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Sudan University of Science and Technology College of Graduate Studies

Effect of different levels of Watermelon (*Citrullus lanatus*) seeds powder on Chemical and sensory properties of set Yoghurt during storage

أثر إضافة مستويات مختلفة من مسحوق بذور البطيخ علي الخصائص الكيميائية والحسية للزبادي الجامد أثناء فترة التخزين

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(إِنَّمَا قَوْلُنَا لِشَيْءٍ إِذَا أَرَدْنَاهُ أَنْ نَقُولَ لَهُ كُنْ فَيَكُونُ)

صدق الله العظيم

(سورة النحل 40)

Dedication

In the name of God, The most compassionate and Merciful. This work is dedicated with mercy and love, to...... To my parents, for their prayers and encouragement me. To my lovely great family my brothers and sisters, for their support. My gratitude and love to my small family my husband, for his endless support and patience all period of my study, and to my lovely son Albraa.

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ABSTRACT

This study was conducted at the Department of Dairy Science and Technology, College of Animal Production Science and Technology, Sudan University of Science and Technology during May 2017 to evaluate the effect of adding different levels of Watermelon (Citrullus lanatus) seeds powder on physicochemical and sensory properties of set Yoghurt during storage. Ten liters of fresh cow's milk were purchased from dairy farm, College of Animal Production Science and Technology. Water melon seeds were purchased from popular market of Omdurman, and then they were milled. The milk was heated at 90-95°C for 5min and then cooled to 45°C; Therefore divided into four equal portions. Four treatments were carried out. The first treatment is yoghurt with no additive (a control); in the second, third and fourth treatments 5%, 7% and 10% of watermelon seeds powder were added and mixed with cow's milk respectively. Three % of starter cultures were added to each treatment, then packed in plastic cups (200ml capacity) in triplicates for each treatment and incubated at 45°C until coagulation occurred. Yoghurt samples were cooled and stored in a refrigerator (4°C) for 10 days. Chemical composition and sensory evaluation were carried out for the yoghurt samples in all treatments at intervals of 1, 5 and 10 days. The results indicated that watermelon seeds powder and storage period had highly significant effect (P<0.01) on chemical composition (protein, fat, titratable acidity, total solid, ash). The sensory characteristics of the yoghurt samples found to be affected significantly (P<0.01) by the watermelon seeds powder on color, flavor and taste; however the texture and overall acceptability were not affected significantly (P>0.05). Storage period had no significant (P>0.05) effect on sensory characteristics of the yoghurt samples. The best sensory evaluation was for the control followed by yoghurt with 5% of watermelon seeds powder samples. According to the sensory evaluation of yoghurt samples it would be concluded that watermelon seeds can be added up to 5% into milk for preparation of yoghurt

مستخلص البحث

أجريت هذه الدراسة لتقييم تأثير إضافة مستويات مختلفة من بدرة بذور البطيخ (Citrullus lanatus) علي الخصائص الفيزيوكيميائية والحسية للزبادي الجامد أثناء فترة التخزين. تم إجراء هذه التجربة في وحدة تصنيع الالبان بكلية علوم وتكنولوجيا الإنتاج الحيواني جامعة السودان للعلوم والتكنولوجيا خلال شهر مايو 2017م. تم الحصول على 10 لتر من لبن الابقار من مزرعة الألبان بكلية علوم وتكنولوجيا الإنتاج الحيواني وتم الحصول على بذور البطيخ من السوق الشعبي أم درمان ومن ثم طحنت. تم تسخين أو غلى اللبن في درجة حرارة 90 – 95 ٥م لمدة 5 دقائق وتبريده ل45 ٥م ؛ وتم تقسيمه لأربعة أقسام متساوية كالآتي: المعاملة الاولى لبن أبقار من غير اضافة كعينة ضابط أما المعاملة الثانية والثالثة والرابعة تم إضافة 5%. 7%. 10% من بدرة بذور البطيخ على التوالي. تمت إضافة البادئ لكل معاملة بنسبة 3%. ثم تمت التعبئة في عبوات بلاستيكية سعة 200 مل بثلاث مكررات لكل معاملة وتم تحضين المعاملات في الحضان بدرجة حرارة 45 ٥م حتى حدوث التخثر. بعد ذلك تم التبريد والتخزين في الثلاجة بدرجة حرارة 4 ٥م لمدة 10 ايام تم إجراء التحليل الكيميائي والتقييم الحسى للعينات في اليوم الاول والخامس والعاشر. أظهرت النتائج أن بدرة بذور البطيخ وفترة التخزين لهما أثر معنوي كبير (P<0.01) على التحليل الكيميائي للعينات (البروتين. الدهن. الحموضة. المواد الصلبة الكلية. الرماد). واوضحت النتائج أن لبدرة بذور البطيخ أثر معنوى كبير (P<0.01) على التقييم الحسى للعينات من حيث اللون. النكهة و الطعم؛ بينما لم يكن لها أثر معنوى (P>0.05) على الملمس والقبول العام. أيضاً اظهرت النتائج أنه ليس لفترة التخزين أثر معنوى (P>0.05) على التقييم الحسى للعينات. أوضحت نتائج البحث أن أفضل تقييم حسى كان لعينات الضابط تليه عينات الزبادي المضاف إليه 5% من بدرة بذور البطيخ. وبناءً على التقييم الحسى للعينات يستنتج أنه يمكن إضافة بدرة بذور البطيخ بنسب حتى 5% للبن المراد تصنيع الزيادي منه.

CHAPTER ONE INTRODUCTION

Milk (mammal milk) is a white liquid produced by the mammary glands of mammals. It is the primary source of nutrition for young mammals before they are able to digest other types of food (Pehrsson *et al.*, 2000).

The nutritional value of milk is particularly high due to the balance of the nutrients that it composes. The composition varies among animal species and breeds within the same species, and also from one dairy to the other, depending on the period of lactation and diet. Milk contains several groups of nutrients; organic substances are present in about equal quantity and are divided into elements builders: proteins, and energy components: carbohydrates and lipids; it also comprises functional elements, such as traces of vitamins, enzymes and dissolved gases, and contains dissolved salts, especially in the form of phosphates, nitrates and chlorides of calcium, magnesium, potassium and sodium (Guetouache *et al.*, 2014).

Dairy processing involves conversion of raw milk into fluid milk products, and an array of dairy products such as butter, yoghurt, and fermented milks, cheeses, dry milk powders, dry whey products, ice cream, and frozen and refrigerated desserts (Chandan *et al.*, 2008).Yoghurt has long been known in human history as a way of preserving milk. Despite the distinctive acidity of natural yoghurt, the consumption of yoghurt or other cultured milk products is believed to have additional health promoting benefits to the host (Lee and Salminen, 2009). Yoghurt represents a very significant dairy product worldwide in modern times, it is a semi- solid fermented product made from a heat-treated standardized milk mix by the activity of a symbiotic blend *Streptococcus*

thermophilus and *lactobacillus delbrueckii* subsp. *bulgaricus* (Clark and Plotka, 2004; Ozer, 2010).

In certain countries, the nomenclature of yoghurt is restricted to the product made exclusively from the two lactic cultures, whereas in other countries it is possible to label a product as yoghurt if it is made with yogurt cultures and adjunct probiotic cultures. The more common adjunct cultures are *lactobacillus acidophilus*, Bifidobacterium SPP, *lactobacillus gasseri* and *lactobacillus rhamnosus* (Maity and Misra, 2009; Chandan and Nauth, 2012). In recent years, there is an increasing trend towards the production of herbal yoghurts by incorporating natural food additives and health promoting substances such as addition of neem (*Azadirachta indica*) and cinnamon (*Cinnamomum verum*) into yoghurt (Shori and Baba, 2011; Shori and Baba, 2012; Shori and Baba, 2013).

Objectives of the study:

The objectives of the study are to determine:

- 1. The effect of watermelon (*Citrullus lanatus*) seeds powder on the chemical and organoleptic properties of set yoghurt.
- 2. The effect of storage period on the chemical and sensory evaluation of set yoghurt.

CHAPTER TWO LITERATURE REVIEW

2.1. Fermented dairy products:

Milk has been a part of food since the dawn of civilization and also considered as a complete food for human beings. The fermentation of milk is also an ancient technique for the preservation of milk. It is largely used as a means to preserving highly perishable products like milk apart from imparting other benefits to the finished product (Kerr and McHale 2001).

Fermented dairy products are those can be produced via fermentation of lactose by microorganisms especially by lactic acid bacteria. When examining worldwide, various dairy products which are different in name but similar in content can be found and those products are an important part of human diet (Hugenholtz, 2013).

Fermented dairy products have long been an important component of nutritional diet. Historically, fermentation process involved unpredictable and slow souring of milk caused by the organisms inherently present in milk; however, modern microbiological processes have resulted in the production of different fermented milk products of higher nutritional value under controlled conditions, these products represent an important component of functional foods, and intense research efforts are under way to develop dairy products into which probiotic organisms are incorporated to make them more valuable (Panesar, 2011).

2.1.1. Nutritional and therapeutical value of fermented dairy products:

A number of health benefits have been attributed to products containing probiotic organisms. While some of these benefits have been well documented and established, others have shown a promising potential in animal models. More importantly, health benefits imparted by probiotic bacteria are very strain specific; therefore, there is no universal strain that would provide all proposed benefits, not even strains of the same species; moreover, not all the strains of the same species are effective against defined health conditions (Chandan *et al.*, 2008). The following describes some of the proposed health benefits of consumption of fermented/probiotic dairy products.

