بسم الله الرحمن الرحيم

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Assessment of two Types of gum Arabic as Prebiotic

تقييم نوعين من الصمغ العربي كبريبايوتك

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قال تعالي:

(فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ وَلَا تَعْجَلْ بِالْقُرْآنِ مِنْ قَبْلِ أَنْ يُقْضَى إِلَيْكَ وَحْيُهُ وَقُلْ رَبِّ زِدْنِي عِلْمًا).

صدق الله العظيم

سورة طه الآية (114)

Dedication

To my father and mother the greatest parent I know …

With love and full respect

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Abstract

This study was carried out to explore prebiotic effects of two types of gum Arabic on beneficial bacterial groups in intestine of rats and in stimulating *Bifidobacterium longum* BB536 growth in peanut milk. Gum Arabic *Acacia senegal* gum "Hashab", and *Acacia seyal* gum "Taleh" were used in this study. Fourty male Albino rats were acclimatized for two weeks to experimental conditions and randomly assigned into five groups. The control group (n=8) received normal rat diet. Other rats groups received normal rat diets partially substituted with wheat bran (n=8) , gum -*Acacia senegal* (n=8), Gum *Acacia seyal*(n=8) , and a mix of gum (n=8) . The experimental trials were extended for a period of 6 weeks. Peanut milk was prepared by roasting the seeds at 130°C for 20 min and soaking in water (12 h), then blending for 5min before filtering to obtain the milk. The milk was sterilized and supplemented with different types of gum Arabic and then inoculated by 3% active *B. longum BB536* culture to produce the fermented peanut milk. General health of rats indicated significant ($p< 0.05$) differences in feed intake, the weight gain and water consumption between five groups of rats. However, there were no blood hematology abnormalities and no signs of any moderate and mild deficiencies of nutrients as revealed by results on blood biochemistry. Enzymes of liver such as serum ALT, AST, and ALP of fed rats groups were within the standard normal range of rats. Feeding with *Acacia senegal* and mix gum had induced significant ($P <0.05$) increases of bifidobacteria in ceacum of rats. Where as *Lactobacillus* significantly ($P <0.05$) increased in ceacum and colon of all rats groups as compared with the control. In addition, there were noticeable significant ($P <0.05$) decreases in pathogenic bacteria, Salmonella, Staphylococcus, Enterocuccus and Enterobacteriaceae in ceacum and colon of rats groups in comparison to control. There were significant ($P <0.05$) increases in viable count of strain BB 536 by extended fermentation period in four types of formulated

beverages as compared to strain level at the beginning of fermentation. The maximum number of strain BB 536 in all beverages was more than 6 log cfu/ml fulfilling the minimum number required to presence in probiotics foods to exert health benefits. During the refrigeration storage of different formulated beverages, there were significant ($p<0.05$) reduction in *B. longum BB536* viable count pH, TSS, and total sugars in the two weeks storage of all fermented beverages. Overall acceptability of different strain BB 536 fermented peanut milks affirmed that panelists mostly preferred peanut milk containing *Acacia senegal* gum followed by the control and then the milk with mix gum. Therefore, two types of gum Arabic and their mix exerted a prebiotic effect both in *IN VIVO* (stimulated beneficial bacteria and suppressed pathogenic ones in rats) and in *IN VITRO* (stimulating growth of strain BB 536), thus are useful for synibiotic (probiotic and perbiotic in same product) application into non- dairy products as functional foods.

ملخص البحث

أجريت هذه الدراسة لاستكشاف التأثيرات الحيوية لنوعين من الصمغ العربي على الفئران ,وتحفيز نمو *Bifidobacterium longum* BB536 في لبن الفول السوداني. تم استخدام الصمغ العربي اكاشيا سنغال ''هشاب ''واكاشيا سيال'' طلح .''تم أقلمت أربعين فار من ذكور الألبينو لمدة أسبوعين على ظروف التجربة، وتم تقسيمهم عشوائياً إلى خمس مجموعات. مجموعة الشاهد(العدد =8) تناولت العلف العادي للفئران. المجموعات الأخرى تناولت العلف العادي مع الإحلال الجزئي ردة القمح(العدد =8) , ومجموعة تناولت صمغ أكاشيا السنغال العدد =8)، مجموعة تناولت صمغ أكاشيا السيال(العدد =8), ومجموعة تناولت خليط من الصمغ العربي (العدد =8). امتدت فترة التجارب لمدة 6 أسابيع. تم تحضير لبن الفول السوداني بواسطة التحميص عند درجة حرارة 130 درجة مئوية لمدة 20 دقيقة ونقعه في الماء 12 ساعة، ثم خلطه لمدة 5 دقائق قبل الترشيح للحصول على اللبن .عقم اللبن ودعم اللبن بأنواع مختلفة من الصمغ العربي، ثم تلقيحه من قبل 3 ٪ من BB563 المنشطة لإنتاج لبن الفول السوداني المخمرة. وأشارت الصحة العامة للفئران إلى اختلافات كبيرة ($P < 0.05$) في التغذية المتناولة ، وزيادة الوزن واستهلاك المياه بين المجموعات المختلفة من الفئران. ومع ذلك، لم يكن هناك خلل في أمراض الدم ولا توجد علامات على أي نقص معتدل وخفيف في المواد الغذائية. كما يتضح من النتائج على الكيمياء الحيوية في الدم .كانت إنزيمات الكبد مثل ALT ، AST، و ALPمن مجموعات الفئران المغذية ضمن النطاق الطبيعي القياسي للفئران .التغذية بصمغ أكاسيا السنغال وخليط الصمغ العربي قد حفز على زيادة معنوية ($P < 0.05$) Bifidobacteria في القولون الصاعد .في حين أن الزيادة المعنوية ($P < 0.05$) Lactobacillus في القولون الصاعد والقولون في كل مجموعات الفئران بالمقارنة مع مجموعة الشاهد .بالإضافة إلى ذلك، كان هناك انخفاض ملحوظ ($P < 0.05$) في البكتيريا الممرضة السالمونيلا، المكورات العنقودية، Enterocuccus و Enterobacteriacea في القولون الصاعد والقولون في مجموعات الفئران بالمقارنة مع الشاهد.

.كانت هناك زيادة معنوية ($P < 0.05$) في عدد النمو لسلالة BB 536 من خلال فترة التخمير الممتدة في كل أنواع المشروبات المصممة مقارنة ببداية التخمر خلال التخزين المبرد لمختلف المشروبات المصممة، الحد الأعلى للبكتيريا الصديقة *Bifidobacterium longum* BB536 في الأربعة أنواع من المشروبات كان أكثر من log 6 CFU/ ml والذي يوفى الحد الأدنى للعدد المطلوب وجوده في أغذية البكتيريا الصديقة لإطفاء فوائد صحية. أثناء التخزين المبرد لمختلف المشروبات المصممة كان هناك انخفاض معنوي ($P < 0.05$) في اعدادBB536 والجوامد الصلبة الكلية، والسكريات الإجمالية

في التخزين لأسبوعين لجميع المشروبات المخمرة . وقد أوضح القبول العام لمختلف ألبان الفول السوداني المخمر بسلالة BB 536 أن معظم المشاركين يفضلون منتج لبن الفول السوداني مع الأكاشيا سنغال متبوعًا الشاهد ثم اللبن مع مزيج الصمغ العربي. نستنتج أن النوعين من الصمغ العربي وخليط الصمغ العربي قد حفزت نمو البكتيريا الصديقة *Bifidobacterium longum* BB536 في تجربة المعمل وبالتالي هي مفيدة في تطبيقات السنبايوتك (وجود البكتيريا الصديقة ومحفزاتها وقمع المجموعات الممرضة في الفئران)، و مفيدة لتطبيقات الأغذية الوظيفية.

