson, 1999). This action reduces the size of the fat globules (Early, 1998; Tamime and Robinson, 1999). The new fat globules are stabilized by binding to some of the casein that was broken during this processing step (Fox and McSweeney, 1998; Tamime and Robinson, 1999).

As a result, homogenization serves several purposes, including preventing the separation of the cream layer, improving stability, altering the physical attributes, etc. (Tamime and Robinson, 1999; Walstra/etal., 1999).

These effects are dependent upon the amount of fat in the milk, as well as the pressure and temperature used for homogenization and may be minimal in skim milk products (Fox and McSweeney, 1998; Tamime and Robinson, 1999).

this processing step should occur prior to the heat treatment regardless, both the serum proteins and the fat in the milk may be affected by homogenization. Some denaturation of these proteins, primarilyby -lactoglobulin and □ - lactalbumin, may occur along with the resultant effects as previously discussed with the heat treatment.

Heating the homogenized milk at a temperature on the surface of suspended micelles and adsorbed on the fat globules are possible, as interactions with the residual fat globule membrane. Adsorbed casein can also be displaced on the fat globule by adsorbing onto the globule surface (Tamime& Robinson, 1999).

The reduction in size of the fat globules and increase in the adsorption on the casein micelle leads to an increase in total volume, resulting in an increased viscosity and the formation of a softer gel (Spreer, 1998; Tamime and Robinson, 1999). Tamimeand Robinson, 1999).

The interaction between the casein and the fat globules, as well as other protein effects, result in an increase in hydro phobicity and water binding which limits syneresis (Tamime and Robinson, 1999).

2.4.4 Starter Cultures:

Starter cultures generally are lyophilized and distributed in dry or frozen state, where the diary starters are culture of active bacteria harmless grown in milk or whey which are used to impart certain characteristic and predictable qualities to various milk product. these culture may be strain of one species of micro organisms ,called single strain culture or a number of strain and / or species called multi – strain or mix strain culture (Abdelkrim, (2010).

2.4.5 Yoghurt additives:

the Additives involved in yoghurt manufacturing could be fruits either natural or synthetic flovour or artificial sweeteners, colouring agents or stabilizer (Tamime and Robinson, 1999) .

2.4.6 Inoculation and incubation:

After heat treatment , the milk base is cooled to the incubation temperature used for growth of the starter culture , an optimum temperature of the thermo philus lactic acid bacteria , streptococcus sub sp . thermo philus and lactobacillus delbrueckii sub sp. Bulgaricus is around 40 - 45 ^{o} C , the inculcation amount vary between 0.5-2.5 % but the optimum value is 2% . Bacterial fermentation converts lactose into lactic acid, which reduces the ph of milk, during acidification of milk, the pH decreases from6.7 to <4.6(Lee and Luce 2010).

2.4.7 Cooling

When yoghurts have reached the desired ph. yoghurts are partially cooled 2o ^{o} C before fruit or flavoring ingredients are added. Yoghurt products are often blast chilled to 5 ^{o} C in the refrigerated cold store to reduce further acid development (Tamime and Robinson, 1999) and (Rehab, 2013).

2.5 Stabilizers in yoghurt manufacturing

stabilizers can be categorized according to the manufacturing process (Tamime and Robinson, 1999).

selection of the stabilizer or stabilizer combination to be used in a food system greatly depends on several variables as functional properties of the stabilizer, intended use and outcome, interactions with other ingredients, and legal aspects are only a few considerations.

In yogurt, stabilizers are added for two main reasons;as thickening or gelling agents and to stabilize the yogurt matrix in this capacity,the hydrocolloids, which are generally added to the milk prior to fermentation, can improve the viscosity, maintain the yoghurt structure, inhibit syneresis, alter the mouth feel, and, in the case of yogurt with added fruit, help keep the fruit in suspension (Early, 1998).

2.5.1Gelatin:

Poppe, (1997) declared that gelatin is one of the hydrocolloids or water-soluble polymers that can be used as a gelling, thickening or stabilizing agent; it is a totally digestible protein, containing all the essential amino acids except tryptophan Gelatin is an ingredient compatible with the milk proteins and contributes good palatability to the end product, giving a fat-like sensory perception because of its unique property of melting at mouth temperature. It eliminates syneresis and considerably reinforces the mechanical resistance of the gels making it possible to obtain a wide range of texture (Fiszman and Salvador, 1999).

Food industry gelatin is used not only for its functional properties, but also because of its importance as a source of protein in the daily diet. The primary properties of gelatin include gel formation, and surface effects such as emulsion and foam formation. Increases in the concentration of gelatin in yogurt and variations in the pH of acid-heat-induced gels caused changes in the shape of the force/displacement curves. Potentially, the use of different concentrations of gelatin would make it possible to obtain a wide range of textures including creamy, slightly gelled and firm, “moldable” gel of yogurt One of the most important properties of gelatin is its ability to form thermo reversible gels. Gelatin swells in cold liquid absorbing water. When heated to temperatures of approximately 50 to 60°C it dissolves and forms a gel when cooled. This sol- gel conversion is reproducible and can be repeated several times (Fiszman and Salvador, 1999).

This versatile property is used in many food applications. The gelling power of gelatin is determined by its bloom value, which is a standardized measurement of the firmness of a standard gel under precisely determined conditions. The bloom values of gelatin range from 80 to 300 g. In general, viscosity increases with increasing bloom strength (Schott, 2001).

2.5.2Gum Arabic:

Natural gums are organic substances of rather indefinite composition, gums from the same species of tree are chemically identical with respect to same components found in them while gum from different species chemically and physically differ (Mantell, 1965).

Gum Arabic is the most important polysaccharide gum in commerce .its name is probably originated from the fact that the Arab in early history were the famous traders and vendors of this substance The gum is an exudates from smalls, thorny shrub-like trees of the genus Acacia, a leguminous tree found in many parts of the world, growing particularly in warm semi –arid regions and often on sandy soil (Ali, 1996). The highest quality gum that constitutes the great bulk of the trade is derived from Acacia Senegal which grows in the Sudan and Senegal regions, and to a much lesser extent in Nigeria, Uganda, Tanzania and recently Niger (Anderson and Miller 1991).

In the Sudan the term (gum belt) is applied to that part of the country in which various types of gum produced by Acacia Senegal (hashab). The gum belt covers parts of eleven states : north kordofan , south kordofan , west kordofan , : north Darfur , south Darfur , west Darfur , kassala , Gadaref , Sinnarand Blue Nile (Ali ,1996).

The principle use of gum Arabic is in food stuffs and is extensively used in the confectionery industry It also acts as an emulsifier, keeping the fat uniformity distributed throughout the products; it is also used as an additive and stabilizer in bakery products, as an emulsifier and fixative in flavor industry, stabilizer in dairy products and as foam stabilizer in beverages. It is used in coating polished food material and for the preparation of dry powdered stable vitamins (Ali, 1996).

2.5.3 Guar Gum

Guar gum is found in the endosperm of the seeds of the guar plant, Cyamopsistetragonoloba, which is milled in order to obtain guar gum (Meer1977; Wielinga 2000)

Guar gum is used as a thickener and stabilizer in the food industry as a result of its hydration and water-binding properties. It is used as a stabilizer at a Concentration of 3.0% in ice cream, ice pops, and sherbet. It improves the body, texture, chewiness, and heat-shock resistance by binding free water (Wielinga 2000).

Also Meer 1977 and Wielinga 2000) indicated that the guar gum is also used in cheese products. In cold-pack cheese it is used at a concentration of 3.0% in order to prevent syneresis and weeping. In soft cheeses it is added to increase the yield of curd and to give the curds a better texture. Concentrations of .25-.35 are added to pasteurized cheeses in addition to locust bean gum and act as a stabilizer. Guar gum is also added to dessert and pastry products, such as pie fillings, icings, cake and donut mixes. It is added to pie fillings to thicken but prevent shrinking and cracking of the filling.

Guar gum can also be used in dietetic beverages or low carbohydrate products due to its suspending ability and improving body of thin and watery products. (Meer 1977).

Moreover Deis 2001) stated that an advantage of guar gum is its cold water solubility which allows viscous pseudo plastic solutions to form when hydrated in cold water.