2.1.1.1. Alleviation of lactose intolerance:

Panesar *et al.*, (2006) stated that inability of adults to digest lactose, or milk sugar is prevalent worldwide; consumption of lactose by those lacking adequate levels of lactase produced in the small intestine can result in symptoms of diarrhea, bloating abdominal pain and flatulence. Gilliland (1985) documented that many lactose intolerant individuals are better able to consume fermented dairy products, such as yoghurt, with fewer symptoms than the same amount of unfermented milk. Yoghurt was found to be helpful in the digestion of lactose because the lactic acid bacteria used to make yoghurt produce lactase and digest the lactose.

2.1.1.2. Protection against gastrointestinal infection:

Gastrointestinal infections including diarrhea result from a change in the gut micro flora caused by an invading pathogen. Gandhi, (2000) suggested that viable lactic acid bacteria interfere with the colonization and subsequent proliferation of food borne pathogens, thus preventing the manifestation of infection therefore, *L*.

bulgaricus, L. acidophilus, S. thermophilus and B. bifidum have been implicated in the reduction of pathogenic organisms.

The beneficial effects of lactic acid bacteria and cultured milk products have also been attributed to their ability to suppress the growth of pathogens either directly or through production of antibacterial substances. Antibiotics have been reported to kill normal bacteria as well, often resulting in disruption of the bacterial flora, leading to diarrhea and other intestinal disturbances. Replenishing the flora with normal bacteria during and after antibiotic therapy seems to minimize disruptive effects of antibiotic use. Probiotics have been reported to effective in prevention of various gastrointestinal infections (Gilliland, 1985).

2.1.1.3. Anti-carcinogenic effect:

Hosono *et al.*(1986) reported that fermented milk products can protect against certain types of cancers, consumption of yoghurt, Gouda cheese, butter milk protect against breast cancer, he also stated that lactic acid bacteria exert anticarcinogenic effect either by prevention of cancer initiation or by suppression of initiated cancer, anti-carcinogenic effects of yoghurt and milk fermented with *L. acidophilus;* different potential mechanisms by which lactic acid bacteria exert antitumor effects have been suggested such as changes in fecal enzymes thought to be involved in colon carcinogenesis, cellular uptake of mutagenic compounds, reducing the mutagenicity of chemical mutagens and suppression of tumors by improved immune response.

2.1.1.4. Immune system stimulation:

The immune system provides the primary defense against microbial pathogens that have entered our bodies (Gandhi, 2000). Animal and some human studies have shown an effect of yoghurt or lactic acid bacteria on enhancing levels of certain immunoreactive cells or factors; Milk components such as whey protein, calcium, certain vitamins and trace elements are also capable of influencing

immune system; Studies have shown that cytokine production, phagocytic activity, antibody production, T-cell production etc. are increased with yoghurt consumption or with lactic acid bacteria (Panesar, 2011).

2.1.1.5. Lowering of serum cholesterol:

Research reports indicated that fermented milk products to have hypocholesteraemic effect (Grunewald, 1992). It has been reported that *L. acidophilus* has exhibited the ability to lower serum cholesterol levels; this promotes the potential healthful aspects of dairy products fermented with *L. acidophilus* (or other lactic acid bacteria), since hypercholestermia is considered to be one of the major factors contributing to cardiovascular disease (Panesar, 2011).

2.1.1.6. Alleviation of Constipation:

Constipation is common problem in subjects consuming the western diet and also in elderly people. Panesar *et al.*, (2009) reported benefits include alleviation of constipation using *L. acidophilus* NCDO 1748, *L. casei Shirota* and *Lactobacillus GG*.

2.1.1.7. Antihypertensive activity:

Casein hydrolysate, produced by an extracellular proteinase from *L. helveticus* (CP790) has been reported to show antihypertensive activity in rats. Two antihypertensive peptides have also been purified from sour milk fermented with *L. helveticus* and *Saccharomyces cerevisiae*. These two peptides inhibit angiotensin-converting enzyme that converts angiotensinogen I to angiotensinogen II, which is a potent vasoconstrictor (Maeno *et al.*, 1996) It has been reported that consumption of certain lactobacilli, or products made from them, may reduce blood pressure in mildly hypertensive people (Panesar, 2011).

2.1.1.8. Antiallergenic qualities:

Probiotics may help prevent allergic reactions in individuals at high risk of allergies, such as food allergies. Probiotic bacteria help to reinforce the barrier function of the intestinal wall, thereby possibly preventing the absorption of some antigens (Panesar *et al.*, 2009, Kirjavainen *et al.*, 2003).

2.1.2. Type of fermented dairy products:

2.1.2.1. Cultured dairy products produced by mesophilic lactic starter cultures: -

2.1.2.1.1. Cultured buttermilk:

Natural buttermilk is a leftover liquid by-product made during the churning of butter. Cultured buttermilk is the sour end product obtained after fermentation of skim or partially skim milk inoculated with LAB cultures (e.g., Lactococcus, Lactobacillus, Streptococcus, and Leuconostocs). Additionally, non-lactic starters can also be used as a co-inoculant with LAB for the production of buttermilk (Kaur *et al.*, 2014). It is similar to yoghurt in the sense that it is cultured using live beneficial bacteria and can be consumed as a thick and creamy beverage (Conway *et al.*, 2014).

2.1.2.1.2. Sour cream or cultured cream:

It is a fermented fat-rich product with a pleasant acidic taste and flavor similar to that of the cultured buttermilk. The starter culture used in its production is similar to that for cultured buttermilk. The main characteristics of this product are thick and heavy body due to high-fat and solids contents with a clean acidic and slight diacetyl flavor; the cream is first standardized to a desired fat content, followed by heating to approximately 70°C, at which temperature it is homogenized usually twice to produce a smooth product of desired viscosity. The pasteurization is the next step in the manufacturing upon which the product is chilled and inoculated with the defined starter culture. The incubation is performed at 22°C for 14–16 hours. The fermentation is terminated when the ultimate pH is reached (usually 0.7–0.9% lactic acid) by cooling the product down to 4°C. Sour cream is a fat-rich product, which makes manufacturing of low-fat varieties

extremely difficult. To compensate for fat reduction, various thickening agents are used (Chandan *et al.*, 2008).

2.1.2.1.3. Ymer

Ymer is a cultured dairy product of Denmark with a high protein (5–6%) and fat (3.5%) content, the starter culture used for its production is similar to buttermilk culture and consists of *Lc. lactis* subsp. *Lactis* biovar. *diacetylactis* and *Leuc. mesenteroides* subsp. *cremoris*. Currently, ymer is commercially produced using ultrafiltration in order to increase milk solids prior to fermentation. Before fermentation, standardized and concentrated milk is homogenized and pasteurized (90°C for 5 minutes). The base is then cooled, inoculated with the starter, and incubation takes place at 20– 22°C for 20 hours. The final product has a pleasant acidic and diacetyl flavor (Tamime and Marshall, 1997b).

2.1.2.2. Cultured dairy products produced by thermophilic lactic starter cultures:-

2.1.2.2.1. Bulgarian buttermilk:

It is a high acid product, mainly produced in Bulgaria. It is made by fermenting pasteurized (85°C for 30 minutes) milk with *Lb. bulgaricus* alone at 40–42°C for 12–16 hours. The fermentation is stopped when about 1.4% titratable acidity is achieved. The flavor of the final product resembles that of yogurt and is dominated by acetaldehyde (Chandan *et al.*, 2008).

2.1.2.2.2. Dahi:

Dahi or curd is the traditional fermented milk product obtained from pasteurized or boiled milk by souring with natural microflora or by the harmless lactics or other bacterial culture. Dahi is popular throughout the Indian subcontinent. It is consumed either in the main course of meal, as a refreshing beverage or as dessert. It is assumed that over 50% of total milk produced in Nepalese households is converted into dahi: only a small amount of the milk produced is sold. The conversion of milk into dahi is an important intermediary step in the manufacture of nauni, ghee (Kharel *et al.*, 2010)

It is produced using mixed mesophilic cultures of *Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris, Lactococcus lactis* subsp. *diacetylactis* along with Leuconostoc species; along with taste dahi has many health benefits (Lin and Yen, 1999; Agarwal and Bhasin, 2002). Mild dahi is made from mesophilic lactococci- leuconostocs may be used as adjunct organisms for an added kuttery aroma and flavor. Sour Dahi contains additional cultures belonging to a thermophilic group (Mistry, 2001). Which are generally thermophilic organisms grow rapidly at 37-45°C (98.6-113F) producing Dahi in less than 4hours (Salampessy and Kailasapathy, 2011).

2.1.2.2.3. Acidophilus milk:

Acidophilus milk is a sour product that has been allowed to ferment under conditions that favor the growth and development of thermophilic lactic acid bacteria. This type of fermented milk is produced by development in milk of *Lacatobacillus acidophilus*, it is claimed that acidophilus milk has therapeutic and health-promoting properties. It is also claimed that the growth of *Lacatobacillus acidophilus* under the condition existing in the intestinal tract can replace undesirable putrefactive fermentation with a beneficial lactic fermentation (De Roos and Katan, 1998).

For the preparation of acidophilus milk, optimized level of skim milk powder (3%) was added to pasteurized toned milk and reheated to at 40-45°C; the milk was inoculated with starter culture (*L. acidophilus*) 7 percent, and then incubated at 40°C for 4-4.5 hours then cooled to 5°C and stored for about 2 hours for inducing cold gelation, then stirred for better homogenous consistency (Amiri *et al.*, 2010).