CHAPTER ONE

INTRODUCTION

Gum Arabic (GA) is dried exudates obtained from the stems and branches of *Acacia senegal* or *Acacia seyal* which are cultivated in the Sudan as a cash crop in agro forestry systems (Abdul-Hadi *et al.,* 2010). Gum Arabic production covers a wide area as it is considered as a principal national product.

Gum Arabic contain more than 80% fiber and due to its low viscosity and its high solubility in water, it is easy to complement food stuff like beverages, dairy products, snack-bars, biscuits, confectionery and meat products with high amounts (up to 50%) of gum Arabic, in addition it is tasteless and odorless therefore giving no off-flavor (McLean-Ross *et al.,* 1983).

GA is a administrated as prebiotic in human in a dose dependent manner, This effect is indicated by an increases in the numbers of the bifidobacteria and Lactobacilli (Calame *et al.*,2008). In intestinal tract gum Arabic is indigestible to both human and animal. It is not degraded in the small intestine, but fermented in the large intestine by microorganisms to short-chain fatty acids, particularly propionic acid (Badreldin *et al*., 2008). Gum Arabic is rich in dietary fiber that is derived from dried exudates of A. senegal. It contains a high molecular weight (lipoprotein) heterogeneous gum polysaccharides (Abd-Razig *et al*., 2010).

The chemical composition of GA is complex and consists of a group of macromolecules characterized by a high proportion of carbohydrates (97%), which are predominantly composed of D-galactose and L-arabinose units and a low proportion of protein (<3%) (Montenegro *et al.,* 2012).

Exceptionally often the modern diet does not contain enough dietary fiber and contain too much fat and sugar. Therefore the goal is often to improve the "normal" diet with dietary fiber in order to improve the nutritional value, stimulate the natural digestion and thus to ensure a healthy condition. (Kohimeler *et al.*1993). No enough studies clearly addressed prebiotic effects of Gum Arabic (Gibson and Roberfroid, 1995). Although gum Arabic contains a considerable amount of fiber and is even sold as a fiber supplement, typically marketed under the name “Acacia fiber.” Dietary fiber, particularly from gum Arabic, therefore, it is essential to evaluate the prebiotic effect of the different types of gum Arabic in Sudan.

In this respect, the use of peanut milk and gum Arabic supplemented will complement probiotic growth at the same time can be a successful non-dairy carriers for *Bifidobacterium* strain . This study aim to assess different types of gum Arabic as prebiotic in *invivo* and in *vitro* modle.

The objectives of this study were to:

1. Determine the proximate composition of gum Arabic and peanut.
2. Evaluate the general health of experimental rats fed with normal diet supplemented with gum Arabic .
3. Assess the prebiotic effect of gum Arabic on the intestinal microbial community of fed rats.
4. Evaluate the growth of *Bifidobacterium longum* BB536 in peanut milk beverage supplemented with gum Arabic.
5. Determine physico-chemical properties and sensory characteristics of peanut fermented beverage.
6. Evaluate the survival of *Bifidobacterium longum* BB536 strain during refrigeration storage of the fermented beverage.

CHAPTER TWO

LITERATURE REVIEW

2.1. Prebiotics

Prebiotic is mainly indigestible food item that usefully affect the host by selectively stimulating the growth and activity of one or a partial number of bacteria in the large intestine, and thus improves the host health (Gibson and Roberfroid, 1995). Prebiotics must be resistant to enzymatic digestion and absorption in the gastrointestinal tract and must be fermented by intestinal bacteria in colon (Roberfroid, 2007).Prebiotics such as galactooligosaccharide , fructooligosaccharide, xylooligosaccharide, beta-glucans and inulin are a non-digestible food ingredients that encourage growth or activity of probiotic bacteria (Birkett and Francis, 2010; Nauta *et al*., 2010 and Paineau *et al.*, 2010). Among prebiotics gum Arabic (GA) is one of the prebiotics when mixed with probiotic in synbiotic products enhance the growth and survival of probiotic bacteria in fermented dairy products (Desai *et al.*, 2004).

2.2. Sources of prebiotics

Prebiotics are mainly derived from fiber, like inulin. Often, yogurt marketed as probiotic yogurt will contain inulin (prebiotic) and live active cultures (probiotic). Other good sources of prebiotics include Acacia gum, dandelion greens, garlic, asparagus, beans, oats, and chicory root (Gibson and Roberfroid, 1995).

Traditional dietary sources of prebiotics include soybeans, inulin sources (such as Jerusalem artichoke, Jicama, and chicory root), raw oats, unrefined wheat, unrefined barley, and yacon. Some of the oligosaccharides that naturally occur in breast milk are supposed to play an important role in the development of a healthy immune system in infants (Gibson and Roberfroid., 1995).

2.3. Dietary fibers

Dietary fibers are part of the plant cell which cannot be digested by the human enzymes. Two various groups of dietary fibers are recognized: soluble and non-soluble dietary fiber. They are remarkable by their solubility in water and show different physiological effects. The benefit of dietary fiber for a healthy diet is widely known. Different diseases, such as constipation, coronary heart diseases and cancer have been correlated to an unhealthy diet, low in dietary fiber (Kohimeler *et al*.1993).

2.4. Gum Arabic

A gum, is any water-soluble polysaccharide that is extracted from marine and land plants, or from microorganisms that acquire the ability to contribute viscosity or gelling ability to their dispersions (Abu Baker *et al.,* 2007). The most fundamental property of a gum is its water solubility and high viscosity in aqueous dispersions. For this reason, resins, latexes and other hydrophobic gums are not regarded as true gums. Through the advantage of natural gum over the artificial counterparts is the bio compatibility, low cost, low toxicity and relative widespread availability (Abu Baker *et al.,* 2007).

Gum Arabic is obtained from a tree of genus; Acacia, subfamily; Mimosodieae, family; Leguminosae. It is known as gum Acacia, a natural gum taken from two species of the Acacia tree; *Acacia senegal* and *Acacia seyal* (ITC, 2008). Gum Arabic is a pale to orange-brown solid which break along a glassy fracture. The best grades are whole, spheroidal-tear shaped, orange, brown, with a matt surface texture. It is a national and international exported commodity, (ITC, 2008). Gum export has been the mainstay of the Sudanese economy for over 400 years, (Abdel Nour, 1999) it was used at least 4000 years ago when it was shipped as an article of trade by Egyptian fleets (Lawal, 1998).