The viscosity of Guar gum is dependent upon factors such as time, temperature, concentration, pH, ionic strength, and type of agitation. Maximum viscosity is reached during the temperature range of 25-40ºC, with higher temperatures increasing the rate at which maximum viscosity is achieved. However, too high temperature will degrade the gum and normal function will not be carried out. Guar gum is stable over a wide range of PHs, with its optimal rate of hydration between pH 7.5-9. The maximum viscosity will remain stable between the pH range of 1 to 10.5.Another advantage of guar gum is its ability to be compatible with salts over a wide range of electrolyte concentrations. For instance, guar gum with borate ions, the borate ions act as cross-linking agents with guar gum to form structural gels. It is also a good emulsifier due to the amount of gelatos substituent. Guar gum exhibits stability during freeze-thaw cycles as it is able to retard ice crystal growth by slowing mass transfer across solid and liquid interfaces (Chaplin 2003).

CHAPTER THREE

Material and Methods

3 .1 Material

3.1.1 Sources of Milk

Fresh cow milk was obtained from Animal Production Department Farm, College of Agricultural studies, Sudan University of Science and Technology (Khartoum North, Shambat). Fresh milk samples were taken in clean plastic containers to national food research center laboratory for determination of physiochemical properties , while the skim milk powder was purchased from the local market.

3.1.2 stabilizers used in the study

Gelatin, Gum Arabic and Guar Gum were purchased from the local market, Gelatin chemical composition was 84% to 90% protein, 8% to 12% water, and 2% to 4% minerals. the chemical composition of Gum Arabic 9.55%, moisture, fibers 87%, crude protein 2%, ash 0.54% and fat 0.98%. the chemical composition of guar gum 75%, moisture 9.55%, fibers 7.6%, crude protein 2.16%, ash 0.54% and fat 0.78%.

3.1.3 Yoghurt manufacturing

Four liters of raw milk was transported to the lab of Animal Production at Sudan University of Science and Technology. The milk pasteurized by (85 °C for 30 minutes) . The pasteurized milk was removed from the water bath and after pasteurization , the milk was immediately cooled in water to 45°C then milk divided into four groups (one liter for each) and 27.3gms of skim milk powder was added to each pasteurized liter, to each liter treated with one stabilizers (gelatin , Gum Arabic , Guar Gum) and control yoghurt without stabilizers .based upon some studies (0.4% gelatin, 0.4% Gum Arabic , 0.4% Guar Gum) ,the inoculated usual use 15% starter culture of (Streptococcus Thermopiles, Lactobacillus delbrueckii) for 3 hours at 45 °C in incubator . The yogurt was cooled by refrigerated at 4 °C C for 24 hours before the chemical composition and sensory evaluation.

3.2 Physical-chemical analysis of milk

Milk constituents and chemical characteristics were analyzed constituents (fat, protein, lactose, TS, SNF, Ash, Ca, P and moisture). . The milk Physical characteristics (pH, Acidity and density) were analyzed.

3.2.1 Fat content:

The fat content was determined by Gerber method as described by AOAC (1995) Ten ml, of sulphuric acid (specific gravity 1.820 were measured into Gerber butyrometer . Mixed well, 10 .94/ml, of milk was gently added into the butrymeter tube. One milliliter of amyl alcohol was added and a lock stopper was inserted securely with the stopper´s end up .The Gerber tube was grasped and shacked with precaution until the sample of milk completely digested. The Gerber tubes were centrifuged at 1100 revolution per minute (rpm) for 4 min .The butyrometer was then placed in a water bath at 65°C for 3 min .The fat percent was finally read out directly from the column.

3.2.2 Protein content:

The protein content was determined by kjeldahl method according to AOAC (1990) as follows :

Digestion: ten milliliters of milk were weighed and poured in dry kjeldahl flask and 2 gm of $CuSO_4$ o were added. Concentrated sulphuric acid (25 ml)was added to flask .the flask were heated until a clear solution was obtained (2-3 hrs) and left for another 30 min. the flask were removed and allowed to cool.

Distillation: the digested sample was poured in a volumetric, flask and diluted to 100 ml with distilled water .Five milliliters were distilled using 10 ml of 40% NaOH. The distillate was received in a conical flask (100 ml) containing 25 ml of 2% boric acid plus 3 drops of indicator (bromocresol green + phenolphthalein red) the distillation was continued until the volume in the flask was 75 ml, then the flask was removed from the distillatory.

Titration: the distillate was titrated with 0.1 N HCL until the end point (red color) was obtained .the protein content was calculated from the following equation:

N (%) $T\times 0.1 \times 20 \times 0.014\times 100$

Where:-

T =Reading of titration.

W =Weight of the original sample

$6.38\times$ Protein (%) =N

3.2.3 Total solids (ts):

The Total solids content was determined according to the modified method of AOAC (1990) three grams of the sample were weighed into a dry clean flat –bottomed aluminum dish, and heated on steam bath for

10-15 min. the dish was placed in an oven at 105°C overnight , cooled in a desiccators and weighed quickly. Weightings were repeated until the difference between two successive reading was <0.1 mg the total solids content was calculated from the following equation:

TS (%) w1× 100

Where:

w1 =weight of sample after drying

W0= weight of weight of sample

3.2.4: Solid-non fat content:

Solids-not- fat content was determined from the following equation : NF(%)= %TS - % fat.

3.2 .5 Ash Content:

The ash content was determined according AOAC (1990). Five grams of the sample were weighed into a suitable crucible and evaporated to dryness on steam bath , then placed in a muffle furnace 550°C until ash is carbon free (2-3 hrs)cooled in a desiccators and weighted.

3.2.6 Lactose Determination by Anthrone Method Preparation of solution:

The standard solution was prepared by dissolving 5mg lactose in to 95ml of distilled water to give 5% (w/v) solution of monohydrate. One ml of this solution was diluted with 500ml volumetric flask to give 75mg lactose /ml standard solution. The Anthrone reagent was prepared by dissolving 150mg of Anthrone into 100 ml of 70% (w/v) sulfuric acid. The solution was then cooled and stored overnight (Richard1959)**.**

Procedure:

One ml of milk was pipetted into a 500ml flask with distilled water. The solution was then mixed thoroughly and 0.5ml was transferred to boiling tube (sample) standard stock solution (0.5ml) was transferred to a second boiling(blank).To each tube 10ml ice cooled An throne reagent was added. The tubes were then transferred to boiling water bath for 6 min then transferred to an ice bath and held for 30 min. The optical density (O.D) was read at 625nm Lactose content (in mg/100 ml) was calculated as follows:

$$\text{Lactose}=\frac{\text{O.D(S)}-\text{O.D(B)}\times 4.75}{\text{O.D(SD)}-\text{O.D(B)}}$$

Where: O.D (S) =Optical density of sample. O.D (SD) =Optical density of standard. O.D (B) = Optical density of blank

3.2.7 pH and Titratable Acidity

Milk pH was measured using a pH meter. The acidity of milk was determined according to AOAC (1990). Ten milliliters of sample were placed in a white porcelain dish, and five drops of phenolphthalein indicator were added . Titration was carried out using 0.1 N NaOH until a faint pink color which lasts for 30 seconds was obtained. The titration figure was divided by 10 to get the percentage of lactic acid.

3.3 Chemical Analysis of yoghurt

3.3.1 Fat Content:

The fat content was determined by Gerber method as described by AOAC (1995) .10 ml, of sulphuric acid (specific gravity 1.820 at 155 °C) were measured into Gerber butyrometer. And mixed well, 10 .94ml, of yoghurt was gently added into the butyrmeter tube. One milliliter of amyl alcohol was added and a lock stopper was inserted securely with the stopper´s end up .The Gerber tube was grasped and shacked with precaution until the sample of yoghurt completely digested. The Gerber tubes were centrifuged at 1100 revolution per minute (rpm) for 4 min.The butyrometer was then placed in a water bath at 65 °C for 3 min .The fat percent was finally read out directly from the column.