2.1.2.3. Cultured dairy products produced by mixed fermentation: -

2.1.2.3.1. Kefir:

Kefir is a fermented milk drink produced by the actions of bacteria and yeasts contained in kefir grains, and are reported to have a unique taste and unique properties (Farnworth, 2005). It originates in the Caucasus Mountains in the former Soviet Union, in Central Asia and has been consumed for thousands of years. It is the product of fermentation of milk with kefir grains and mother cultures prepared from grains (Libudzisz and Piatkiewicz, 1990). The fermentation of grains results in numerous components of the kefir including lactic acid, acetic acid, CO_2 , alcohol (ethyl alcohol) and aromatic compounds; That make its unique organoleptic properties are occurred: fizzy, acid taste, tart and refreshing flavor (Otles and Cagindi, 2003).

The traditional method of making kefir is occurred by directly adding kefir grains. The raw milk is boiled and cooled to 20-25 C and inoculated with 2-10% (generally 5%) kefir grain. After a period of fermentation, 18-24 hours at 20-25 C, the grains are separated from the milk by filtering with a sieve and can be dried at room temperature and kept at cold temperature for being used in the next inoculation. Kefir is stored at 4 C for a time then is ready for consumption (Karagozlu and Kavas, 2000).

2.1.2.3.2. Kumys (Kumiss, Koumiss):

Koumiss is a fermented milk drink. It is consumed in the countries of the Caucasus region – Kazakhstan, Azerbaijan, Turkey (kumyz) (Tooner, 1994). Traditional koumiss is produced from mare's milk, and in Mongolia it is also produced from camel's milk. In Europe and North America, a koumiss-like product is made from full or skimmed cow's milk (Mann, 1989; Di Cagno *et al.*, 2004). It contains 2% alcohol, 0.5-1.5% lactic acid, 2-4% milk sugar and 2% fat. Koumiss is recommended for the treatment of tuberculosis, asthma, pneumonitis,

cardiovascular diseases and gynaecological diseases. It is also suitable for weight gain and increasing robustness and energy (Yaygin, 1992; Kinik *et al.*, 2000).

2.1.2.3.3. Skyr:

Skyr is Icelandic fermented milk, the modern variant of the traditional and homemade product with the same name. Its properties are close to quark or concentrated yoghurt, and sometimes it is categorized as being a concentrated, homogenous, fresh cheese product (Hilmarsdottir and Arnadottir, 1989).

Skyr is made from skimmed milk with added rennet and fermented with a specific mixed starter culture consisting of different undefined strains of thermophilic lactic acid bacteria including *Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus* and *Lactobacillus helveticus* and lactose-fermenting yeasts (Gudmundsson, 1987; Magnusson, 1988).

2.1.2.3.4. Viili:

Viili is the modern variant of the traditional product filbunke. It is very popular in Finland with an annual consumption of 4.5 kg per person. A flavoured variant with a fruit preparation at the bottom is also available together with a variant including the probiotic bacteria *Lactobacillus rhamnosus* GG. It has a lower fat content, is made from homogenized milk and does not have any mould added (Leporanta, 2003).

2.2. Yoghurt:

Yoghurt is one of the most popular fermented dairy products worldwide which has great consumer acceptability due to its health benefits other than its basic nutrition. In general, yogurt is considered as a nutrition-dense food due to its nutrient profile and is a rich source of calcium that provides significant amounts of calcium in bio-available form. In addition, it provides milk proteins with a higher biological value and provides almost all the essential amino acids necessary to maintain good health. Yogurt is considered as a probiotic carrier food that can deliver significant amounts of probiotic bacteria into the body which can claim specific health benefits once ingested. Moreover, yogurt is reported to claim improved lactose tolerance, immune enhancement and prevention of gastrointestinal disorders. Because of these known health benefits of yogurt, consumer demand for yoghurt and yoghurt related products has been increased and became the fastest growing dairy category in the global market (Weerathilake *et al.*, 2014).

Yoghurt starter culture consists of a blend of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp *Bulgaricus*. Yoghurt is mainly of two types i.e. set yoghurt and stirred yoghurt. Yoghurt properties can be enhanced by the addition or treatment with various additives. It can be supplemented with various useful ingredients. Addition of herbs or their active components like oils could be an effective strategy to improve functionality of milk and milk products with respect to the health benefits, food safety and bio preservation (Aswal *et al.*, 2012). Yoghurt is a functional food. The functional food includes probiotics, prebiotics and synbiotics. Probiotics can be defined as "live microbial feed supplements that beneficially affect the host animal by improving its intestinal microbial balance" (Champagne and Gardener, 2005).

2.2.1. Health benefits of yoghurt:

Yogurt is considered as a nutrient dense food that contains essential nutrients such as protein, vitamins and minerals necessary for growth. Consumption of dairy products such as yogurt helps to improve the overall quality of the diet while increasing the chances of achieving nutritional recommendations such as Recommended Dietary Allowances of each nutrient in daily basis. For instance, milk products including yogurt is a rich source of calcium in bio-available form which is reported to provide 41% of the recommended daily requirement of Calcium for a 5-year old through a serving of 50 g of yogurt (Mckinley, 2005).

Lactose is the main carbohydrate found in milk which is a disaccharide composes of one molecule of glucose and galactose. Lactose is broken down to its simple sugars due to the action of the enzyme, lactase inside the gut. Inadequacy of secretion or interferences to the digestion process of lactase may pass undigested lactose into the large intestine which will then be fermented by colonic microflora that results gastrointestinal symptoms such as flatulence, diarrhea and abdominal pain. This phenomenon is called as the lactose intolerance. It has been reported that the lactose intolerance is associated with low calcium intake and bone mineral density most probably unnecessary exclusion of milk and dairy products from the diet (Mckinley, 2005; Buchowski *etal.*, 2002). Therefore, it can be concluded that yoghurt is effective for the individuals with lactose intolerance to attain all the benefits of milk products without causing discomforts associated with hypolactasia (Weerathilake *et al.*, 2014).

It is generally accepted that the optimum balance in the intestinal microflora is associated with good nutrition and health. Further, Lactobacilli and Bifidobacteria are known to be the primary microbial strains associated with this balance; therefore available research findings suggested that maintaining favorable microbial profile through regular consumption of bio-yogurt results numerous therapeutic benefits (Mckinley, 2005). Yogurt acts as a probiotic carrier food that is considered as an easy food to incorporate probiotics which results high probiotic viability. Bio-yogurt is considered to be an ideal source for the delivery of viable probiotic strains, *L. acidophilus* and *Bifidobacterium bifidum* which are the most common probiotics used in the dairy industry. However, in order to attain the probiotic effect, it is reported the need of consuming adequate amounts of viable probiotic cells regularly which is known as the therapeutic minimum (LourensHattingh, and Viljoen, 2001). Consumption of probiotics seems to be helpful to maintain good health, restore body vigor and combat intestinal disorders through the therapeutic and beneficial effects associated with them. Probiotics reported to have the therapeutic effects such as prevention of urogenital infections, alleviation of constipation, protection against diarrhea, prevention of infantic diarrhea, prevention of hypercholesterolemia, protection against colon/bladder cancer and prevention of osteoporosis. On the other hand, probiotics claimed to have other beneficial effects such as maintenance of normal intestinal flora, enhancement of the immune system, reduction of the lactose-intolerance and serum cholesterol levels, and enhance anticarcinogenic activity (Mckinley, 2005).

Moreover, yoghurt is reported to be beneficial for the treatment of Inflammatory Bowel Disease (IBD) that includes gastrointestinal disorders such as Crohn's disease, ulcerative colitis and pouchitis. The VSL#3 (a mixture of four strains of lactobacilli including *L. casei*, *L. plantarum*, *L. acidophilus* and *L. delbrueckii ssp. bulgaricus*, three strains of bifidobacteria including *B. longum*, *B. breve* and *B. infantis* and one strain of *S. thermophilus*) found to be effective in maintaining remission in patients with chronic relapsing pouchitis (Mimura *et al.*, 2004), and for the prophylaxis of pouchitis in patients who had ileo-pouch anal anastomosis for ulcerative colitis (Mckinley, 2005; Gionchetti *et al.*, 2003).

Yogurt consumption is also reported to be effective in cytokine production, T-cell function and natural killer-cell activity, and thereby result an overall immunological enhancement (Mckinley, 2005).

2.2.2. Varieties of yoghurt:

There are different varieties of yogurt that can be categorized according to the physical and chemical nature, added flavors and post incubational processes:

2.2.2.1. Based on the chemical composition of the product:

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Based on the fat content of yogurt, it can be categorized into three major varieties namely, regular yogurt, low-fat yogurt and non-fat yogurt. Regular yogurt is produced from the full fat milk which should contain at least 3.25% of milk fat. On the other hand, low-fat yogurt and non-fat yogurt are produced from low fat milk or partially-skim milk, and skim milk respectively (Weerathilake *et al.*, 2014). **2.2.2.2. Based on the physical nature of the product:**

Dairy Consultant (2013) reported that the physical nature of yogurt can be solid, semi-solid or fluid. Yogurts that are solid in nature (jelly-like texture) are called as set yogurt that is incubated and cooled in the final packaging. Whereas yogurts which are in semi-solid state and fluid nature called as stirred yogurt and fluid/drinking yogurt, respectively. Stirred yogurts are produced by incubating the mix in a tank followed by breaking by stirring prior to cooling and packaging. Drinking yogurts usually go through a homogenization process in order to reduce the particle size that assured hydrocolloidal distribution and stabilization of the protein suspension.