The gum is made up of highly branched polysaccharides, and also a complex mixture of saccharides and glycoprotein, which gives it most useful property, it is perfectly edible (Smolinske, 1992). It is very soluble in water and is a neutral or slightly acidic salt of a complex polysaccharide containing calcium, magnesium and potassium salt (Abdul-Hadi *et al.*, 2010). Gum Arabic is unique in that it is produced by trees, only when they are in an unhealthy condition. Healthy trees have not been observed to provide gum. Most authorities believe that the formation of gum exudates is a pathological condition resulting from a microbial infection of the injured tree. (Badreldin *et al.*, 2008; Abdul-Hadi *et al.*, 2010). Natural factors that tend to lower the vitality of the tree, such as poor soil, require of moisture and hot weather, improve gum yields, others consider the production of gum to be a normal metabolic process in the plant, with the quantity and quality produced is a function of environmental conditions (Badreldin *et al.*, 2008; Abdul-Hadi *et al.*, 2010). The gum has a wide range of industrial utilization, especially, in areas of foodstuff, textiles and pharmaceutical industries. In food products, it serves as a stabilizer, emulsifier, and binding agent for chewing gums, ice creams and jams. In pharmaceuticals, the gum is a binder in tablets, pills, throat pastilles and cough drops (Abdul-Hadi *et al*., 2010). In the textile industry, it is used for textile stiffening and as a binder for textile printing. It is also used in the plastic industry (Verbeken *et al.*, 2003). In various industries it is used in producing ink, watercolors, paints, carbon papers, pottery glazes etc. (Montenegro *et al.*, 2012) The main use of gum Arabic remain in confectionery, solid gum, soft gum and gum pastilles. Gum Arabic provides income for farmers and merchants also provide a good foreign exchange for the nation because there is a world demand for the commodity (ITC, 2008; Unanaonwi, 2011).

Despite the verity that GA is widely used as a vehicle for drugs in experimental physiology and pharmacological experiments and is assumed

to be an "inert" substance, some recent reports have claimed that GA possesses antioxidant, nephroprotectant and other effects (Badreldin *et al.*, 2008). Among all the types of gums marketed worldwide, gum Arabic is without doubt the most well known and the most in demand both at the levels of the producing regions and internationally (Lelon *et al*., 2010). This often leads to a downgrading of offers because some suppliers mix in other elements into the product in order to fulfill their obligation. It also resulted in the over-exploitation of resources as producers seek to increase the availability of gum Arabic (Lelon *et al*., 2010).

2.5. Chemical composition and structure of gum Arabic

The chemical composition of GA may vary slightly depending on its origin, climate, harvest season, tree age and processing conditions, such as spray drying (Flindt *et al.*, 2005; Hassan *et al.*, 2005; Siddig *et al.*, 2005). There are some differences between the chemical compositions of the GA taken from *Acacia senegal* and *Acacia seyal*. Both gums have the same sugar residues, but *Acacia seyal* gum has a lower content of rhamnose and glucuronic acid and a higher content of arabinose and 4-O-methyl glucuronic acid than *Acacia senegal* gum, put *Acacia seyal* gum contains a lower proportion of nitrogen, and specific rotations are also completely different (Montenegro *et al.*, 2012).The determination of the last parameters may clearly spot the difference between the two species (Osman *et al.*, 1993; Montenegro *et al.*, 2012).

The main amino acids present in the protein of an Arabino Galactan gum Arabic and an Arabino galactan-Proteine Complex (AGP) were hydroxyl proline, serine and proline, where as in Glycoprotein (GP), aspartic acid was the most abundant (Badreldin *et al.*, 2008).

2.6. pH

The hydrogen ion concentration (pH) of gum Arabic solutions is normally around 4.5-5.5. However maximal viscosity is found at pH 6.0. Gum Arabic has excellent emulsifying properties (Verbeken *et al*., 2003).

2.7. Gum Arabic enzymes

One of the major functional characteristics of gum Arabic is its ability to act as an emulsifier for essential oils and flavors. Prolonged heating of gum Arabic solution causes 32 protinaceous components to precipitate out of solution, thus influencing the gums emulsification properties. Gum Arabic contains both oxidases and peroxidases that are inactive by heating the gum solution to 80 ºC or higher for one hour. The peroxide content varies, but the enzyme can be in actived without affecting the viscosity by heating to 100ºC (Williams, 2000). The gum Arabic, enzymes such as oxidases, peroxidases, and pectinases, some of which have antimicrobial properties (Tyler *et al.*, 1977; Kirtikar and Basu, 1984)

2.8. Applications of gum Arabic

Exudates gum is used in a great number of uses, mainly situated in the food area, there are also considerable non-food applications. Gum Arabic is being widely used for industrial purposes such as a stabilizer, a thickener, an emulsifier and an encapsulating in the food industry and to a lesser extent in textiles, ceramics, lithography, cosmetic and pharmaceutical industry (Montenegro *et al.*, 2012). In the food industry, Gum Arabic is primarily used in confectionery, bakery, dairy, and beverage as a microencapsulating agent (Montenegro *et al.*, 2012).

Gum Arabic readily dissolves in cold and hot water the solutions are characterized by a low viscosity, allowing the use of high gum concentrations in various applications (Verbeken *et al*., 2003).

2.9. Safety of gum Arabic

The first case of an industrial sensitization of gum Arabic in a candy factory was reported more than seven decades ago (Badreldin *et al.*, 2008). That was followed by a number of reports causing dermatitis and asthma (Ilchyshyn and Smith., 1985). In 1998, a carbohydrate specific IgE was identified in a chocolate confectioner with cough and dyspnea (Fotisch *et al.*, 1998). Sander *et al.*, (2006) found that sensitization to gum Arabic carbohydrate structures occurs causing in atopic patients with pollen sensitization without obvious exposure to gum arabic, and that allergy to gum Arabic is mediated preferentially by Igt antibodies directed to polypeptide chains of gum Arabic.

Gum Arabic (10% in the drinking water) for either 3 or 14 days decreased urinary excretion of inorganic phosphate and increased calcium and magnesium and also decreased plasma concentrations of 1.25-dihydroxy vitamin D in healthy Mice (Nasir *et al*, 2007). The level of the metabolic disturbances in mice with renal insufficiency has not been determined, but is expected to be more pronounced (Badreldin *et al*., 2008).

Several reports showed that gum Arabic has antioxidant capacity (Montenegro *et al.*, 2012. There are controversial results of it, mainly in vivo studies. For example, gum Arabic (GA) has been reported to cause a protective effect against gentamicin and cisplatin nephrotoxicity (Al-Majed *et al.*, 2002), and doxorubicin cardiotoxicity (Abd-Allah *et al*., 2002) in rats. Ali *et al*., (2009) reported that treatment of rats with GA causes only a slight palliative effect of gentamicin nephrotoxicity. Trommer and Neubert (2005) studied lipid per oxidation antioxidant and reducing effects in vitro of various polysaccharides (including GA). They found that GA reduces lipid per oxidation of skin in a dose-dependent. Ali *et al*.,(2009) reported that administration of GA at concentrations of 2.5%, 5.0% and 10.0% in drinking water for eight following days in rats did not significantly vary the

concentrations of free radical scavenger's glutathione (GSH) and ascorbic acid (AA), and superoxide dismutase (SOD), or lipid per oxidation. Dietary fibers are not digested in the upper parts of the digestive system, but the microbiological flora of the intestine contains enzymes which are able to metabolize them. The pH decreases to a certain limit, due to the digestion of the dietary fiber in the intestine, which is accounted as positive for this organ (Jacobs and Lupton, 1986) and prevents the growth of pathogenic bacteria. (Campbell *et al.,* 1997).