3.3.2 Protein content:

The protein content of samples was determined by kjeldahl method according to AOAC (1995) as follows:

Digestion:

10 ml of yoghurt were weighed and poured in a clean dry kjeldahl flask and 2 gm of cuso4 were added. Concentrated sulphuric acid [25ml] was added to the flask. The flasks were heated until a clear solution was obtained [2-3 hrs] and left for another 30 min. the flasks were removed and allowed to cool.

Distillation:

the digested sample was poured in a volumetric ,flask [100ML] and diluted with distilled water ,then 15ml of 40 percentage Na OH was added to each flasks and the content of the flask was distillated .The distillate was received in a conical flask [100ml] containing 10 ml of 2 percentage boric acid plus 3 drops of indicator [bromocresol green plus phenolphthalein red].the distillation was continued until the volume in the flask was 75ml,then the flask was removed from the distillatory.

Titration:

The distillate was titrated with 0.1N H Cl until the end point [red color] was obtained .the protein content was calculated from the following equation.

$$N\% = \frac{T \times 0.1 \times 0.014 \times 100}{W}$$

T= Reading of titration

W= weight of the original sample

Protein (%) =%NX×6.38

3.3.3 Total solids content:

Total solids (T.S) content was determined according to AOAC (1995). A clean aluminum moisture dishes were dried at 105 °C for 3hrs. Five grams of the sample were weighed in dry clean flat bottomed aluminum dish and heated on a steam bath for 15 min . The dishes were placed into a forced draft oven at 100 °C for 3hrs. Then cooled in a desiccators and weighed quickly .Weighing was repeated until the difference between the two readings was <0.1mg. The total solids (T.S) content were calculated as follows:

$$T.S.\ \% = \frac{W1}{W2} X100$$

Where:

W1=Weight of sample after drying

W2=Weight of sample before drying

3.3.4 solids –non- fat content

The Solids-not- fat content was determined from the following equation:

SNF (%)= % TS% - %F

3.3.5 Ash Content:

The ash content was determined by gravimetric method AOAC (1995). Five grams of the sample were weighed in a crucibles ,then placed in a muffle furnace at 550-600 °C for 3 hours until ashes were carbon free. The crucibles were then cooled in desiccators and weighed. The ash content was calculated using the following equation:

$$\text{Ash \%} = \frac{w1}{w2} \times 100$$

Where: w1 = weight of ash, w2 = weight of sample.

3.3.6 Calcium and phosphorus determination

The samples were analyzed after aching briefly. Two grams of milk were weighted into platinum crucibles and ached in the furnace at 550 °C for 16 hr. Calcium was determined using an atomic absorption spectrometer and phosphorus was measured at 400 nm by spectrophotometer.

3.3.7 Lactose determination was by Anthrone Method (Richard1959)

Preparation of solution:

The standard solution was prepared by dissolving 5mg lactose in to 95ml of distilled water to give 5% (w/v) solution of monohydrate. One ml of this solution was diluted with 500ml volumetric flask to give 75mg lactose /ml standard solution. The Anthrone reagent was prepared by dissolving 150mg of Anthrone into 100 ml of 70% (w/v) sulfuric acid. The solution was then cooled and stored overnight.

Procedure:

One ml of yoghurt was pipette into a 500 ml flask with distilled water. The solution was then mixed thoroughly and 0.5ml was transferred to boiling tube (sample) standard stock solution (0.5ml) was transferred to a second boiling(blank).To each tube 10ml ice cooled Anthrone reagent was added. The tube were then transferred to boiling water bath for 6 min then transferred to an ice bath and held for 30min. The optical density (O.D) was read at 625nm Lactose content (in mg/100ml) was calculated as follows:

$$\text{Lactose}=\frac{\text{O.D(S)}-\text{O.D(B)}\times 4.75}{\text{O.D(SD)}-\text{O.D(B)}}$$

Where: O.D (S) =Optical density of sample. O.D (SD) =Optical density of standard. O.D (B) = Optical density of blank.

3.4 Physical Analysis of yoghurt

3.4.1 pH and Titrable Acidity:

pH was determined by electric pH meter (Hanna instrument pH, 209). 10 ml of yoghurt were pipette into the tube ,then the pH meter was adjusted with buffer PH 4 , the PH meter was placed into the sample, and the PH was directly read.

The acidity of yoghurts was determined according to AOAC (1990).Ten milliliters of sample were placed in white porcelain, and five drops of phenolphthalein indicator were added. Titration was carried out using 0.1 N Na OH until a faint pink color with lasts for 30 seconds was obtained. The titration figure was divided by 10 to get the percentage of lactic acid.

$$T.A\ \%\ \frac{9\times 0.1\text{ml of NaOH}}{\text{Milk weight}}$$

3.4.2 Determination of viscosity

Viscosity was measured by viscometer (hake georz aueegermany).

Two reading were taken by viscometer , at higher and at lower speed.

3.5 Sensory Evaluation

30 panelists were asked to Judge on taste, smell, flavor, colour , viscosity and over all acceptability ranged from 1----10, where we evaluated the best for the parameters assessed in this study , and zero for the worst one in the above mentioned parameters.

Their answer given in the evaluation was formulated as data then evaluated using proper statistical model.

3.6 Statistical analyses

Data generated was subjected to statistical analysis system (SPSS) program. Statistical package of social science version 11.5 using analysis of variance (Independent T test for fresh milk and (CRD) completely randomized design for yoghurt and means separated by Duncan's test Duncan's Multiple range test (DMRT).

CHAPTER Four

RESULTS AND DISCUSSION

4.1 Chemical composition of the milk

Result in Table (3.1) shows the chemical composition (fat, protein, moisture, total solid, ash, acidity, and pH) of fresh cow milk. Those results were on line with Johnson (1980) who found that chemical composition of cow milk were that water 87.20% ,fat 3.70% ,protein 53.50% , Ts 12.80% ,and ash 0,70%.

Generally the milk composition is affected by water availability, stage of lactation and availability of the green fodder as well as the differences in management system under which the herds are kept (Farah ,1996, Backeit,1999).

(table3.1)Raw milk analysis:

The parameters	The contents
Moisture (%)	87.73
Protein (%)	3.2
Fat (%)	3.3
Ash (%)	0.79
Lactose (%)	4.98
T.S	12.27
T.N.F	8.97
pH	6.3
Acidity mg/100g	0.18
Calcium mg/100g	119
Phosphor mg/100g	96
Viscosity	490

4.2 Physic- chemical composition of control yoghurt s:

The differences of physic -chemical of natural yoghurt found in literature may be due to breed, season, nutrition and the addition of skim powder milk.

In this study the average for control yoghurt fat was 3.6% its higher than that reported by (Weerathilake , 2014) 3% and Suleiman (1982) reported 3.1% and the protein content was 4.8% its higher than that reported by Kosikowski (1982) 3.45% and lower than that reported by (Weerathilake , 2014) 5.7% and the lactose was 5.41% its higher than that reported by Kosikowski (1982) 5.15% , and lower than that reported by (Weerathilake, 2014) 7.8%. The total solid was 14.6% its higher than that reported by Kosikowski (1982) 10.98% and by (Weerathilake ,2014) , where the pH was 4.35 after 10 days it’s higher than that reported by Suleiman (1982).

Table (4.1): chemical Analysis of Yoghurt made from different type of stabilizers:

Parameters	Treatments means				Grand Mean	Std. Error
	A Yoghurt+ Gelatin	B Yoghurt+ Gum Arabic	C Yoghurt+ Guar gum	D Yoghurt control		
Moisture (%)	85.10^{a}	84.60^{a}	84.57^{a}	85.32^{a}	84.90	±0.16
Protein (%)	5.23^{a}	4.53^{b}	4.70^{b}	4.87^{ab}	4.83	±0.10
Fat (%)	3.73^{a}	3.53^{a}	3.40^{a}	3.63^{a}	3.58	±0.08
Ash (%)	0.80^{ab}	0.81^{ab}	0.85^{a}	0.76^{b}	0.80	±0.01
Lactose (%)	5.14^{b}	6.52^{a}	6.49^{a}	5.42^{b}	5.89	±0.24
Ts (%)	14.90^{a}	15.40^{a}	15.43^{a}	14.68^{a}	15.10	±0.16
S.N.F (%)	11.17^{b}	11.87^{ab}	12.03^{a}	11.04^{b}	11.53	±0.19
Ca^{++}(mg/100g)	118.67^{b}	129.67^{a}	128.33^{a}	115.33^{c}	123.00	±1.89
P (mg/100g)	108.33^{a}	100.67^{b}	99.33^{bc}	95.00^{c}	100.83	±1.53

Different superscript letters (a to d) within the same raw showed significant differences among the groups (P<0.05).