2.2.2.3. Based on the flavor of the product:

2.2.2.3.1. Plain/Natural Yoghurt.

This is the simplest and the least adulterated form of the yoghurt made by lactic acid bacterial fermentation of pasteurized milk in order to produce its characteristic texture and flavor. In other words, it can be defined as the plain and unsweetened fermented milk product containing no added color or any other additives. Therefore, it is closer to the nutritional value of milk which it is made of, and provides all of the benefits associated with fermentation while supplying fewer amounts of calories. Moreover, plain yogurt gives the pure yogurt taste and contains the richest calcium content among the yogurt products (Weerathilake *et al.*, 2014).

2.2.3.2. Flavoured Yoghurt.

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The flavours are usually added at or just prior to filling into pots. Common additives are fruit or berries, usually as a puree or as whole fruit in syrup. These additives often have as much as 50% sugar in them, however with the trend towards healthy eating gaining momentum; many manufacturers offer a low sugar and low fat version of their products. Low or no sugar yoghurts are often sweetened with saccharin or more commonly aspartame (Aswal *et al.*, 2012).

2.2.2.4. Yogurt related products:

2.2.2.4.1. Dried Yoghurt/ Yoghurt Powder.

Yoghurt powder is produced by fermenting non-fat milk using standard yoghurt cultures until attain the desirable pH followed by a step of drying, most probably by freeze-drying (Childs and Drake, 2008). Yoghurt powders add a unique dairy flavor to food applications including beverages, confections and dips. The main intension of manufacturing yoghurt powder is to store the product in a stable and readily utilizable state, and it can be utilized to replace fresh yoghurt for beverage and dip, and in confectionary industry as a coating material for coating of dried fruit, nuts, pretzels, cereal and other snack items (Childs and Drake, 2008; Krasaekoopt and Bhatia, 2012).

2.2.2.4.2. Frozen Yogurt.

Frozen yoghurt is inoculated and incubated in the same manner as stirred yoghurt. However, cooling is achieved by pumping through a Whipper / chiller / freezer in a fashion similar to ice-cream. The texture of the finished product is mainly influenced by the whipper/ freezer and the size and distribution of the ice crystals produced (Aswal *et al.*, 2012).

2.2.2.4.3. Herbal Yoghurt:

In recent years, there is an increasing trend towards the production of herbal yoghurts by incorporating natural food additives and health promoting substances. Bio yoghurts prepared from cow milk and camel milk with *Cinnamonum verum* reported to inhibit enzymes such as α -amylase and α -glucosidase related to diabetes whereas the higher counts of Lactobacilli were observed in the herbal yoghurts prepared with camel milk than that of the plain yoghurt (Shori and Baba, 2011; Shori and Baba, 2012). Also preparing yoghurts rich in poly- and mono-unsaturated fatty acids which were manufactured from reconstituted skimmed milk powder using vegetable oils (olive, groundnut, sunflower or maize) to replace the milk fat (Barrantes *et al.*, 1996).

2.2.3. Chemical composition of yoghurt:

Yadav and Shukla, (2014) stated that the chemical composition of plain yoghurt were protein 3.414%, fat 3.424%, Moisture 75.61%, acidity 0.81% and ash 0.684% and the composition of cinnamon yoghurt protein 3.45%, fat 3.414%, moisture 78.5%, acidity 0.87% and ash 0.618% when they study the effect of cinnamon extract on physicochemical analysis of prepared Herbal yogurt.

Oladipo *et al.*, (2014) reported that the protein content range from 4.78 to 5.23%, fat 0.59% - 0.73%, ash 0.69% - 0.83%, total solid 14.61% - 17.15%, moisture 82.85% - 85.39% and titratable acidity range from 0.159% to 0.187% in a proximate analysis result of possibility of using different milk source to produce yoghurt.

In study to evaluate the physicochemical, nutritional and organoleptic characterization of a skimmed goat milk fermented with the probiotic strain *Lactobacillus plantarum* C4 found that the acidity from 0.876% to 1.08%, total solid 9.88% - 17.8% and the protein concentration from 3.29% to 3.99% up to 6.65%. (Montoro *et al.*, 2018).

Ndife *et al.*, (2014) reported that The results of physico-chemical analysis obtained were increase in the proximate values for moisture (80.10-85.23%), fat

(1.50-3.13%), fiber (0.2-2.18%) and ash (0.53-1.01%); when they produce yoghurt from milk substitutions with coconut-cake.

Mohammad and El-Zubeir (2011) stated that the average composition of plain yoghurt made from fresh milk was; total solid 15.04%, fat 3.53%, protein 4.42%, ash content 0.82% and titratable acidity 1.33%; in a study of chemical composition and microbial load of set yoghurt from fresh and recombined milk powder in Khartoum Sudan.

2.2.4. Yoghurt microbiology:

Yoghurt is produced by symbiotic actions of two lactic acid bacteria, namely *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* which ferment lactose to lactic acid, which gives it its sour taste (Tamime and Robinson, 2004; Kumar and Mishra, 2004), but other products may require a different blend. For example, Bulgarian buttermilk is produced using *L. delbrueckii* subsp. *bulgaricus* alone, whilst dahi in India is produced using a mixed starter culture containing *S. thermophilus, Lactococcus lactis* biovar *diacetylactis* and *Lactococcus lactis* subsp. *cremoris*. Bio-yoghurts are made with different and defined starter cultures containing the yoghurt organisms (single or mixed) and/or *Lactobacillus, Bifidobacterium* and *Enterococcus* species (Tamime and Marshall, 1997a).

The reasons for selecting the combinations of starter cultures used during the manufacture of yoghurt and related fermented milk products are to achieve the desired flavour characteristics of the product, mainly lactate, aroma compounds (acetaldehyde, acetoin and diacetyl) and exopolysaccharide (EPS) and to provide the consumer with a wide choice of therapeutic products (Wacher-Rodarte *et al.*, 1993).

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The synergistic effect between *S. thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* may be easily explained. ST is stimulated by the bioavailability of free amino acids and peptides in culture media. On the other side, LDB is able to attack and proteolyze milk proteins; consequently, needed amino acids and peptides can be easily found if lactobacilli can spread freely in milks (Oliveira *et al.*, 2009; Oliveira *et al.*, 2011).

2.2.5. Storage of yoghurt:

Recently, in study prepared by Mudawi *et al.*, (2014) to investigate the effect of incubation and storage temperatures on quality of set yoghurt made from cow milk during storage life of 10 days, they found that Storage temperature of 6° C gave the best quality with regard to (total acidity, whey volume, viscosity and total score for sensory quality) compared to storage temperature of 10° C which gave large amount of separated whey and bitterness.

2.2.6. Factors affecting the physical and sensory properties of yoghurt:

2.2.6.1. Dry matter fortification:

The physical and sensory properties of yoghurt gels are greatly influenced by the total solids content of the yoghurt milk, especially the protein content. The solid-like properties values of yoghurt increases with an increase in the total solids content obtained by the addition of skim milk powder or by ultrafiltration (Biliaderis *et al.*, 1992; Lee and Lucey 2010). Increased yoghurt viscosity is observed when the total solids content of milk is increased (Wacher-Rodarte *et al.*, 1993). The oral viscosity of yoghurt or perceived thickness also increases with an increase in total solids content of milk (Sodini *et al.*, 2004). Addition of whey protein concentrates to milk followed by high heat treatment led to increase the solid- like values and decreased gelation time in yoghurt (Lucey *et al.*, 1999). The increased solids content in yoghurt milk as a result of fortification also creates increased buffering that requires additional acid development by the starter cultures to achieve a similar pH target (Lee and Lucey 2010).

Most yoghurt products are sweetened (not plain). The use of sucrose increases the total solids of the mix and strengthens the gel network. A range of sweeteners are used commercially, especially for low calorie products. Another option is to use β -galactosidase to hydrolyse lactose as the products are glucose and galactose, which are much sweeter than lactose (Lee and Lucey 2010).

2.2.6.2. Heat treatment:

When milk is heated at 70°C, the major whey proteins, such as, β -lactoglobulin, are denatured. During denaturation β -lactoglobulin interacts with the κ -casein on the casein micelle surface (and any soluble κ -casein molecules, i.e. κ -casein that dissociates from the micelle at high temperatures) by disulfide bridging, which results in increased gel firmness and viscosity of yoghurt (Lucey *et al.*, 1997).

Heat treatment of milk for 15 min at $\geq 80^{\circ}$ C results in significantly increased denaturation of β -lactoglobulin compared with milk heated at 75°C for a similar time (Lucey *et al.*, 1997). The extent of denaturation of whey proteins during the heat treatment of milk affects the firmness and viscosity of acid milk gels (Dannenberg and Kessler, 1988).

The higher solid- like properties value of yoghurts made with milk heat treated at \geq 78°C for 30 min was largely due the increased covalent cross-linking of proteins by the denatured whey proteins associated with the casein micelles (Lee and Lucey, 2003; Lee and Lucey 2010).

In stirred yoghurts, Cayot *et al.* (2003) reported that the consistency index of stirred acid gels, calculated from the Ostwald model, increased as milk heating

temperature increased from 70 to 100°C. An increase in milk heating temperature resulted in an increase in apparent viscosity of stirred yoghurts; an increase in heat treatment resulted in an increase in oral viscosity and perceived mouth coating attributes, as well as, a decrease in the chalkiness attribute of stirred yogurt (Lee and Lucey, 2006).