The fermentation of dietary fibers results in short chain fatty acids (SCFA). Propionate, Acetate, and butyrate are the main fermentation products. The SCFA are metabolized by the cells of the walls of the intestine, providing them with an important energy source. Because of its highly branched chemical structure the fermentation of Gum Arabic is very slow and gas production is delayed in time, displace all along the large bowel without irritating a feeling of flatulence. Soluble dietary fibers help decrease the total cholesterol and the LDL-cholesterol, which has a negative influence on coronary heart diseases. Amount of 25g of gum Arabic per day reduce the total cholesterol significantly and have therefore a positive influence on the prevention of coronary heart diseases (McLean-Ross *et al.,* 1983).

The higher amount of living cells also leads to a higher fecal volume and a longer passage through the ceacum allowing a better digestion and absorption of other nutrient (Bliss.,1996). Gum Arabic also has an influence on the growth of the Epithelium cells of the digestive system.

Gum Arabic has the property to bind cations, especially divalent cations as calcium and magnesium (Tulung *et al.*,1997) .Due to this effect the amount of calcium and magnesium in the ceacum rises significantly. The result is a supply of these cations in the large bowel, where they are efficiently absorbed The absorption of minerals in the bowel is increased through Gum Arabic and

the nitrogen metabolism is positively influenced by gum Arabic (Tulung *et al.*,1997).Gum Arabic has an influence on the balance of nitrogen in fecal and urinary excretion (Assimon, and Stein, 1994). It decreases the urinary nitrogen excretion and increases the fecal nitrogen excretion. With a normal diet Gum Arabic decreases the urea production and urea recycling (Assimon, and Stein, 1994). All this leads to a decrease in the workload on the kidneys, (Assimon, and Stein, 1994) Due to the fermentation in the bowel and the production of volatile fatty acids, which can be partly absorbed, there is a small intake of energy from Gum Arabic (Phillips,1998). The caloric value of Gum Arabic is suggested to be 1.5 kcall (Phillips,1998).

2.10. Probiotics.

The Joint FAO /WHO, 2001). Working Group defines probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host. Probiotics are a type of living bacteria that actually benefit health when taken in an appropriate amounts (FAO/ WHO, 2001). These useful friendly bacteria, to be found in the gastrointestinal tract, come in a variety of forms with more than 400 different bacteria living in the human gastrointestinal tract; the most common forms of intestinal probiotics are *L. Acidophilus* and *Bifidobacteria bifidum*. These bacteria perform a balancing agent for non-friendly, pathogenic, gut bacteria such as the Candida fungus or *E. Coli* (Birada *etal*,2005). Studies show that *L. acidophilus* actually creates a natural form of antibiotics in the body (Biradar.*et al*, 2005). Stress, unhealthy lifestyles, and most importantly, unhealthy acidic diets, destroy natural amounts of probiotics (Asahara *et al.*, 2004).

In the last decade, the use of probiotics in fermented dairy product and feed product applications has a noticeable interest and development. These organisms play significant role in lowering the pH of the large intestine through the release of lactic and acetic acid (Asahara *et al*., 2004).

2.11. History of probiotics

At the beginning of the 20th century, the Russian Nobel Prize Laureate Elie Metchnikoff associated the observed longevity of Bulgarian peasants with their high consumption of live microbes in fermented milk products, as he reported in his book the prolongation of life (Metchnikoff, 1907). The concept of probiotics was already successful in Asia for many years when the first probiotics fermented milk products were eventually introduced in Europe.

2.12. Health benefits of probiotics

Vitamin production, availability of minerals and trace elements, production of important digestive enzymes such as β –galactosidase for alleviation of lactose intolerance, barrier, restoration, antagonistic effects against: Infectious diarrhea, antibiotic – associated diarrhea, irradiation – associated diarrhea, cholesterol –lowering effect. Stimulation and improvement of the immune system, enhancement of bowel motility, relief from constipation. Anti-carcinogenic effects in the colon, maintenance of mucosal integrity. Reduction of inflammatory allergic reactions, adherence and colonization resistance, antioxidateive activities (Kullisaar *et al*., 2002).

In many cases, the health-promoting mechanisms of probiotic action are not sufficiently known. However, the majority of them are based on the positive effect they exert on the immune response, i.e. on their immunomodulatory activity (Isolauri *et al*., 2001). In most cases, this is due to stimulation of natural immunity (Newburg 2005; Galdeano and Perdigon 2006). In doing so, they modulate primarily the production of cytokines and antimicrobial peptides (Trebichavský and Šplíchal, 2006). This is the mode of action of not just typical sour milk, functional foodstuffs, such as sour milk, kefir or yoghurt (Meydani and Ha 2000) produced by the food-processing industry, but also that of the dietary supplements containing the probiotic bacteria in pure form. However, the final are the products of pharmaceutical industry and

compared to functional food products, they have a standard composition, and known immunomodulatory characteristics, verified both experimentally and in controlled clinical studies (Clancy, 2003). In terms of their quality and efficiency they are also under regular pharmaceutical control. It will be therefore more precise to call them immunobiotics, in order to distinguish them, from classical probiotics in functional foodstuffs (Clancy 2003).

2.13. Criteria of Selection of appropriate probiotic

Different aspects have to be considered in probiotic selection safety criteria for any successful probiotic have been defined in several reviews (Lee and Salminen, 1995; Adams, 1999) include the following specifications:

Strains uses are preferably of human origin. They are isolated from healthy human GI tract. They have a history of being non-pathogenic. They have no history of association with diseases such as infective endocarditis or GI disorders. They do not deconjugate bile salts (bile salt deconjucation or dehydroxylation would be a negative trait in the small bowel (Marteau *et al*., 1995). They do not carry transmissible antibiotic resistance genes.

While in selecting a preferable probiotic strain several aspects of functionality have to be considered: Acid tolerance and tolerance to human gastric juice. Bile tolerance (an important property for survival in the small bowel). Adherence to epithelial surfaces and persistence in the human GI-tract. Immunostimulation, but no pro-inflammatory effect. Antagonistic activity against pathogens such as *Helicobacter pylori*, *Salmonella sp., Listeria monocytogenes* and *Clostridium difficile*. Antimutagenic and antigarcinogenic properties.

Feeding trials with different probiotic strains have shown that the probiotic strain usually disappears from the GI-tract within a couple of weeks after the ingestion is discontinued (Fukushima *et al.,* 1998; Johanston *et al.*,1998;

Alander *et al.,* 1999 ; Donnet-Hughes *et al.*,1999). The role of the probiotic persistence in the human GI-tract has therefore been questioned. However, even temporary persistence, which has been noted for several ingested probiotic strains, may enhance their chances for beneficial functions in the GI-tract, and is therefore considered a desirable trait (Shah, 2000b). Necessary safety and functional criteria the aspects related to probiotic production and processing are also of utmost importance, such as:. Good sensory properties. Phage resistance viability during processing and stability in the product and during storage.

Good viability and activity of probiotics are considered prerequisites for optimal functionality. However, several studies have shown that non-viable probiotics can have beneficial effects such as immune modulation and carcinogen binding in the host (Ouwehand and Salminen, 1998; Salminen *et al*.; 1999). Thus, for certain probiotic strains it might be sufficient that they grow well during initial production steps (to obtain high enough numbers in the product) but they do not necessarily need to retain good viability during storage (Salminen *et al*.; 1999).