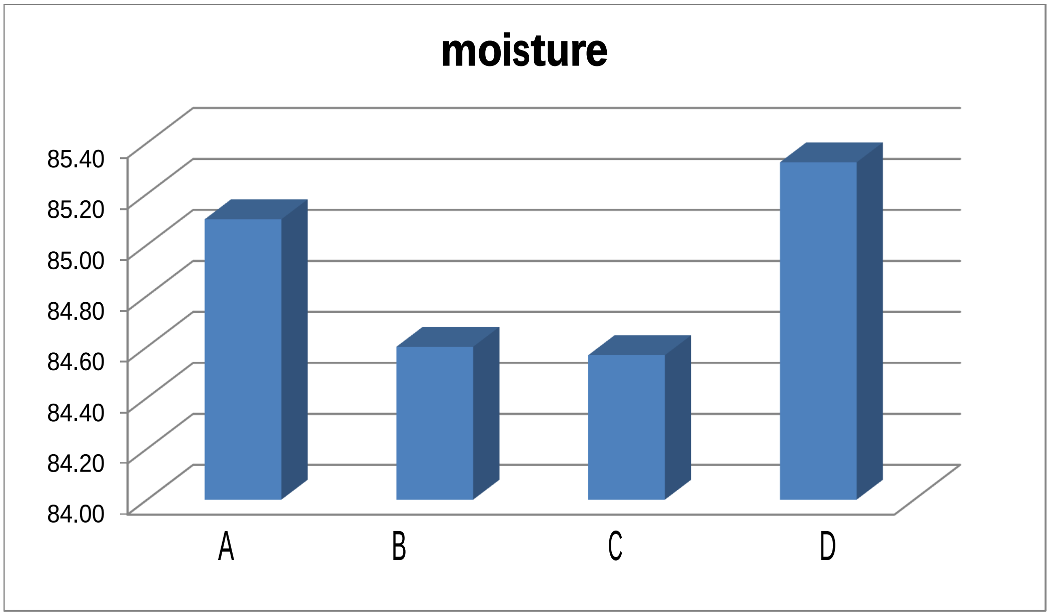
* *significant* at*(P<0.05).*

** highly *significant* at (p*<0.01).*

4.2.1Moisture content

Figure (4.1) shows the changes in moisture content of yoghurt made from different type of stabilizers. There was no significant difference ($p \leq 0.05$) between all types of yoghurt the highest moisture content (85.323) was obtained in control yoghurt at day zero of processing followed by yoghurt made with Gelatin (85.100), then yoghurt made with Gum Arabic (84.60) while the lowest moisture content (84.56) was obtained by yoghurt made with Guar gum. Guar gum is used as a thickener and stabilizer in the food industry as a result of its hydration and water-binding properties. It is used as a stabilizer at a Concentration of 3.0% in ice cream, ice pops, and sherbet. It improves the body, texture, chewiness, and heat-shock resistance by binding free water (Wielinga 2000).

Figure (4.1): **Effect of type of stabilizers on moisture content**



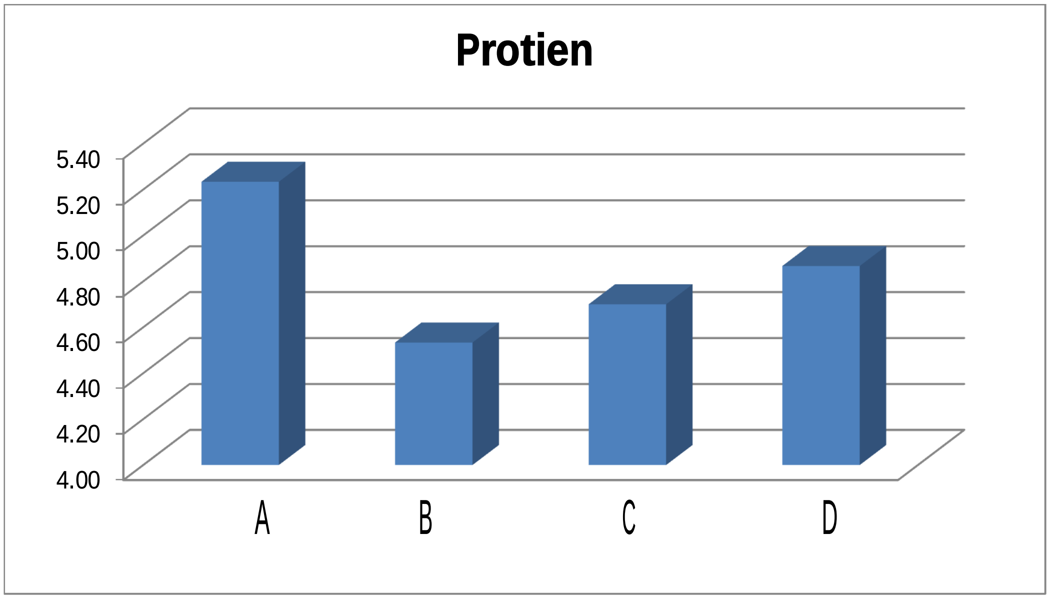
A= Gelatin ***B***= Gum Arabic ***C***= Guar gum ***D*** control yoghurt

4.2. 2 Protein content:

Figure (4.2) shows the changes in protein content of yoghurt affected by different type of stabilizers. there is no significant differences ($p \leq 0.05$) . the highest protein content (5.23%) was obtained by yoghurt made with Gelatin , followed by control yoghurt (4.86), then yoghurt made with Guar gum (4.70) while the lowest protein content (4.53%) was obtained by yoghurt made with Gum Arabic .

In the food industry gelatin is used not only for its functional properties, but also because of its importance as a source of protein in the daily diet (Fizman and Salvador, 1999).

Figure (4.2): **Effect of type of stabilizers on Protein content**

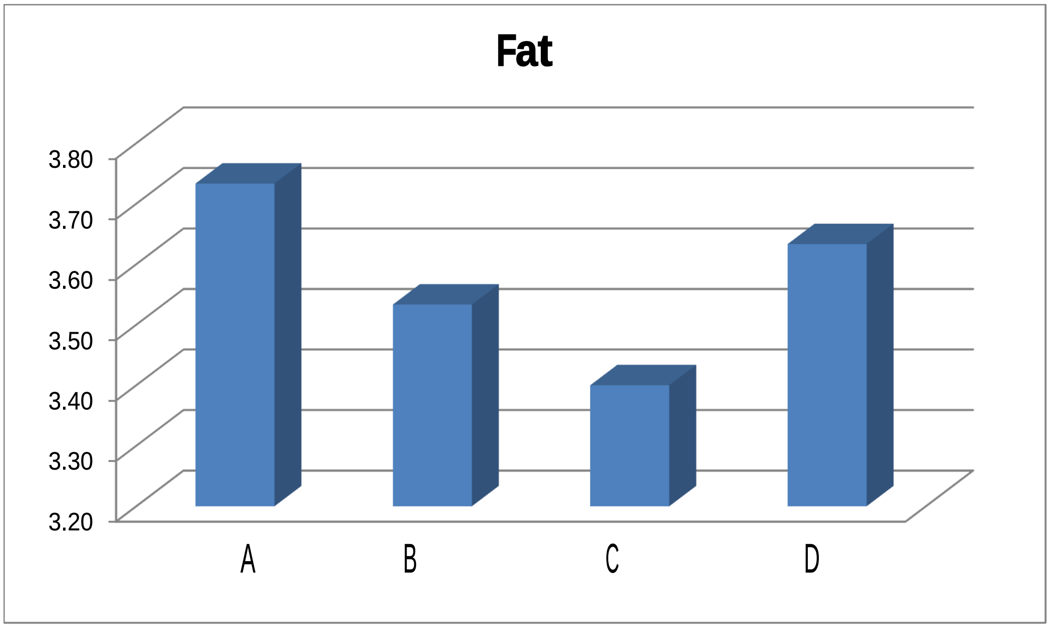


A= Gelatin B= Gum Arabic C= Guar gum D= control yoghurt

4.2.3 Fat content:

Figure (4.3) shows the changes in protein content of yoghurt affected by different type of stabilizers .there is No significant differences ($p \geq 0.05$). the highest fat content (3.73) was obtained by yoghurt made with Gelatin, followed by control yoghurt (3.63), then yoghurt made with Gum Arabic (3.53) and the lowest fat content (3.40) was obtained in yoghurt made with Guar gum . Gelatin is an ingredient compatible with the milk proteins and contributes good palatability to the end product, giving a fat-like sensory perception because of its unique property of melting at mouth temperature. It eliminates syneresis and considerably reinforces the mechanical resistance of the gels making it possible to obtain a wide range of texture (Fiszman and Salvador, 1999).