2.2.6.3. Incubation temperature:

Physical properties and microstructure of yoghurt are influenced by incubation temperature. The use of high incubation temperature resulted in a decrease in gelation time and the solid- like properties values at pH 4.6, and whey separation compared with yoghurt gels incubated at low temperature (Lee and Lucey, 2003). Yoghurt gels formed at high temperature are weak and have a coarse gel network due to extensive rearrangement resulting in the formation of large pores and greater whey separation (Lucey, 2004). At lower incubation temperature, there is an increase in the voluminosity of casein particles, which results in an increase in the area of the junctions between aggregated casein particles. Increased contact area between casein particles could contribute to the increased stiffness of gels observed at low temperature (Walstra, 1998).

Higher viscosity was observed in stirred yoghurts that had been incubated at lower temperature 40°C compared to gels incubated at high temperature >40°C (Sodini *et al.*, 2004; Lee and Lucey, 2006).

As incubation temperature increased, there was a decrease in the sensory attributes, such as mouth coating and smoothness of stirred yoghurt (Martin *et al.*, 1999).

Recently, a novel two-stage incubation temperature method was proposed. Peng *et al.*, (2010) reported that if incubation temperature was changed after gelation, the textural properties of yoghurt became similar to those of yoghurts made at that new temperature for the entire fermentation process. It may be possible to use high incubation temperature for the initial stage of fermentation to facilitate rapid growth of the starter cultures and then slowly reduce the incubation temperature at some stage to achieve better textural properties.

2.3. Watermelon seed:

Watermelon (*Citrullus lanatus*) is of the cucurbitaceous family. Cucurbit seeds are source of food particularly protein and oil (Hassan *et al.*, 2008b). Dehulled cucurbit seeds were reported to contain about 50% fat and 35% protein (Martins, 1998).

2.3.1. Watermelon (Citrullus lanatus):

As a member of the cucurbitaceae, watermelon is related to the cantaloupe, squash and pumpkin and other plants that grows on vines on the ground. Watermelon is a good source of carotenoid and lycopene. Lycopene has been found to be protective against a growing list of cancer (Cho *et al.*, 2004)

Watermelon is also expectedly high in citrulline; an amino acid the body make use of to make another amino acid, arginine (used in the urea cycle to remove ammoniacal from the body) (Collins *et al.*, 2007). Watermelon fruit contained many smooth compressed seeds that thickened at the margin and of black or yellow-white color (Sodeke, 2005).

2.3.2. Watermelon seeds as food:

Watermelon seeds are known to be highly nutritional; they are rich sources of protein, vitamins B, minerals (such as magnesium, potassium, phosphorous, sodium, iron, zinc, manganese and copper) and fat among others as well as phytochemicals (Braide *et al.*, 2012). The seeds of watermelons are known to have economic benefits especially in countries where cultivation is on the increase. The seeds are for instance used to prepare snacks, milled into flour and used for sauces.

Oil from the seeds are used in cooking and incorporated into the production of cosmetics (Jensen *et al.*, 2011).

2.3.2.1. Nutrient Composition of watermelon seeds:

Available literature on composition of watermelon seeds showed different percentages of protein, fat, fiber, moisture, ash and carbohydrate contents in comparison to the present results (Lakshmi and Kaul, 2011). The possible reason for such a difference could be attributed to varietal and regional/soil differences. The crude fiber in watermelon proximately between 39.09 - 43.28%, which help in expand the inside walls of the colon, easing the passage of waste, thus making it effective against constipation; it lowers cholesterol level in the blood and reduce

the risk of various cancers (Tabiri *et al.*, 2016).

Tabiri *et al.*, (2016) reported the proximate crude fat (oil content of the seed) between 26.50 - 27.83%. Whereas Madaan and Lai (1984) reported oil content values of 41.0-56.6% in melon seeds. The percentage of fat extracted reported by Oyeleke *et al.*, (2012) was 47.9% from watermelon seeds. While that Lakshmi and Kaul (2011), also showed a fat content of 46.83% for the seed meal. Oil provides concentrated energy in diet and enhanced palatability. It is worthy to note that cheap and novel source of oils that would be useful domestically and perhaps industrially. Cucurbit seeds are source of particularly protein and oil (Hassan *et al.*, 2008a; Wilcox, 2006).

The proximate crude protein content values are 16.33 - 17.75% (Tabiri *et al.*, 2016). The production of food protein in sufficient amounts possesses many problems especially since they are more expensive to produce than carbohydrate or lipids. In order to satisfy this steady growing demand for protein, new protein sources must be explored (Wilcox, 2006).

The moisture content of watermelon seed approximately between 7.40 - 8.00%, and the carbohydrate content were 9.55 - 15.32% (Tabiri *et al.*, 2016). Also

Loukou *et al.*, (2007) recorded the carbohydrate value 9.87% for *Citrullus lanatus* seeds.

The ash percentages of the samples indicated the total inorganic content of the samples from where the mineral content could be obtained. The ash content was 2 - 3% and that means the watermelon seeds are rich in mineral and could aid in digestion, formation of strong bones and teeth as well as hemoglobin formation; Calcium is an important mineral required for bone formation and neurological function of the body. The calcium content ranged from 0.11-0.16 mg/100 g. iron content 2.72 - 4.6 mg/100 g, the recommended dietary allowance for iron in adult and children is 10 mg/day while female adult is 15 mg/day. Iron is required for blood haem formation. The seeds studied could therefore contribute to iron needs of consumers. Magnesium content ranged from 0.14 – 0.17 mg/100 g, also is very important in calcium metabolism in bones and also involved in prevention of circulatory diseases. It helps in regulating blood pressure and insulin release (Tabiri *et al.*, 2016).

2.3.2.2. Phytochemicals of Watermelon seeds:

The screening of the seeds showed the presence of saponins, tannins, triterpenoids, and glycosides as well as alkaloids whilst flavonoids, anthracene glycosides and cyanogenic glycosides were absent. Tannins which is a major plant polyphenol when isolated from edible or non-edible plants have shown strong biological activity in the form of anti-tumour, anti-mutagenic, anti-diabetic, anti-proliferative, anti-bacteria and anti-mycotic properties (Arapitsas, 2012). The treatment of sore throat, hemorrhage and wound healing has also been linked to tannins (Abdul-Mumeen, 2013). But if ingested in excessive quantities, tannins inhibit the absorption of minerals such as iron and calcium which may lead to aneamia or osteoporosis if prolonged (Varadharajan *et al.*, 2012).

Glycosides which were also present in the seeds are known to have antidiarrhoeal (Tiwari *et al.*, 2011). Alkaloids are known to be ranked the most efficient therapeutically important plant secondary metabolite and are widely used worldwide as a basic agent for analgestic, antisplasmodic and bacterial effects, alkaloids were found to have the highest concentration in the watermelon seeds (Oseni and Okoye, 2013).

Cyanogenic glycosides which were absent in watermelon seeds are known to be toxic. Cyanogenic glycosides when enzymatically hydrolysed, release cyanohydric acids known as prussic acid. This acid is extremely toxic due to its ability of linking with metals such as Fe²⁺, Mn²⁺ and Cu²⁺ which are functional groups of many enzymes thereby inhibiting processes like reduction of oxygen in the cytochrome respiratory chain, electron transport in the photosynthesis, and the activity of enzymes like catalase, oxidase Therefore its absence is preferred (Francisco and Pinotti, 2000).

Triterpenoids were also present in all the samples and are known to help heal wounds faster (Tabiri *et al.*, 2016).

2.3.2.3. Total Phenol Content of watermelon seeds:

There is considerable amount of phenols in watermelon seeds, the presences of phenols in fruits and vegetables have attracted attention over the years due to its antioxidant activity. It has the ability to scavenge free radicals and neutralize it effects thereby preventing diseases like cancer and cardiovascular diseases (Tabiri *et al.*, 2016).

It is well known that there is a strong relationship between total phenol content and antioxidant activity, as phenols possess strong scavenging ability for free radicals due to their hydroxyl groups. Therefore, the phenolic content of plants may directly contribute to their antioxidant action (Mohammed *et al.*, 2011).

2.3.2.4. Antioxidant activity of watermelon seeds:

Antioxidants are known to quench free radicals, thus are essential components of anti-ageing formulations. Antioxidants also offer protection against damage to tissues due to the detrimental effects of environmental and other agents and encourage collagen growth by combating harmful effects of free radicals (Rodriguez *et al.*, 2006). Consumption of the seeds may reduce the chances of getting cardiovascular diseases and cancers due to the appreciable amount of total phenols found in the seeds and its antioxidant activity. Therefore, watermelon seeds may provide considerable medicinal, health and economic benefits if freshly consumed or utilized in food products (Tabiri *et al.*, 2016).

CHAPTER THREE MATERIALS AND METHODS

3.1. Study area:

This study was conducted during May 2017 at the laboratories of the Department of Dairy Sciences and Technology, College of Animal Production Sciences and Technology, Sudan University of Sciences and Technology.

3.2. Materials:

3.2.1. Milk:

Ten liters of cow's raw milk sample were purchased from the Dairy Farm, College of Animal Production Science and Technology, Sudan University of Science and Technology, Hillat Kuku.

3.2.2. Watermelon seeds powder:

Water melon seeds were purchased from popular market of Omdurman. The watermelon seeds were milled and analyzed to get the approximate chemical composition of the powder.

3.3. Design of the study:

In this study four treatments (A, B, C, D) were conducted. The first treatment is the control with 100% fresh cow's milk was processed into yoghurt. In the second, third and fourth treatments 5%, 7% and 10% of watermelon seeds powder were added and mixed with cow's milk respectively.