2.14. *Bifidobacterium* as probiotics

Bifidobacteria is the predominant species of human colonic and fecal microbiota. It has been extensively introduced into the food industry and pharmaceutical applications (Guar ner and Malagelada, 2003). The prevalence of bifidobacteria in the feces of breast fed infant may have been a major reason for selecting strains of this group for use as probiotics (Lilly and Stillwell, 1965).

Bifidobacteria are considered as important probiotic and used in the food industry to relieve and treat many intestinal disorders (Mayo and van Sinderen, 2010). Bifidobacteria exert a range of beneficial health effects, including the regulation of intestinal microbial homeostasis, the inhibition of

pathogens and harmful bacteria that colonize and/ or infect the gut mucosa , the modulation of local and systemic immune responses , the repression of a number of dietary compounds into bioactive molecules (Mayo and van Sinderen, 2010). Bifidobacteria utilize lactose, galactose and fructose beside glucose, and has the ability to metabolize oligosaccharides beside the simple sugars such as Inulin with special enzymes (Martınez-Villaluenga and Gomez, 2007). These enzymes, which were found in most strains of bifidobacteria and not found in the lactic acid bacteria (Desjardins *et,al.*,1990), include β–glucosidase, α–glucosidase, D-glucosaminidase and β–galactosidase, which are very important in the fermentation of prebiotic with bifidobacteria (Martınez-Villaluenga and Gomez, 2007).

2.15. Species of *Bifidobacterium*

There are several species of *Bifidobacterium* including ***:*** *B. angulatum; B. animalis; B. asteroides; B. bifidum; B. boum; B. breve; B. catenulatum; B. choerinum; B. coryneforme; B. cuniculi; B. dentium; B. gallicum; B. gallinarum; B indicum; B. longum; B. magnum; B. merycicum; B. minimum; B. pseudocatenulatum; B. pseudolongum; B. psychraerophilum; B. pullorum; B. ruminantium; B. saeculare; B. scardovii; B. simiae; B. subtile; B. thermacidophilum; B. thermophilum; B. urinalis; B. sp*. **(**Holzapfel *et al*., 1995**)**

Bifidobacteria spp are high-GC content, Gram-positive bacteria which belong to the Actinobacteria branch and these species naturally colonize the gastrointestinal tract (GIT) of mammals, birds and insects (Ventura *et al*., 2007). Scientists have determined the major probiotic properties of *Bifidobacteria spp* isolated from the human intestine and these properties include the strengthening of the intestinal barrier, modulation of the immune response and antagonism pathogens (Marco *et al*., 2006).

Bifidobacterium spp has been reported to possess various glycosyl hydrolases (GH) and these hydrolases metabolize plant- or milk-derived oligosaccharides including nondigestible ones such as galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS) (Schell *et al*, 2002; Sela *et al.*, 2008). The capability to utilize nondigestible oligosaccharides confer a competitive advantage to *Bifidobacterium spp* in the human gut (Sela *et al.* 2008).

Bifidobacterium longum (B. longum) and various other bifidobacteria strains are often added to probiotic products in combination with other lactic acid bacteria (LAB) (Borriello *et al*.; 2003). Through their long and safe history of application, LAB have acquired the status of "Generally Regarded As Safe" (GRAS), but the safety of bifidobacteria and other LAB strains selected for probiotics still need to be carefully evaluated(Borriello *et al*.; 2003).

The key safety aspects for the use of bifidobacteria and other LAB strains of probiotics include antibiotic resistance, production of harmful metabolites and the potential for virulence. Antibiotic resistance in potential probiotic strains is not considered a risk factor unless resistance is transferred to pathogens or it renders the probiotic untreatable in very rare cases infection (Borriello *et al.*; 2003). Biogenic amines, D-lactic acid, azoreductases and nitroreductases produced by bifidobacteria and other LAB strains are potential health hazards And the safety of some of these compounds have been evaluated (Ruiz-Moyano *et al.;* 2009).

2.16. *Bifidobacterium longum* BB536

Bifidobacterium longum is one of the *bifidobacteria* species found mainly in human feces and it may be considered as the most common species of bifidobacteria, being found both in infant and adult(Ventura *etal.*, 2007). Potential benefits from consumption of *B. longum* include: antagonistic action toward intestinal pathogens, improved lactose utilization, anticarcinogenic action and control of serum cholesterol levels (Borriello *et al.*, 2003).

Scientific studies showed the benefits offered by *Bifidobacterium longum BB536* (Kojima *et al*., 1996; Namba *et al*., 2003). Thus, there is considerable interest in incorporating these healths promoting *bifidobacteria* into food. Nevertheless, probiotic strains, particularly *bifidobacteria* are rarely used outside the diary based industry (Wei *et al*, 2010). The scarcity of animal milk in many countries makes it difficult to provide an adequate *bifidobacteria* intake among many nations (Wei *et al*, 2010).

2.17. Definition of fermentation and its benefits

Fermentation was defined by Gale (1948), as the process leading to anaerobic breakdown of carbohydrates, other major compounds such as organic acids, proteins, and fats. In a broader view, fermentation is an energy yielding process (Kosikiowski, 1982). Human societies all over the world independently discovered the value of fermenting food as a cheap means of preservation, improving nutritional quality and enhancing sensory characteristics. The fermentation of milk, cereals, nut and other substrates to produce beverages with health-promoting properties is indigenous to many regions of Asia, Africa, Europe, the Middle East and South America (Alan *et al*.(2014).

2.18. Type of fermentation

Kosikowski (1982) stated that there are six major fermentation reactions in milk:Lactic acid fermentation, propionic acid fermentation, citric acid fermentation, alcohol fermentation, butyric fermentation, coliform gassy fermentation. Many lactic acid bacteria occur normally in milk and are responsible for its spontaneous souring (Stanier *et al.*,1957).

2.19. Starter culture for milk fermentation

A starter culture can be defined as a microbial preparation of large numbers of cells of at least one microorganism to be added to a raw material to produce a

fermented food by accelerating and steering its fermentation process. The type of starter culture used depends on the desired product. Culture supply companies can provide processors with a variety of cultures modified for their operation that can be purchased frozen or dehydrated, typically as a mixture of several strains (Caplice and Fitzgerald, 1999).

Dairy starters are cultures of safe, active bacteria, grown in milk or whey, which imparts certain characteristics and qualities of various milk products. The culture may be one strain of microorganism species, called a single-strain or a number of strains and species called a multi strain or mixed-strain culture (Kosikowaski, 1982).Starter culture uses in fermented milk products are used not only for acid development, but also to lessen the putrefaction taking place in milk as a result of the presence of spoilage bacteria (Musa, 1994).

Fermented dairy products have different characteristics, and different starter cultures are therefore used in their manufacture. Stareter cultures can be classified according to their preferred growth temperature: Mesophilic bacteria – optimal growth temperatures of 20 to 30 ºC. Thermophilic bacteria - optimal growth temperatures of 40 to 45 ºC (Gosta,2014). The cultures may be of : single – strain type; containing only one strain of bacteria.Multiple – strain type; amixture of several strains, each with its own specific effect (Gosta,2014).