Figure (4.3): **Effect of type of stabilizers on fat content**

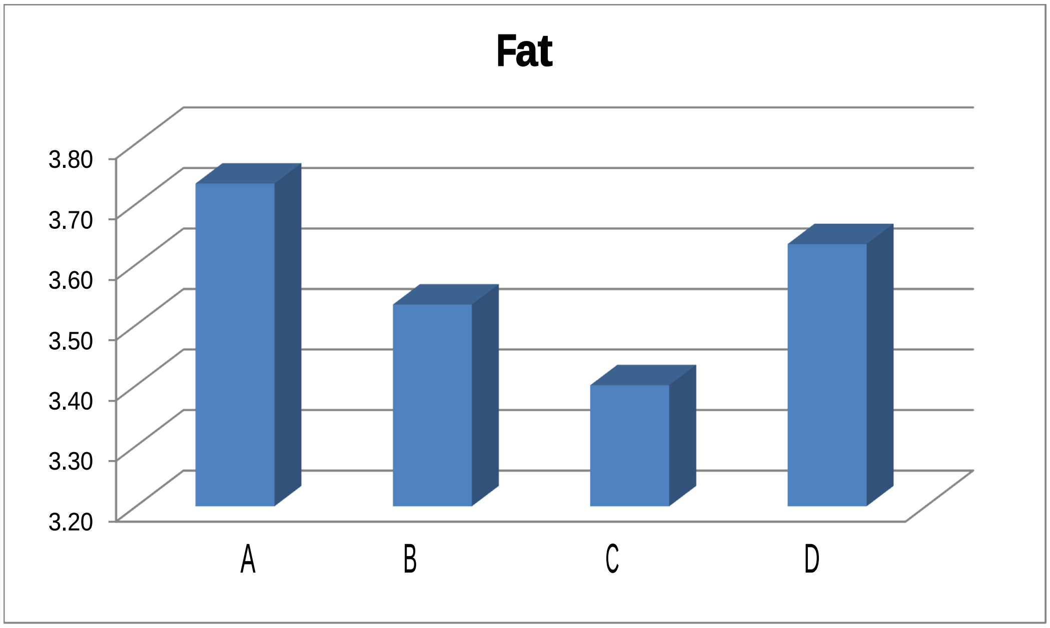


A= Gelatin B= Gum Arabic C= Guar gum D control yoghurt

4.2.4 Ash content:

Figure (4.4) shows the changes in protein content of yoghurt affected by different type of stabilizers .there was no significant differences between deferent yoghurt ($p \geq 0.05$).the highest ash content (0.84) was obtained by yoghurt made from Guar gum, followed by yoghurt made with Gum Arabic (0.81) then yoghurt made with Gelatin(0.79) and while the lowest ash content (0.76) was obtained in control yoghurt .advantage of guar gum is its ability to be compatible with salts over a wide range of electrolyte concentrations. For instance, guar gum with borate ions, the borate ions act as cross-linking agents with guar gum to form structural gels. It is also a good emulsifier due to the amount of gelatos substituent. Guar gum exhibits stability during freeze-thaw cycles as it is able to retard ice crystal growth by slowing mass transfer across solid and liquid interfaces (Chaplin 2003).

Figure (4.4): **Effect of type of stabilizers on Ash content**

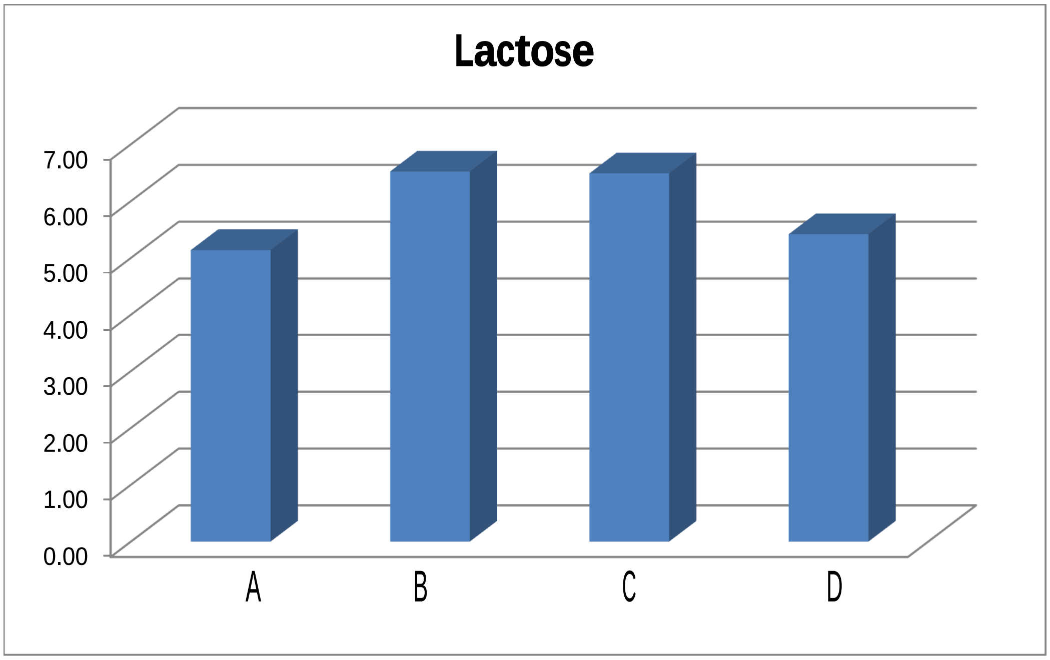


A= Gelatin B= Gum Arabic C= Guar gum *D*= control yoghurt

4.2.5 Lactose content:

Figure (4.5) shows the changes in lactose content of yoghurt made with different type of stabilizers. There are no significant differences ($p \leq 0.05$) the highest lactose (6.52) was obtained by yoghurt made with Gum Arabic. Followed by yoghurt made with Guar gum (6.48), then control yoghurt (5.41) while the lowest lactose content (5.13) was obtained from yoghurt made with Gelatin.The principle use of gum Arabic is in food stuffs and is extensively used in the confectionery industry It also acts as an emulsifier, keeping the fat uniformity distributed throughout the products; it is also used as an additive and stabilizer in bakery products, as an emulsifier and fixative in flavor industry, stabilizer in dairy products and as foam stabilizer in beverages. It is used in coating polished food material and for the preparation of dry powdered stable vitamins (Ali, 1996).