3.4. Methods

3.4.1. Yoghurt making process

Yoghurt prepared according to Lee and Lucey, (2010). Fresh and cow's milk were filtered. Four treatments were carried out, in the first treatment 2.5 liters of pure cow's milk was processed into yoghurt, while in the second treatment 2.5 liter of cow's milk mixed with 125g of watermelon seeds powder processed into yoghurt. In the third treatment 2.5 liters of cow's milk mixed with 175g of watermelon seeds powder were processed into yoghurt. In the fourth treatment 2.5 liters of cow's milk mixed with 250g of watermelon seeds powder were processed into yoghurt. The milk in all treatment was heated at 90-95°C for 5min (Tamime and Robinson, 1999), then cooled to 45°C. The starter culture of (*streptococcus Thermophilus, lactobacillus delbrueckii ssp.Bulgricus*) at the rate of 3% added to the mixtures and stirred thoroughly, then packed in plastic cups (200ml capacity) in triplicates for each treatment and incubated at 45°C until coagulation occurred. Samples from different treatments were stored at refrigerator temperature (4°C) for 1, 5 and 10 days. Chemical and sensory evaluations were carried out for the yoghurt samples at zero days and at the end of each storage period.

3.4.2. Chemical analysis

3.4.2.1. Protein determination

Protein content was determined by Kjeldahl method according to the AOAC (2003) as follows:

Ten grams of each sample were weighed in a crucible and transferred to a digestion flask. Two tablets of Kjeldahl catalyst (mercury) and 3 ml of concentrated sulphuric acid (sp.g.1.84) were added to the sample. The flask was placed on the digestion apparatus, heated strongly until the liquid had become clear.

To the digested sample of yoghurt fifteen ml of NaOH (40%) were added in kjeldahl distillation apparatus. Ten ml of boric acid (2%) and three drops of

indicator (bromo creysol green and methyl red) were added to a receiving flask. The distillation was continued until the distillate in the receiving flask was 75 ml.

The sample in the receiving flask was titrated against HCL (0.1N) until the color was changed to a faint pink. The protein content was calculated as flows:-

 $N \% = T \times 0.1 \times 20 \times 0.014 \times 100$

Weight of sample

Where:-

T = Titration figure
20 = dilution factor
0.014 = atomic weight of N/1000
0.1 N = Normality of HCL

Protein (%) = N (%) \times 6.38

Where:-

N = nitrogen content

6.38 =conversion factor

3.4.2.2. Fat content

The fat content was determined by Gerber methods according to Bradley *et al.*, (1992) as follows:

In a clean dry Gerber tube, 10ml of sulphuric acid (density 1.8 gm / ml at 20 °C) were poured, and then 10.94 ml of yoghurt sample were added, amyle alcohol (1-2ml) was added to tube, followed by the amount of distilled water. The contents were thoroughly mixed till no white particles could be seen. The Gerber tubes were centrifuged at 1100 revolution per minute (rpm) for 4-5 min. The fat column was then read immediately.

3.4.2.3. Titratable acidity

Titratable acidity was determined according to AOAC (1990). Ten ml of yoghurt sample were placed into a clean porcelain dish and one ml of phenolphthalein indicator was added. The sample was titrated against 0.1 N NaOH till a faint color lasted for at least 30 seconds. Then the titratable acidity of each sample was calculated as follows:

Titratable acidity = T/W

Where:

T= Titration figures

W=Weight of samples

3.4.2.4. Total solids content

The total solids content was determined according to the modified methods of AOAC (1990). Three grams of sample were weighed into a dry oven flat bottomed aluminum dish, and heated on steam bath for 10-15 min. The dish was placed in an oven at 105°C for three hours, and then cooled in desiccators and weighted immediately. Weighting were repeated until the difference between the two readings was < 0.1mg. The total solids content was calculated from the following equation.

 $T.S\% = W_1 / W_0 \times 100$

Where:

 W_1 = Weight of sample after drying

 W_0 = Weight of sample before drying

3.4.2.5. Ash content

The ash content was determined according to AOAC (2003). Five grams of the samples were weighed in porcelain crucibles, and then placed in a muffle furnace at 550-600°C for 3 hrs until ashes were carbon free. The porcelain crucibles were then cooled in desiccators and weighed. The ash content was calculated using the following equation:

Ash%= $W_1/W_2 \times 100$ Where: W_1 = Weight of ash W_2 =Weight of sample before ashing

3.4.3. Sensory evaluation:

Sensory profiling of the yoghurt sample was conducted, using conventional profiling, by untrained panelists according to Larmond (1977). Ten panelists were selected among staff and students of the College of Animal production Science and Technology, Sudan University of Science and Technology. The panelists were given a hedonic questionnaire (Appendix NO1) to evaluate taste texture, colour, and flavor and overall acceptability of coded samples different treatments. Those stored for difference period of time (1, 5, 10 days).

3.4.4. Statistical analysis:

Statistical analysis were carried out with SPSS (2008) version 16 General linear model was used for data analysis (Factorial design) to test the effect different treatments and storage period on the quality of set yoghurt. Least significant difference (LSD) was used for mean separation between the treatments. Alpha level 5% was used in this study.

CHAPTER FOUR RESULTS

4.1. Chemical composition of raw material:

The chemical composition of raw milk were 13.89% total solid, 2.25% fat, 3.66% protein, 0.30% acidity and 0.78% ash.

Watermelon seeds powder composition were 5.83% moisture, 19.75% protein, 24.81% fat, 4.92% ash, 12.65% carbohydrate and 31.93% fiber.

4.2. Effect of different levels of watermelon seeds powder on the chemical composition of set yoghurt:

Data in Table 1 showed the effect of watermelon seeds powder on the physicochemical characteristic of set yoghurt.

The results indicated that there is highly significant difference (P<0.01) in protein content of the yoghurt samples. The highest protein content ($4.28\pm0.05\%$) was recorded in the yoghurt with 10% watermelon seeds powder, while the lowest one ($3.73\pm0.06\%$) was found in control yoghurt samples.

Fat content of the yoghurt samples showed high significant difference (P<0.01) among the treatments. The highest fat content $(5.30\pm0.63\%)$ was found in the yoghurt sample with 10% watermelon seeds, while the lowest value $(3.39\pm0.67\%)$ was in control yoghurt sample.

The results revealed high significant variation (P<0.01) in the titratable acidity of yoghurt samples, the highest value was $(1.21\pm0.16\%)$ in the yoghurt sample with 10% of watermelon, while the lowest value was $(1.10\pm0.11\%)$ in control yoghurt sample.

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Table (1) Effect of different levels of watermelon seeds powder onchemical composition of set yoghurt.

	Chemical Composition %						
Treatments	Protein	Fat	Acidity	T.S	Ash		
Α	3.73±0.06 ^d	3.39±0.67 ^c	1.10±0.11 ^b	15.86±6.59 ^b	0.81±0.06 ^d		
В	3.85±0.06 ^c	3.60±0.86 ^c	1.13±0.13 ^b	20.47±1.38 ^a	0.91±0.03 ^c		
С	4.18±0.05 ^b	4.58±1.55 ^b	1.15±0.17 ^b	22.06±6.38 ^a	1.09±0.05 ^b		
D	4.28±0.05 ^a	5.30±0.63 ^a	1.21±0.16 ^a	23.73±8.62 ^a	1.18±0.04 ^a		
Sig	**	**	**	**	**		

Means with different superscript in the same column are significantly (P<0.05) different

A = control yoghurt with no additive

B = yoghurt with 5% of watermelon seeds powder mixed with cow's milk

C = yoghurt with 7% of watermelon seeds powder mixed with cow's milk

D = yoghurt with 10% of watermelon seeds powder mixed with cow's milk

Total solids content of yoghurt sample secured high significant difference (P<0.01) among the treatment (Table 1). The highest total solids content (23.73 \pm 8.62%) was for yoghurt sample with 10% watermelon seeds powder, in contrast the lowest one (15.86 \pm 6.59%) was found in control yoghurt sample.

Also the results indicated that there is highly significant difference (P<0.01) in the ash content of the treatments, the highest one $(1.18\pm0.04\%)$ was recorded in the yoghurt sample with 10% watermelon seeds powder, while the lowest value $(0.81\pm0.06\%)$ for control yoghurt sample.

4.3. Effect of storage period on the chemical composition of set yoghurt:

Data in Table 2 showed the effect of storage period on the chemical composition of set yoghurt.

The results indicated that the storage period had significant effect (P<0.01) on protein content, at day one showed the highest value ($4.06 \pm 0.24\%$) and it was decrease to ($3.96 \pm 0.24\%$) at day 10.

Fat content of the yoghurt samples showed significant (P<0.01) variations during the storage period. The highest fat content was $(4.87 \pm 0.91\%)$ at day 5 and the lowest one $(3.23 \pm 1.09\%)$ at day 1.

The titratable acidity of the yoghurt samples in table 2 recorded significant difference (P<0.01) during the storage period. The highest value $(1.24 \pm 0.11\%)$ at day 10, and the lowest $(0.98 \pm 0.04\%)$ was at day 1

Also the results indicated that the storage period had significant (P<0.01) effect on the total solids content of set yoghurt. Highest value ($25.22 \pm 4.76 \%$) was recorded at day 1, and the lowest one ($14.60 \pm 4.32\%$) at day 10.

Table (2) Effect of the storage period on the chemical composition ofset yoghurt.