Recently, the use of functional starter cultures in the food fermentation industry is being explored (De Vuyst, 2000). Functional starter cultures are starters that possess at least one inherent functional property. In addition to contribute to food safety and to offer one or more organoleptic, technological, nutritional, or health advantages. The implementation of carefully selected strains as starter cultures or co-cultures in fermentation processes can help to achieve the desired property, maintaining a perfectly natural and healthy

product(Pidcock, *et al*.,2002). Examples are LAB that are able to produce antimicrobial substances, sugar polymers, sweeteners, aromatic compounds, useful enzymes, or nutraceuticals, or LAB with health-promoting properties, so called probiotic strains. This represents a way of replacing chemical additives by natural compounds, at the same time providing the consumer with new, attractive food products. Although probiotic strains may also be classified as functional starter or co-cultures for food fermentations (Erkkila¨*et al*., 2001; Jahreis *et al.*, 2002).

2.19.1Types of dairy cultures

The dairies can obtain cultures with selected properties for specific product characteristics such as texture, flavor and viscosity. According to Gosta (2014) the commercial cultures in various form are:

- Deep – frozen, highly concentrated cultures in readily soluble form, for direct inoculation of product.
- Freeze – dried, highly concentrated cultures in powder form, for direct inoculation of the product.
- Deep – frozen, concentrated cultures for propagation of bulk starter.
- Liquid, for propagation of mother culture (nowadays fairly rare).

2.19.2 Stages of propagation

According to Gosta (2014) the process may involve two or more stages. Cultures in various stages of propagation are known by the following names:

- Commercial culture: master culture- the original culture that the dairy buys from the laboratory.
- Mother culture: the culture prepared from master culture at the dairy. The mother culture is prepared daily and is as the name indicates, the origin of all cultures made at the dairy.

- Intermediate culture: an intermediate step in manufacture of large volumes of bulk starter.
- Bulk starter: the starter used in production.

2.19.3. Growth characteristics of starter cultures

During the growth of any dairy starter culture, the cells divide and increase in number up to a certain level and then start to die. This behavior gives rise to the characteristic growth curve, where it can be seen that the rate of cell division is divided into four different sections (Tamime and Robinson, 2007).

- Lag phase- this is the phase that follows immediately after inoculation of the milk. The delayed bacterial activity could be due to adjustment or adaptation of organism to a new medium.
- Log phase – during this phase the cells display maximum activity, i.e. Shortest generation time, as long as optimum condition (nutrients and temperature) is available.
- Stationary phase- at a certain point, the viable cell count remains constant due to a lack of nutrient and an accumulation of waste metabolites (e.g. lactic acid in milk); the death of old cells and the production of new cells is in balance.
- Death phase- the number of viable cells starts to diminish, mainly due to unfavorable growth conditions

2.20. Functional Food

In the manufacturing world, the concepts in nutrition are changing significantly. From a previous emphasis on survival, through hunger satisfaction, and more recently food safety, food sciences now aim at developing foods to promote well-being and health while at the same time reducing the risk of some major diseases. That is because scientific evidence

supports the hypothesis, by modulating specific target functions in the body; diet can have beneficial physiological and psychological effects that go beyond adequate nutritional effects (Roberfroid.1999). Such evidence is already supported by scientific data show both nutritive and non-nutritive components in food have the potential to modulate target functions in the body which are relevant to well-being and health and reduction of disease risk (Roberfroid.1999).

The concept of functional foods includes foods or food ingredients that exert a beneficial effect on host health and/or reduce the risk of chronic disease beyond basic nutritional functions(Huggett and Schliter, 1996).Successful types of functional products that have been designed to reduce high blood pressure, cholesterol blood sugar, and osteoporosis have been introduced into the market (Sanders, 1998). Recently, the functional food research has moved progressively towards the development of dietary supplementation, introducing the concept of probiotics and prebiotics, which may affect gut microbial composition and activities (Ziemer and Gibson, 1998).

2.21. Probiotic foods

Probiotic foods are defined as those that contain single or mixed culture of microorganisms that affect beneficially the consumer's health by improving their intestinal microbial balance (Fuller, 1989). There is significant scientific evidence, based mainly on in vitro studies and on clinical trials using animals, suggesting the potentially beneficial effects of probiotic microorganisms. These include: metabolism of lactose, control of gastrointestinal infections, suppression of cancer, reduction of serum cholesterol, and immune stimulation (Gilliland, 1990; Salminen *et al*., 1998; Fooks *et al.*,1999).

2.22. Milk

Milk is considered a staple in many people diet. It is consumed as a beverage, poured on cereal and added to smoothies, tea or coffee. Milk defined as a normal secretion of the mammary glands of mammals. Moreover, (Musa, 1994) define it as an emulsion of fat in a watery solution of sugars and mineral salt, with protein in a colloidal suspension. Hargrove and Alford (1980) defined milk as a dynamically balanced mixture of protein, fat, carbohydrates, salt, and water co-existing as emulsion, colloidal suspension and solution.

However, Milk is a fresh product, and scarce in many countries with little or no dairy production of their own. Fresh milk has a very limited shelf life and is easily spoiled by bacteria, enzymes and exposure to direct sunlight (Musa, 1994). On other hand fermented milks are widely produced in many countries. This type of process is one of the oldest methods used to extend the shelf-life of milk, and has been practiced by human beings for thousands of years. The exact origin(s) of the manufacture of fermented milks is difficult to establish, but it is safe to assume that it could date to more than 10 000 year ago as the way of life of humans changed from food gathering to food production. Furthermore, the fermentation of fresh milk by added starter of *Streptococcus thermophilus* (formerly known as *S. salivarius* subsp. *thermophilus*) and *Lactobacillus delbrueckii* subsp. *bulgaricus* are normally used because of the associative growth that exists between these two microorganisms, whilst in some countries, *Lactobacillus helveticus* and *Lactobacillus delbrueckii* subsp. *lactis* (ie. non-traditional organisms) are sometimes mixed with the starter culture (Pederson, 1979).

Dairy products are highly accepted by consumers and play an important role as carriers for probiotics. Effective probiotic strains must retain their functional health characteristics, including the ability to survive transit

through the stomach and small intestine and to colonize the human gastrointestinal tract (Tuomola *et al*., 2001).

Nevertheless probiotic bacteria grow slowly in milk because they lack proteolytic activity. Therefore the usual practice is to add yogurt bacteria (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) to the probiotic products to reduce the fermentation time (Shihata and Shah, 2000).

However, yoghurt bacteria produce lactic acid during storage (known as postacidification), which is claimed to affect the viability of probiotic bacteria (Dave and Shah, 1998). There is a large collection of historical data indicating that lactobacilli and bifidobacteria are safe for human use (Adams and Marteau, 1995; Naidu *et al*., 1999). In addition, if a probiotic strain is of human origin and thus a member of the normal commensal flora, the organism can generally be considered safe for use (Salminen *et al.*, 2001).