Figure (4.5): **Effect of type of stabilizers on Lactose content**



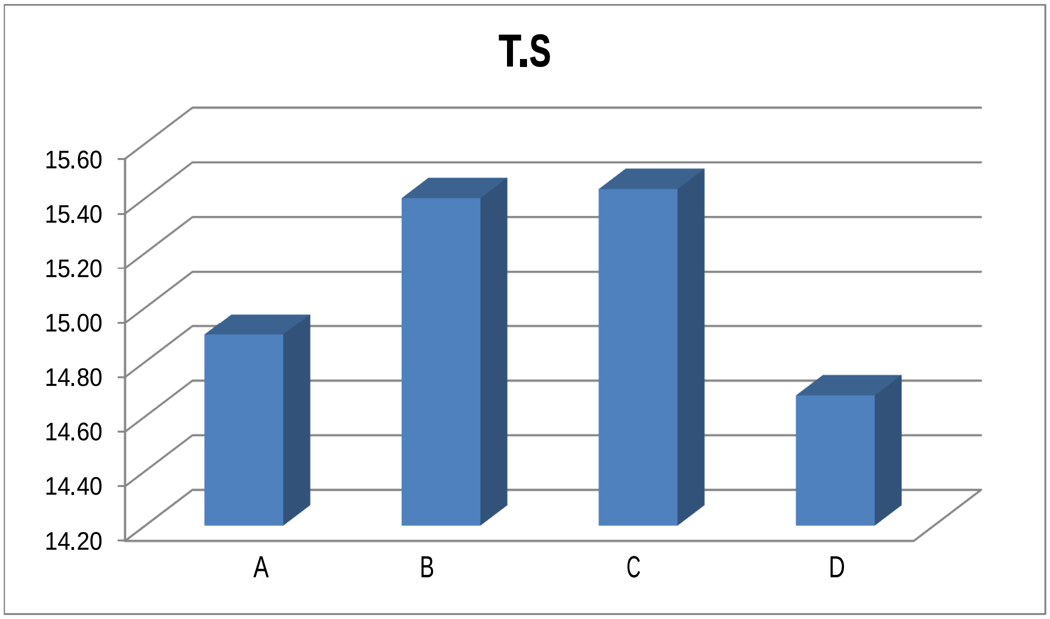
A= Gelatin B= Gum Arabic C= Guar gum D control yoghurt

4.2.6 T.S.S content:

Figure (4.6) shows the changes in T.S.S content of made with different type of stabilizers. no significant differences ($p \leq 0.05$) .the highest T.S.S (15.43) was obtained by yoghurt made with Guar gum. followed by yoghurt made with Gum Arabic (15.40) then yoghurt made with Gelatin (14.90) and while the lowest ash content (14.67) was obtained from control yoghurt.

the water availability, stage of lactation, and availability of green fodder as well as the differences in management system under which the animal are kept were the main factors affecting the composition of milk (Farah, 1996,Backeit,1999).

Figure (4.6): **Effect of type of stabilizers on T.S.S content**

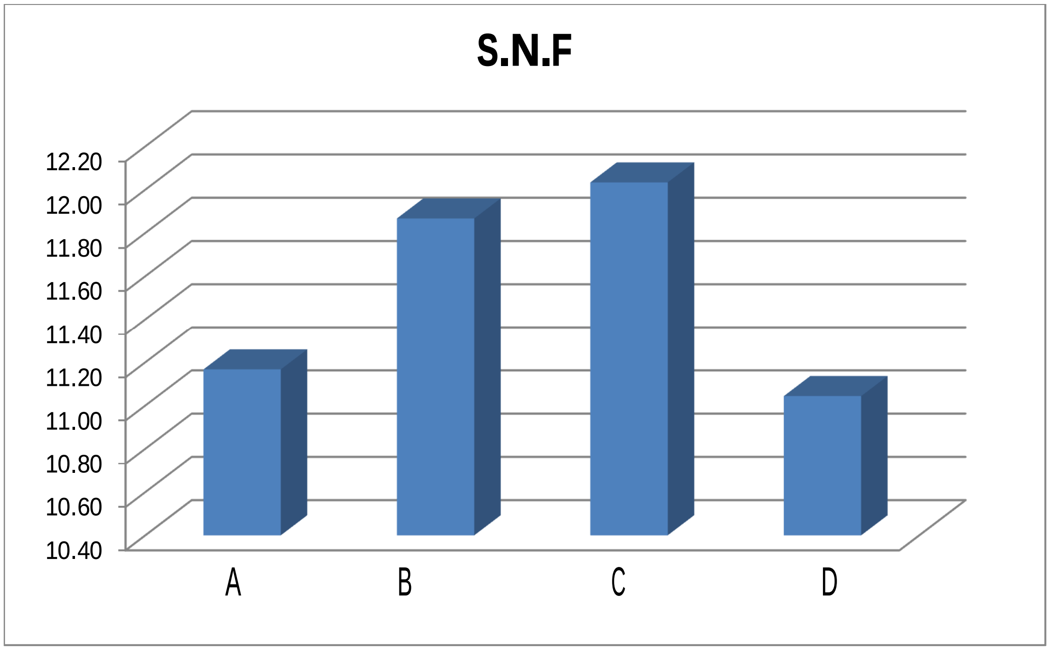


A= Gelatin B= Gum Arabic C= Guar gum D control yoghurt

4.2.7 S.N.F content

Figure (4.7) shows the changes in S.N.F content of yoghurt affected by different type of stabilizers highly significant differences ($p \leq 0.05$). the highest S.N.F (12.03) was obtained by yoghurt made from Guar gum.. followed by yoghurt made from Gum Arabic (11.86) then yoghurt made from Gelatin(11.16) and while the lowest ash content (11.04) was obtained from control yoghurt. Guar gum can also be used in dietetic beverages or low carbohydrate products due to its suspending ability and improving body of thin and watery products. (Meer 1977).

Figure (4.7): **Effect of type of stabilizers on S.N.F content**

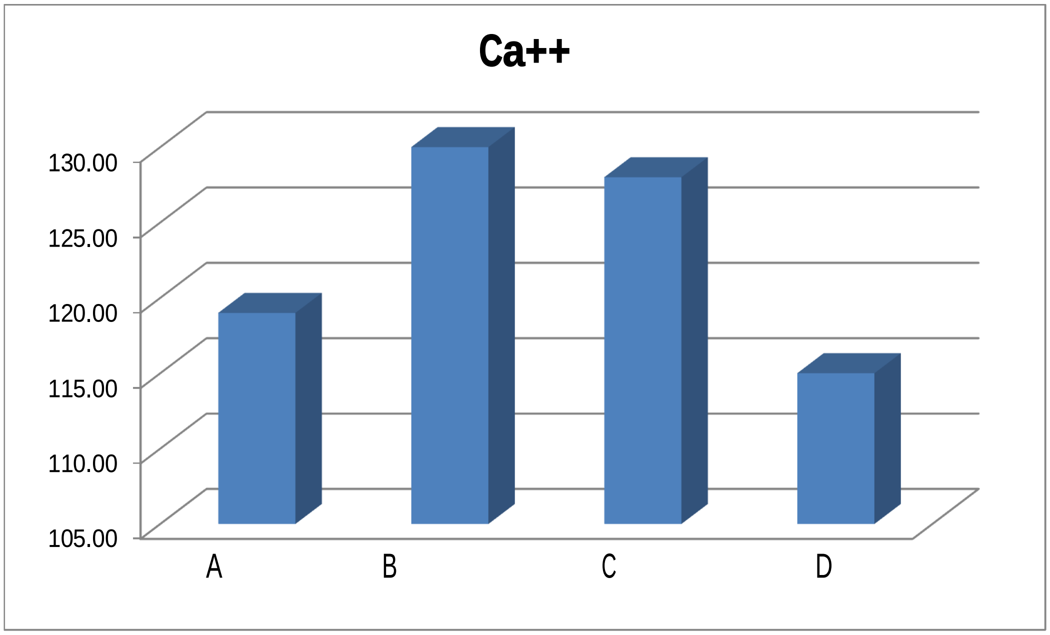


A= Gelatin B= Gum Arabic C= Guar gum D control yoghurt

4.2.8 Calcium content:

Figure (4.8) shows the changes in Ca content of yoghurt affected by different type of stabilizers. Highly significant differences ($p \leq 0.05$) .the highest Ca (129.67) was obtained by yoghurt made from Gum Arabic followed by yoghurt made from Guar gum (128.33).) then yoghurt made from Gelatin(118.67) and while the lowest ash content (115.33) was obtained from control yoghurt. Essential minerals are present in milk products in varying levels depending on technological treatments of the products, the type of milk base used and the accuracy of analysis (Miller et al., 2000).