Storage	Chemical Composition %						
period	Protein	Fat	Acidity	T.S	Ash		
Day1	4.06 ± 0.24^{a}	3.23± 1.09 ^b	0.98 ± 0.04 ^b	25.22 ± 4.76^{a}	1.05 ± 0.15^{a}		
Day5	4.02 ± 0.23^{b}	4.87 ± 0.91^{a}	1.22 ± 0.09^{a}	21.77 ± 6.27^{b}	1.00 ± 0.15^{b}		
Day10	3.96 ± 0.24^{c}	4.56± 1.07 ^a	1.24 ± 0.11 ^a	$14.60 \pm 4.32^{\circ}$	$0.94 \pm 0.16^{\circ}$		
Sig	**	**	**	**	**		

Means with different superscript in the same column are significantly (P<0.05) different

Ash content of the yoghurt samples showed significant (P<0.01) variations during the storage period (Table 2). The highest Ash content $(1.05 \pm 0.15\%)$ was at day 1 and the lowest $(0.94 \pm 0.16\%)$ was at day 10.

4.4. Effect of storage period and different levels of watermelon seeds powder on chemical composition of set yoghurt:

Data in Table 3 showed the effect of storage period and different levels of watermelon seeds powder on chemical composition of set yoghurt.

The result indicated that the storage period and different levels of watermelon seeds powder had no significant (P>0.05) effect on protein and the total solids contents. The highest protein content $(4.34 \pm 0.03\%)$ was recorded in yoghurt sample with 10% watermelon seeds powder at day 1, and the lowest one $(3.69 \pm 0.06\%)$ was for control yoghurt sample at day 10. While the highest total solids content $(31.18 \pm 3.37\%)$ was for the yoghurt samples with 10% watermelon seeds powder at day 1, however, the lowest value $(9.56 \pm 2.16\%)$ was for the control yoghurt sample at day 10.

The results revealed that the storage period and different levels of watermelon seeds powder had significant (P<0.05) effect on the fat, titratable acidity and the ash contents of yoghurt samples. The highest fat content ($5.87 \pm 0.14\%$) was for the one with 10% watermelon seeds powder at day 10; the lowest value ($2.65 \pm 0.35\%$) was for the one with 5% watermelon seeds powder at day 1. The highest titratable acidity ($1.34 \pm 0.03\%$) was for yoghurt samples with 10% watermelon seeds powder at day 10% watermelon seeds powder at day 10% watermelon seeds powder at day 1. The highest titratable acidity ($1.34 \pm 0.03\%$) was for yoghurt samples with 10% watermelon seeds powder at day 1. The one with 5% watermelon seeds powder at day 1. The highest because the powder at day 10 and the lowest value ($0.95 \pm 0.06\%$) was for the one with 5% watermelon seeds powder at day 1.

Storage period	Treat.	Chemical Composition %						
		Protein	Fat	Acidity	TS	Ash		
Day 1	Α	3.77 ± 0.05	2.76 ± 0.81	0.96 ± 0.03	21.89 ± 0.96	0.86 ± 0.01		
	B	3.90 ± 0.03	2.65 ± 0.35	0.95 ± 0.06	21.97 ± 4.95	0.95 ± 0.00		
	С	4.23 ± 0.03	2.80 ± 0.96	0.99 ± 0.01	25.85 ± 1.22	1.15 ± 0.02		
	D	4.34 ± 0.03	4.70 ± 0.74	1.00 ± 0.03	31.18 ± 3.37	1.23 ± 0.00		
Day 5	Α	3.74 ± 0.06	3.81 ± 0.47	1.19 ± 0.05	16.05 ± 5.37	0.83 ± 0.00		
	B	3.87 ± 0.00	4.48 ± 0.52	1.10 ± 0.03	20.47 ± 0.50	0.90 ± 0.00		
	С	4.19 ± 0.02	5.85 ± 0.65	1.29 ± 0.07	24.78 ± 7.06	1.09 ± 0.02		
	D	4.28 ± 0.01	5.35 ± 0.10	1.28 ± 0.06	25.79 ± 6.91	1.19 ± 0.01		
Day 10	Α	3.69 ± 0.06	3.60 ± 0.08	1.12 ± 0.08	9.56 ± 2.16	0.73 ± 0.02		
	В	3.79 ± 0.03	3.68 ± 0.25	1.18 ± 0.06	14.22 ± 3.59	0.86 ± 0.00		
	C	4.14 ± 0.02	5.10 ± 0.84	1.33 ± 0.04	15.56 ± 3.92	1.05 ± 0.02		
	D	4.23 ± 0.01	5.87 ± 0.14	1.34 ± 0.03	19.06 ± 0.69	1.13 ± 0.01		
Si	ig	NS	*	**	NS	**		

Table (3) Effect of different levels of watermelon seeds powder and storage period on chemical composition of set yoghurt.

Means with different superscript in the same column are significantly (P<0.05) different

A = control yoghurt with no additive

B = yoghurt with 5% of watermelon seeds powder

C = yoghurt with 7% of watermelon seeds powder

D = yoghurt with 10% of watermelon seeds powder

The highest ash content $(1.23 \pm 0.00\%)$ was for the yoghurt samples with 10% watermelon seeds powder at day 1 and the lowest value $(0.73 \pm 0.02\%)$ was for the one at day 10 (Table 3).

4.5. Effect of watermelon seeds powder on the sensory characteristics of set yoghurt: -

Data in Table 4 indicated the addition of watermelon seeds powder had significant (P<0.01) effect on the color of set yoghurt (Table 4), the highest value (6.67 ± 2.62) was for the control yoghurt sample while the lowest one (4.50 ± 2.14) was for the one with 10% watermelon seeds powder.

The results showed that watermelon seeds powder had significant (P<0.01) effect on the flavor of set yoghurt. The highest scores (5.77 ± 2.33) was for the control yoghurt sample; however, the lowest value (3.97 ± 2.01) was for the one with 10% watermelon seeds powder.

Statistical analysis revealed that watermelon seeds powder had no significant (P>0.05) effect on texture and overall acceptability of the set yoghurt (Table 4).

The taste of the yoghurt samples was found to be affected significantly (P<0.01) by the watermelon seeds powder addition. The highest taste score (5.87 \pm 2.54) was recorded for the control yoghurt and the lowest value (4.13 \pm 1.85) was for the yoghurt with 7% watermelon seeds powder.

4.6. Effect of storage period on the sensory characteristics of set yoghurt:

The data in Table 5 showed that no significant (P>0.05) variations were observed in the color, flavor, texture, taste and overall acceptability of the yoghurt samples due to storage period. Slight improvement in the color of the yoghurt samples was noticed as the storage period progressed. At day 10 the color value (5.92 ± 2.65) was a maximum.

Table (4) Effect of the different levels of watermelon seeds powderon sensory characteristics of set yoghurt.

Treatments	Sensory Attributes						
	Color	Flavor	Texture	Taste	Overall acceptability		
Α	6.67 ± 2.62^{a}	5.77 ± 2.33^{a}	5.37 ± 2.44	5.87 ± 2.54^{a}	5.33 ± 2.68		
В	5.40 ± 2.33^{b}	$4.20{\pm}~2.14^{b}$	5.47±2.61	5.13 ± 2.19^{ab}	4.63 ± 2.02		
С	4.53 ± 2.91^{b}	4.00 ± 2.07^{b}	5.40 ± 3.03	4.13 ± 1.85^{b}	4.67 ± 2.02		
D	4.50 ± 2.14^{b}	3.97 ± 2.01^{b}	4.67 ± 2.47	4.17 ± 1.93^{b}	4.10 ± 1.77		
Sig	**	**	NS	**	NS		

Means with different superscript in the same column are significantly (P<0.05) different

A = control yoghurt with no additive

B = yoghurt with 5% of watermelon seeds powder

 $C=yoghurt \ with \ 7\% \ of watermelon seeds powder$

D = yoghurt with 10% of watermelon seeds powder

The flavor of the yoghurt samples (Table 5) did not change during the storage period, the highest flavor score (4.87 ± 2.34) was at day 5 and the lowest value was at day 10. The texture of the yoghurt samples did not change during the storage period, the lowest value (4.50 ± 2.46) was reported at day 5. The Taste of the yoghurt samples changed slightly during the storage period as the storage period progressed the Taste scores decreased the highest score (5.00 ± 2.31) was at day 1.

The overall acceptability of the yoghurt samples improve slightly during the storage period as the storage period progressed the overall acceptability scores increased the highest score (4.92 ± 1.97) was at day 10.

4.7. Effect of watermelon seeds powder and storage period on the sensory characteristics of set yoghurt: -

Data in Table 6 showed the effect of storage period and different levels of watermelon seeds powder on sensory characteristics of set yoghurt.

The result indicated that the storage period and different levels of watermelon seeds powder had no significant (P>0.05) effect on color, flavor, texture, taste and overall acceptability of the yoghurt samples.

The color of control yoghurt samples at day 1 secured highest score (6.90 \pm 2.68) while, the lowest one (3.10 \pm 2.84) was in the yoghurt samples with 7% watermelon seeds powder at day 5. Although the control yoghurt samples showed highest flavor score (6.00 \pm 2.31) at day 1, while the lowest one (3.50 \pm 1.27) was in the yoghurt samples with 10% watermelon seeds powder at day 10. The highest texture score (6.40 \pm 2.67) was for the control yoghurt samples at day 1, and the lowest value (4.00 \pm 2.16) was for the one at day 5. The control yoghurt sample at day 1 was recorded the highest score (6.50 \pm 2.80), while the lowest one (3.80 \pm 1.69) was for that with 10% watermelon seeds powder at day 1. The highest (6.20

 \pm 2.66) overall acceptability score was for the control yoghurt samples at day 1, and the lowest one (3.10 \pm 1.66) was for the yoghurt samples with 10% watermelon seeds powder at day 1.