2.22.1. Fermented milk products

Fermentation is an ancient form of food preservation, which also improves the nutritional content of foods. In many regions of the world, fermented beverages have become known for their health-promoting attributes(Alan *et al*., 2014). On the basis of recent developments, it is probable that fermented beverages will continue to be a significant component within the functional food market (Alan *et al*.,2014).The global functional beverage market is a growing sector of the food industry as modern health-conscious consumers show an increasing desire for foods that can improve well - being and reduce the risk of disease. Fermented milks, especially yoghurt-style products, are the most popular functional beverages (Leatherhead, 2011). Fermented milk products can be made with milk (or skimmed milk) from various sources, including cow, camel, goat, sheep, yak and even coconut milk, and can be either pasteurized or unpasteurized ,that require specific lactic acid bacteria, to develop their

characteristics flavor and texture (Webb *et al.*,1980). Fermented milks are usually fluid or semi fluid in nature, and all contain lactic acid in varying proportions. Fermentation in milk modifies its properties resulting in cultured beverages, such as yoghurt and kefier, etc (Webb *et al.*,1980).Fermented milk products are unique in the sense that the required organoleptic properties depend on the unique characteristics of certain component of milk e.g. curdling properties of calcium caseinate-phosphate complex, blends of lactose and flavor characteristics of lipolyzed milk fat (Webb *et al.*,1980). Fermented milk products, depend on starter culture not only for acid development, but also for accumulation of desirable intermediates for example, volatile acid, action (dimthyl ketol, methyl carbinol) and diacetyl (diketobutan, biacetyl),which act as flavoring agents (Peppler and Robert, 1977). Many fermented milk beverages are produced as a result of back-slopping, whereby a small portion of already fermented milk is used to begin a new fermentation. In this way, cultures from the LAB naturally present in the raw milk are passed from household to household and between generations. While the consumption of spontaneously fermented milk is common to many different regions, the exact microbial differences between these products have not been ascertained (Alan *et al.*,(2014).

.2.23.Non-dairy fermented beverages

Another important class of fermented beverages are those made from cereals and nuts, which are popular in tropical regions and on the continent of Africa in particular. As with many milk-based products, the natural microbial component is used to ferment grains including maize, millet, barley, oats, rye, wheat, rice or sorghum. The grains are often heated, mashed and sometimes filtered. Back-slopping is again quite common, but the microbial populations responsible for the fermentation of these beverages are not as well characterized (Alan *et al.*,2014 ; Zorba, *et al.*, 2003).

In addition to milk and cereal-based fermentations, there are also other forms of fermented beverages. One example is kombucha, which is a fermented sweetened tea that was originally popular in China but is now enjoyed worldwide, it is fermented by a symbiotic mixture of bacteria (typically acetic acid bacteria, with small quantities of LAB) and yeast, which are implant within a cellulosic matrix that floats above the fermentate, similar to the mother cultures of vinegar (Akpinar *et al*., 2010).

2.24. Milk alternatives

Cow milk possesses a notable nutrient profile. It's rich in high-quality protein and important vitamins and minerals, including calcium, phosphorus and B vitamins. Though, cow milk is not a suitable option for everyone. There are different reasons it might be looking for an alternative, including:

2.24.1. Milk allergy: Some 2–3% of kids under the age of three are allergic to cow milk. This can cause a range of symptoms, including rashes, vomiting, diarrhea and severe anaphylaxis. Around 80% of kids outgrow this allergy by age 16 (Lifschitz and Szajewska, 2015)

2.24.2. Lactose intolerance: An estimated 7.5% of the world's population is intolerant to lactose, the sugar found in milk. This condition happens when people have a deficiency in lactase, the enzyme that digests lactose (Friedman, 2015).

2.24.3. Dietary restrictions: Some people choose to exclude animal products from their diets for ethical or health reasons. For example, vegans exclude all products that come from animals, including cow milk. Potential health risks: Some people choose to avoid cow milk due to concerns over potential contaminants, including antibiotics, pesticides and hormones (Friedman,

2015; Palupi *et al*., 2012; McDermott *et al*.,2002). There are many non-dairy options available :

2.24.4. Soy Milk

Soy milk is made with any soybeans or soy protein isolate, and often contains thickeners and vegetable oils to improve taste and consistency. In terms of nutrition, soy milk is a close non-dairy substitute for cow milk. It contains a similar amount of protein, but around half the number of calories, fats and carbohydrates. It is also one of the few plant-based sources of high-quality protein, which provides all the essential amino acids (House *et al.*, 2010).

2.24.5. Almond Milk

Almond milk is made with either whole almonds or almond butter and water. It has a light texture and a slightly sweet and nutty flavor Compared to cow milk, it contains less than a quarter of the calories and less than half fat. It is also significantly lower in protein and carbohydrates. It is one of the lowest-calorie non-dairy milks available and is a great option for those wanting or needing to lower the number of calories they're consuming. Also, it is a natural source of vitamin E, although almond milk is a much less concentrated source of the beneficial nutrients found in whole almonds, including protein, fiber and healthy fats (Schlemmer *et al.*,2009). Almonds also contain phytic acid, a substance that binds to iron, zinc and calcium to reduce their absorption in the body. This may somewhat decrease your body's absorption of these nutrients from almond milk (Schlemmer *et al.*,2009).

2.24.6. Coconut Milk

Coconut milk is made from water and the white flesh of brown coconuts. Coconut milk contains one-third the calories of cow milk, half the fat and significantly less protein and carbohydrates. It may not be the best option for those with increased protein requirements, but it would suit those looking to

reduce their carb intake (Assunção.,2009 ; Cardoso.,*et al*.2015). On the other hand, a recent review of 21 studies found that coconut oil may raise levels of total and low-density-lipoprotein (LDL) cholesterol to a greater extent than unsaturated oils (Eyres., *et al*.2016).

2.24.7. Oat Milk

In its simplest form, oat milk is made from a mixture of oats and water. Oat milk contains a similar number of calories to cow's milk, up to double the number of carbohydrates and about half the amount of protein and fat. Interestingly, oat milk is high in total fiber and beta-glucan, a type of soluble fiber that forms a thick gel as it passes through the gut. The beta-glucan gel binds to cholesterol, reducing its absorption in the body. This helps lower cholesterol levels, particularly LDL cholesterol, the type associated with an increased risk of heart disease (Hou *et al*.,2015). One study in men with high cholesterol found that consuming 25 ounces (750 ml) of oat milk daily for five weeks lowered total cholesterol by 3% and LDL cholesterol by 5% (Onning *et al*.,1999) . Research has shown that beta-glucan may help increase feelings of fullness and lower blood sugar levels after a meal (Rebello *et al*.,2014 ; Nazare *et al*.,2009). Oat milk is also cheap and easy to make at home.

2.24.8. Peanut milk

Since the early 1950s, various reports have been published clearing that peanut milk and its products can be made by different methods. The chemical composition of peanut milk depends on the producer desire, but generally, peanut milk has a high protein level (Diarra *et al*.,2005; Kouane *et al*., 2005). Low-cost edible products with high nutritional value could be successfully made from peanut milk (Diarra *et al*.,2005). In this regard, researchers have interested in fermented peanut milk products like yoghurt, butter milk and ripened cheese analogs (Diarra *et al*.,2005;Yadav *et al*.,2010). Also, peanuts

can act as an activated substrate for probiotic bacteria (Kabeir *et al.,* 2009). Peanut (*Arachis hypogaea*) is an important source of protein for an average Brazilian citizen's diet (Wetzel *et al*.,2005).

2.25. Peanut

2.25.1. Scientific classification : Kingdom *Plantae,* Family *: Fabaceae,* Genus: Arachis, Species : *A.hypogaea* (Alper and Mattes, 2003).

2.25.2. History of peanut

The domesticated peanut is an amphidiploids or all otetraploid meaning that it has two sets of chromosomes from two different species, thought to be *A. duranensis and A. ipaensis.* These likely combined in the wild to form the tetraploid species *A. monticola*, which gave rise to the domesticated peanut .This domestication might have taken place in Paraguay or Bolivia where the wildest strains grow today. Many pre-Columbian cultures, such as the Moche, depicted peanuts in their art (Seijo *et al*., 2007).