Figure (4.8): **Effect of type of stabilizers on Calcium content**

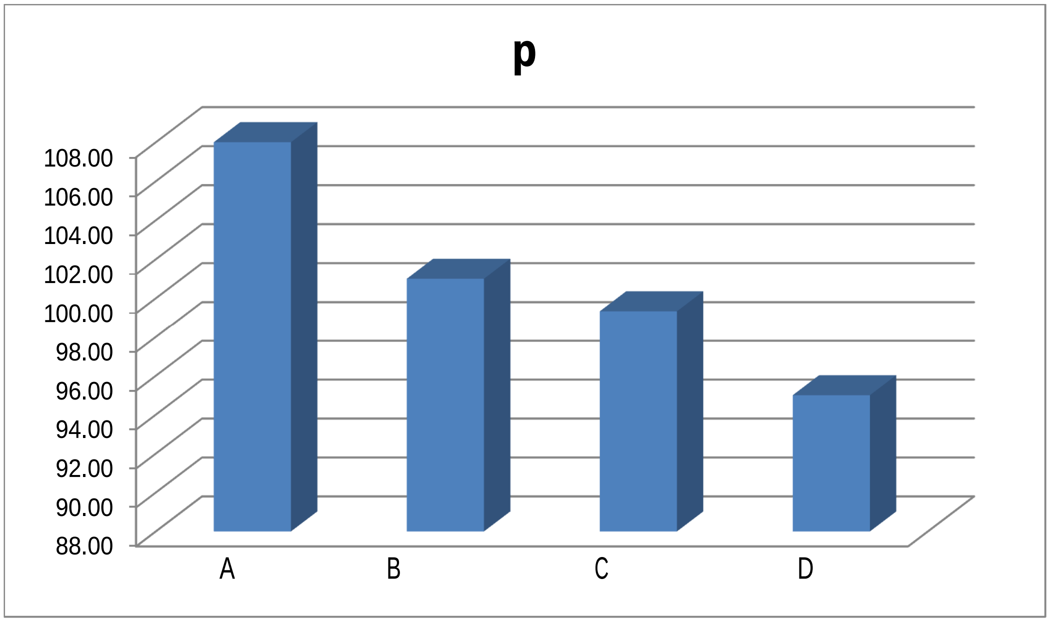


A= Gelatin B= Gum Arabic C= Guar gum D= control yoghurt

4.2.9 Phosphorus content:

Figure (4.9) shows the changes in P content of yoghurt affected by different type of stabilizers. Highly significant differences ($p \leq 0.05$).the highest P content (108.3) was obtained by yoghurt made from Gelatin. followed by yoghurt made from Arabic gum (100.67) then yoghurt made from Guar gum(99.33 while the lowest P content (99.3) and while the lowest ash content (95.00) was obtained from control yoghurt. Phosphorus plays a vital role in the structure of cell membranes and virtually all metabolic processes (Miller, 2008).

Figure (4.9): **Effect of type of stabilizers on Phosphorus content**



A= Gelatin B= Gum Arabic C= Guar gum D= control yoghurt

Table (4.2): Physical Analysis of Yoghurt made from different type of stabilizers:

Parameters	Treatments means				Grand Mean	Std. Error
	A Yoghurt+ Gelatin	B Yoghurt+ Gum Arabic	C Yoghurt+ Guar gum	D Yoghurt control		
pH	5.70^{b}	5.60^{b}	5.40^{c}	5.87^{a}	5.64	±0.06
Acidity	0.73^{c}	0.75^{b}	0.78^{a}	0.72^{d}	0.75	±0.01
Viscosity	726.67^{bc}	775.00^{ab}	798.00^{a}	710.00^{c}	752.42	±15.53

Different superscript letters (a to d) within the same raw showed significant differences among the groups (P<0.05).

* *significant* at*(P<0.05).*

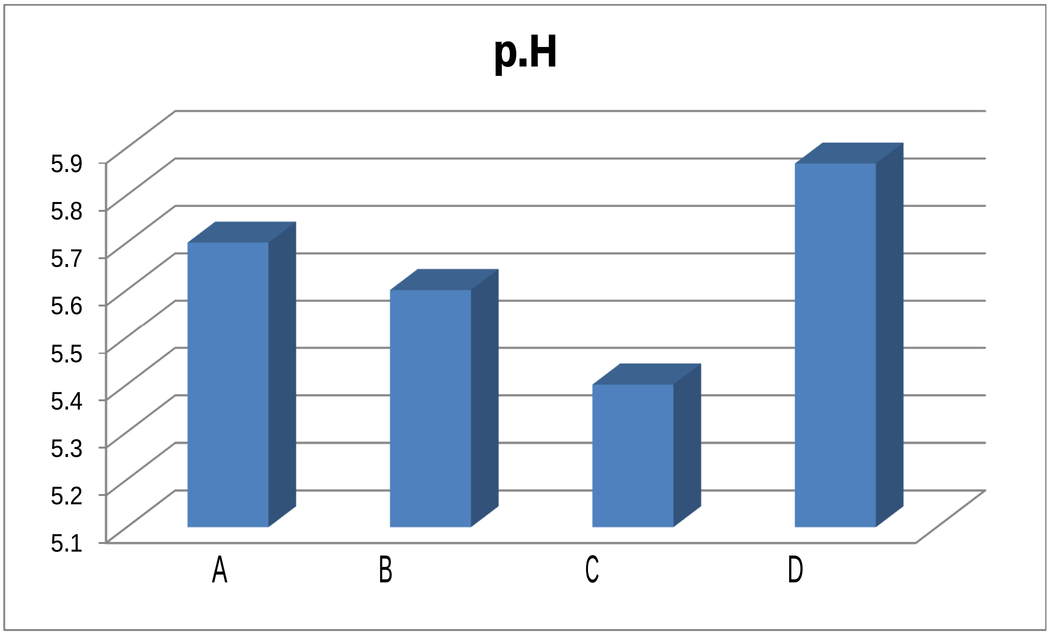
** highly *significant* at (p*<0.01).*

4.2.10 PH Value:

Figure (4.10) shows the changes in pH of yoghurt affected by different type of stabilizers.

Highly significant differences ($p \leq 0.05$).the highest pH value (5.86) was obtained by control yoghurt. Followed by yoghurt made from by yoghurt made from Gelatin (5.70). then yoghurt made from Arabic gum (5.60) and while the lowest pH value (5.40) was obtained from yoghurt made from Guar gum. which slower the rate of fermentation by lactic acid bacteria that found in starter culture (Galal et al .,2004;Gouda et al., 2004).