 Table (5) Effect of storage period on the sensory characteristics of set yoghurt.

Storage	Sensory Attributes					
period/day	Color	Flavor	Texture	Taste	Overall	
Day 1	5.25 ± 2.48	4.35 ± 2.24	5.75 ± 2.69	5.00 ± 2.31	4.55 ± 2.34	
Day 5	4.65 ± 2.68	4.87 ± 2.34	4.50 ± 2.46	4.75 ± 2.35	4.57 ± 2.22	
Day 10	5.92 ± 2.65	4.22 ± 2.14	5.42 ± 2.66	4.72 ± 2.09	4.92 ± 1.97	
Sig	NS	NS	NS	NS	NS	

Means with different superscript in the same column are significantly (P>0.05) different

Storage period	Treat ment	Sensory Attributes						
		Color	Flavor	Texture	Taste	Overall		
Day 1	Α	6.90 ± 2.68	6.00 ± 2.31	6.40 ± 2.67	6.50 ± 2.80	6.20 ± 2.66		
	В	5.40 ± 2.27	3.60 ± 2.17	600 ± 2.71	5.00 ± 2.05	4.60 ± 1.78		
	C	4.70 ± 2.31	4.20 ± 2.15	600 ± 3.26	4.70 ± 2.00	4.30 ±2.31		
	D	4.00 ± 1.94	3.60 ± 1.65	4.60 ± 2.01	3.80 ± 1.69	3.10 ± 1.66		
Day 5	Α	6.50 ± 2.63	5.90 ± 2.02	4.00 ± 2.16	5.40 ± 2.63	4.30 ± 2.98		
	B	4.80 ± 2.20	4.90 ± 2.13	4.80 ± 3.05	5.30 ± 2.11	4.50 ± 1.58		
	C	3.10 ± 2.84	3.90 ± 2.33	4.90 ± 2.51	3.80 ± 2.35	4.80 ± 2.39		
	D	4.20 ± 2.15	4.80 ± 2.74	4.30 ± 2.31	4.50 ± 2.27	4.70 ± 2.00		
Day 10	Α	6.60 ± 2.80	5.40 ± 2.80	5.70 ± 2.00	5.70 ± 2.31	5.50 ± 2.27		
	B	6.00 ± 2.58	4.10 ± 2.13	5.60 ± 2.12	5.10 ± 2.60	4.80 ± 2.74		
	C	5.80 ± 3.12	3.90 ± 1.91	5.30 ± 3.46	3.90 ± 0.99	4.90 ± 1.37		
	D	5.30 ± 2.31	3.50 ± 1.27	5.10 ± 3.14	4.20 ± 1.93	4.50 ± 1.27		
Si	ig	NS	NS	NS	NS	NS		

Table (6) Effect of the different levels of watermelon seeds powderand storage period on sensory characteristics of set yoghurt.

Means with different superscript in the same column are significantly (P>0.05) different

A = control yoghurt with no additive

B = yoghurt with 5% of watermelon seeds powder mixed with cow's milk

C = yoghurt with 7% of watermelon seeds powder mixed with cow's milk

D = yoghurt with 10% of watermelon seeds powder mixed with cow's milk

CHAPTER FIVE DISCUSSION

Watermelon seeds are the most important cash crop in Sudan mainly in western Sudan during the lean years they can be used as stable food mainly the people make porridge from these seeds.

Addition of different levels of watermelon seeds powder had significant effect (P<0.01) on the chemical composition of set yoghurt (Table 1). The high protein content of the yoghurt samples with watermelon seeds powder (C, D and B) probably due to the high protein content of watermelon seeds powder. Tabiri et al., (2016) reported that watermelon seeds contained high protein content. This result was agreed with the statement of Ehirim and Onyeneke, (2013) who reported that protein content of yoghurt blends increased as percentage goat milk increased. Also in agreement with Oladipo et al., (2014) who stated that high crude protein of tiger-nut milk yoghurt could probably be due to high crude protein of tiger-nut. The fat content increase gradually with an increase of the percentage of watermelon seeds powder this may be due to the fact of watermelon seeds contain at about 26.50 - 27.83% crude fat (Tabiri et al., 2016). This result was agreed with that of yoghurt samples with guava seed powder (Maurya, 2015). The results of high titratable acidity for the yoghurt samples with watermelon seeds powder (Table 1) could be due to more availability of carbohydrate in watermelon seeds powder to fermenting microbes. These results are in line with Maurya (2015).

Statistical analysis shows significant difference in total solids among the control and experimental yoghurt samples (p<0.01). The high total solids contents in the yoghurt samples with watermelon seeds powder might be due to considerable contents of total solids in the watermelon seeds powder. This result was agreed with that by Roy *et al.*, (2015) who reported that the total solids

increased with an increase of banana pulp in yoghurt. The ash content also increased as the proportion of watermelon seeds powder increased in the yoghurts. This could be due to the fact that watermelon seeds powder has high ash content and minerals (Tabiri *et al.*, 2016). The high ash values in the yoghurt with watermelon seeds powder agreed with the results of baobab substituted yoghurts by Eke *et al.*, (2013).

The protein content of the yoghurt samples decreased significantly (P<0.01) as the storage period progressed (Table 2). The decrease in the protein content could be due to the proteolytic activity of the starter cultures. This result was in agreement with that by Mohammad and El-Zubeir (2011) and Maurya (2015). The fat % firstly increase till day 5 and then decrease afterwards, the decrease of fat content might be due to the increase action of lipolytic bacteria during storage. Kumar (2013) reported that fat content of herbal ice cream decreased during storage. The titratable acidity increase as storage period progressed this result was in line with Maurya (2015), Mohammad and El-Zubeir (2011) and Al-Otaibi and El-Demerdash (2008). The total solids decreased as the storage period increase this possibly due to the proteolytic and lipolytic activities of the culture during storage. This result was in disagreement with Hassan and Amjad (2010) who said that with the passage of time, total solids mass could be increased. Abubakar et al., (2005) conducted a study on physicochemical properties of yoghurt prepared from three base materials; cow milk, whole milk and powdered milk. They estimated that total solids were increased in the three samples. Ash % of the yoghurt samples decreased as storage period progressed; this might be due to the increase rate of synersis during storage period which might increase the loss of minerals in the whey.

The interaction among the addition of different levels of watermelon seeds powder and storage period had no significant effect on the protein content (Table 3). The decrease in protein % could be due to the breakdown activity of the culture. The Fat content had significantly affected (p<0.05) it was decrease during storage except in the yoghurt with 10% watermelon seeds powder it was increase. The increase in fat % was in agreement with Hassan and Amjad (2010), and Mutlu and Guler (2005) who observed that the fat content of bio-yoghurt ranged from 3.1 to 4.5% during storage. The total solids % decrease during storage period in all sample with no significant effect.

The organoleptic evaluation and acceptability of the different yoghurt samples are shown in Table 4. The statistical analysis revealed that the watermelon seeds powder had significant (p< 0.01) negative effect on color and flavor, this result agreed with Maurya (2015) who said that guava seed powder and yoghurt culture had negative effect on the yoghurt, and with Sivakumar (2014) who reported that control and flax seeds fortification yoghurt samples varied significantly in color and appearance. The taste followed the same trend. Slightly decrease in texture and overall acceptability with no significant effect.

The storage period was not affected the sensory characteristics of the yoghurt samples (Table 5) significantly (P>0.05). This result was not in line with Cinbas and Yazici (2008) who are recorded that blueberry and sugar mass fractions and storage time had a significant effect on the physicochemical and sensory properties of yoghurts. And also was disagree with Roy *et al.*, (2015) who indicated that there was significant (p<0.05) difference in sensory attributes during storage period.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1. Conclusion:

From the results of the study it would be concluded that:

- Possibility of manufacturing yoghurt from milk incorporated with watermelon seeds powder.
- Chemical analysis of the yoghurt samples showed that the watermelon seeds powder yoghurt had higher fat, protein and mineral content as compared to those in control yoghurt.
- The sensory evaluation of yoghurt prepared by incorporating different levels of watermelon seeds powder show that the watermelon seeds can be added up to 5% into milk for preparation of yoghurt.
- Storage period significantly (P<0.01) affected the chemical composition of the yoghurt samples.
- Storage period has no significant effect on the sensory characteristics of yoghurt samples.

6.2 Recommendations:

- Further work will be required for determination the maximum level of watermelon seeds powder added to yoghurt.
- Studies about evaluation of microbiological quality of watermelon seeds powder yoghurt.

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APPENDIX Sensory evaluation

Samples No.	Color	Flavor	Texture	Taste	Overall acceptability
1-					
2-					
3-					
4-					
Color: Extremely Accept Acceptable 7 Slightly accept 5 Moderately accept Not acceptable 1 Taste: Extremely Accept Acceptable 7 Slightly accept 5 Moderately accept Not acceptable 1 Flavor: Extremely intense Intense 7 Moderately intense Slightly intense 3 Poor 1 Texture: Very soft 9 Soft 7 Slightly soft 5 Tough 3	ot 3 table 9 ot 3 e 9 se 5				
Very tough 1 Overall acceptal Extremely intense Intense 7 Moderately intense Slightly intense 3	e 9 se 5				

Poor 1