Figure (4.10): **Effect of type of stabilizers on P.H content**

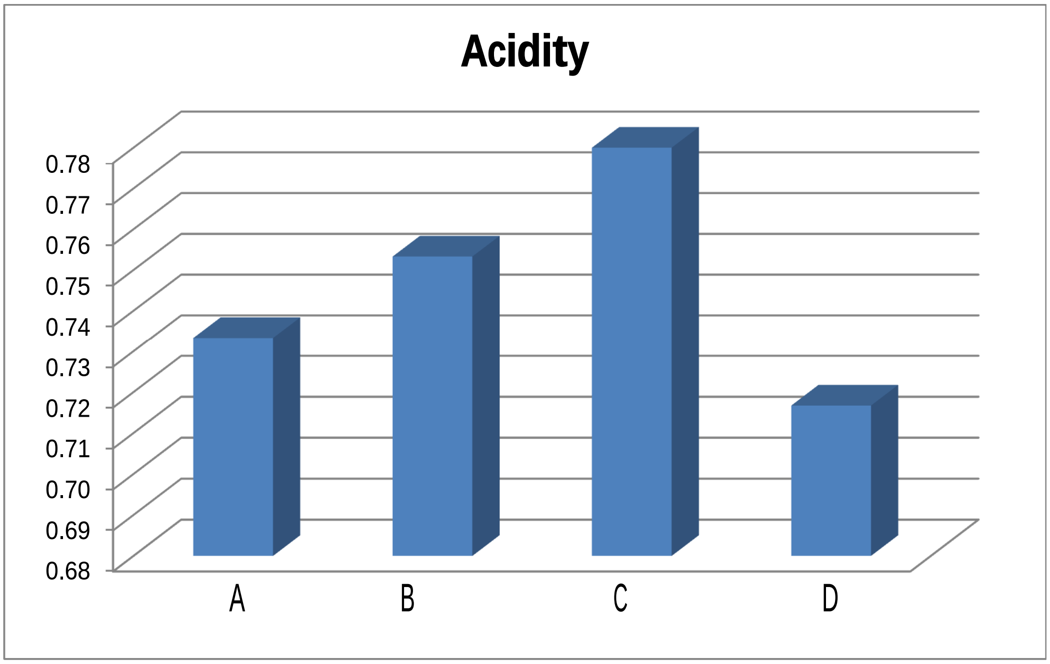


A= Gelatin ***B***= Gum Arabic ***C***= Guar gum ***D*** control yoghurt

4.2.11 Titratable Acidity:

Figure (4.11) shows the changes in titratable acidity of yoghurt affected by different type of stabilizers. highly significant differences ($p \leq 0.05$).The highest titratable acidity (0.78) was obtained by yoghurt made from Guar gum. followed by yoghurt made from Gum Arabic (0.75) then yoghurt made from Gelatin(0.73) and while the lowest titratable acidity (0.71) was obtained from control yoghurt .the pasteurization and incubation temperatures revealed significant effects for the titratable acidity and pH. Also the high values of the titratable acidity and lower level of pH for the processed yoghurt revealed that the temperatures is the most important factor in controlling the growth of the microorganisms in the milk and dairy products (olson, 1980).

Figure (4.11): **Effect of type of stabilizers on Acidity content**



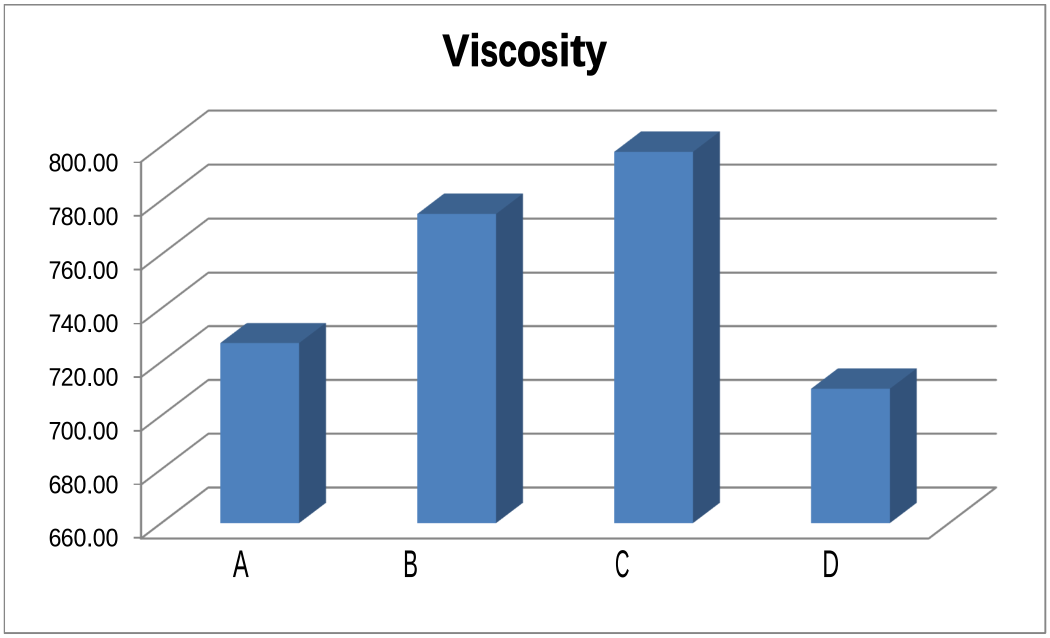
A**=** Gelatin ***B*****=** Gum Arabic ***C*****=** Guar gum ***D***= control yoghurt

4.2.12 Viscosity:

Figure (4.12) shows the changes in viscosity of yoghurt affected by different type of stabilizers .Highly significant differences ($p \leq 0.05$). The highest viscosity value (798.00) was obtained by yoghurt made from Guar gum. Followed by yoghurt made from Gum Arabic (775.00) then yoghurt made from Gelatin (726.67) and while the lowest ash content (710.00) was obtained from control yoghurt. Guar gum is used as a thickener and stabilizer in the food industry as a result of its hydration and water-binding properties. It is used as a stabilizer at a concentration of 3.0% in ice cream, ice pops, and sherbet. It improves the body, texture, chewiness, and heat-shock resistance by binding free water (Wielinga2000). Its viscosity is dependent upon factors such as time, temperature, concentration, pH, ionic strength, and type of agitation. Maximum viscosity is reached during the temperature range of 25-40ºC, with higher temperatures increasing the rate at which maximum viscosity is achieved. (Chaplin 2003).

Increase in fat and milk protein may cause increase in viscosity but whereas this components were equal in all samples so increase in viscosity was only due differences in type of stabilizers (Tarkashv and, Yadolah, 2005).

Figure (4.12): **Effect of type of stabilizers on Viscosity content**



A= Gelatin ***B***= Gum Arabic ***C***= Guar gum ***D*** control yoghurt

4.3 Visual observation:

The findings obtained from this study showed that the samples of yoghurts The best viscosity were obtained by yoghurt made with Guar gum, where it revealed thicker and more homogeneous.

4.4 Sensory evaluation:

The sensory evaluation of yoghurt made with different type of stabilizer significant ($p \leq 0.05$) in taste, flavor, smell, and overall acceptability, but had no significant ($p \geq 0.05$) recorded on the texture and color, Table (4.3).

The best value for taste, flavor, smell, texture and overall acceptability were obtained by yoghurt made with Gelatin followed by yoghurt made with both Arabic gum and control yoghurt and the last one was yoghurt made with Guar gum Table (4.3).

Table (4.3): Sensory Evaluation of Yoghurt made from different type of stabilizers:

Parameters	**Treatment means± SE**			
	A	**B**	**C**	**D**
Taste	7.2667	6.7667	5.0333	7.3333
Color	7.6000	7.4333	6.5000	6.9667
Flavor	7.1667	6.2667	4.9000	6.6333
Smell	7.3333	7.1333	5.2333	7.0000
Texture	6.9000	7.2000	6.4333	6.8333
Overall acceptability	7.3333	7.1000	5.9667	7.1333

A*=** Gelatin ***B*=** Gum Arabic ***C*=** Guar gum ***D= control yoghurt

4.5 Economical impact of using different stabilizers

The results in table (4-4) showed that he cost of yoghurt according to the type of stabilizer used, where he price of one kilogram of each were 50 SD, 60 SD and 60SD for guar gum, gum Arabic and gelatin respectively, so that the price of the cup of yoghurt made with guar gum is slightly cheaper than that other produced from other stabilizers of this study.

Table (4-4): Economical impact of using different stabilizers:

Stabilizers	**A**	**B**	**C**	**Level of sig**
Cost KM/ SD	60	60	50	NS
The cost of stabilizer (10 gms) of yoghurt 250gms in SD	0.6	0.6	0.5	NS

Where: - N S: no significant

A**=** Gelatin ***B*****=** Gum Arabic ***C*****=** Guar gum ***D***= control yoghurt

CHAPTER FIVE

Conclusions and Recommendations

5.1 Conclusion

From the results obtained we can conclude the following:

1.Yoghurt made with Gelatin was super in terms of sensory characteristics , obtained the highest, Ash ,Protein, Fat, SNF, , pH, Calcium and Phosphorus.,, while the lowest protein content was obtained in yoghurt made with Gum Arabic .

2. Yoghurt made with Guar gum the highest Ash, T.S.N.F and viscosity, while the lowest viscosity was obtained in yoghurt made with Gelatin.

3. Yoghurt made with Gum Arabic the highest Lactose, T.S.S, while the lowest lactose was obtained in control yoghurt.

4. the addition of stabilizers altered the chemical composition and enhance nutritive value of yoghurt.

5. Although yoghurt made with Gelatin scored the highest overall a acceptability.

5.2 Recommendations

1. Recombination of fresh milk with skim milk powder will improve the chemical and sensory characteristics of yoghurt.
2. Stabilizers powder shall be used without blending in water in yoghurt manufacture.
3. Adding stabilizers shall be used (0.4% Guar Gum).

CHAPTER Six

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