Archeologists have dated the oldest specimens to about 7,600 years, found in Peru .Cultivation spread as far as Mesoamerica, where the Spanish conquistadors found the tlalcacahuatl (the plant's Nahuatl name, whence Mexican Spanish cacahuate and French cacahuète) being offered for sale in the marketplace of Tenochtitlan (Mexico City). The plant was later spread worldwide by European traders .In the United States, a US Department of Agriculture program encouraged agricultural production and human consumption of peanuts in the late 19th and early 20th centuries. George Washington Carver is well known for his participation in that program to develop hundreds of recipes from peanuts (Seijo *et al*., 2007).

2.25.3. Peanut Applications

Most peanuts grown in the world are used for protein and oil production. Therefore Peanut is one of the most important oil and protein producing crops

in the world .Peanut (*Arachis hypogaea* L.) is a major source of edible oil and protein meal and is therefore considered to be highly valuable in human and animal nutrition. Peanuts may be consumed raw, roasted, pureed, or in a variety of other processed forms. Peanuts are often a major ingredient in mixed nuts because of their relative cost compared to Brazil nuts, cashews and walnuts, (Bonnie, 1988). Although peanut butter has been a tradition on camping trips because of its high protein content and because it resists spoiling for long periods of time, the primary use of peanut butter is at the home . Large quantities of peanut butter are also used in the commercial manufacture of sandwiches, candy, and bakery products. Boiled peanuts are a preparation of raw, unshelled green peanuts boiled in brine and often eaten as a snack. Peanuts are also used in a wide variety of other areas, such as cosmetics, nitroglycerin, plastics, dyes and paints (Bonnie, 1988).

2.25.4. Nutritional value of peanut

Peanuts are highly nutritious, providing over 30 essential nutrients and phyto nutrients. Peanuts are a good source of niacin, folate, fiber, vitamin E, magnesium and phosphorus. They are naturally free of trans-fats and sodium, and contain about 25% protein the highest proportion than in any true nut. Peanuts are used to help fight malnutrition (Griel *et al.*,2004).for it is being high-protein, high-energy and high nutrient peanut-based pastes developed to be used as a therapeutic food to aid in famine relief. The World Health Organization, UNICEF, Project Peanut Butter and Doctors Without Borders have used these products to help save malnourished children in developing countries (Griel *et al.*,2004).

2.25.5. Protective nutrients of peanut

Peanuts, along with other legumes are considered a part of meat and meat alternative group in the Food Guide Pyramid. Peanuts have an excellent nutritional profile due to which it is widely used in the diets for weight

management and meeting appropriate protein levels in the body. People who eat peanuts tend to intake more key nutrients critical to health. In more than 15,000 people who consumed peanuts and peanut products, it was found that levels of vitamin A, vitamin E, folate, magnesium, zinc, iron, calcium, and dietary fiber were higher than those who did not consume peanuts (Griel, 2004).

2.25. 6. Composition of peanut

Peanut is a rich source of protein, fats, and fiber that make up peanuts. These major components are all the healthy types when it comes to peanuts. The protein is plant-based; the fat is unsaturated, and the fiber is the main type of complex carbohydrate in peanuts. It makes sense that three healthy components come together in peanuts with their help benefits (Johnston *et al.*, 2005).

2.25.7. Vitamins and minerals

Peanuts and peanut butter contain numbers of vitamins and minerals that we need daily in our diets (Swain *et al*., 2008) integral to growth, development, metabolism, and immunity. All of the nutrients in peanuts through multiple mechanisms are likely to have synergistic effects toward improving the health status (Janige *et al.*, 2006).

2.25.8. Bioactive components of peanut

Many compounds in peanuts and in their skins that may have added health benefits beyond basic nutrition. Peanuts have been touted as a functional food with numerous functional components. Peanuts contain many bioactive compounds, such as flavonoids, phenolic acids, plant sterols, and stilbenes (Francisco & Resurreccion, 2008). Yang *etal,* 2009 determined that of ten nuts, including legume peanuts, peanuts had the third highest total flavonoid content. Flavonoids confer many benefits. In foods, flavonoids are responsible

for colour, taste, prevention of fat oxidation, and protection of vitamins and enzymes (Yao *et al.,* 2004). In humans, flavonoids were reported to be protective against cardiovascular diseases, cancers, and age-related diseases (Rosenberg *et al.,* 2002; Prasain *et al.,* 2010).

These bioactive components have been recognized for having disease preventative properties and some are antioxidants while others are thought to promote longevity. They are together with vitamins, minerals, and healthy fats, protein, and fiber promotes health. Therefore peanuts are bioactive food in a shell (Francisco, 2008). Peanuts also have significant levels of phytosterols. Phytosterols are well known for their ability to reduce cholesterol and new research showed that they are cancer-preventative. Flavonoids are a class of compounds also found in peanuts that reduce inflammation and inhibit platelets from sticking to the arteries (Griel *et al.,* 2004)

2.25.9. Antioxidant capacity of the bioactive component

The numerous bioactive components in peanuts contribute to good health by their antioxidant capacity. Peanuts have a higher antioxidant capacity .When peanuts are consumed with their skins, their antioxidant capacity doubles. And roasting can at times actually increase this capacity as well roasted peanuts with skins. For example, roasted peanut with skin have a higher antioxidant capacity than blueberries (Francisco, 2008).

2.25.10 Peanut and disease prevention

Peanuts have provided complex nutritive value to many diets and improve health. Peanuts, peanut butter, and peanut oil all help to prevent chronic diseases, including heart disease, diabetes and cancer (Jiang *et al.,* 2006).

Peanuts, peanut butter and peanut oil have potent lipid lowering effects and may act to reduce inflammation, which is one of the underlying mechanisms

that trigger chronic disease. The unique nutrient profile and bioactive components of peanut play a beneficial role in many areas of health and disease prevention (Jiang *et al.*, 2006).

There has been no research conducted on the survival of probiotic bacteria in non-dairy soy, almond, or peanut beverages nor were sensory evaluations conducted on these beverages. Isanga and Zhang (2007) conducted a study investigating the production and sensory properties of peanut milk stirred yoghurt. Samples were prepared from a blend of 70% peanut milk with 30% reconstituted whole milk, 70% peanut milk and 30% reconstituted whole milk with yoghurt flavouring, and pure reconstituted whole milk control (Isanga and Zhang, 2007). The results of this study indicate that peanut milk-based yoghurt had a good sensory texture, appearance, and flavour (Isanga and Zhang, 2007).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1. Gum Arabic

Gum Arabic (*Acacia senegal* and *Acacia seyal)* was obtained from Natural Gums Research Center (Sudan University of Science and Technology). Spheroidal gum tears were milled and sieved to obtain a fine powder.

Wheat bran (40% Canadian wheat+ 60% Russian wheat) and Refined flour were obtained from Seen and Sayga Flour Mills (Khartoum North- Sudan) respectively.

Sunflower oil (Shams- Savola Group Company) was purchased from the local market (Khartoum North- Sudan). Dried meat obtained from Arabian Company for Poultry (Sudan). Vitamins and minerals (Combivit MA).

3.1.2. Albino rats

Male albino rats (4 weeks-old) were obtained from the National Center for Research (Medical and Aromatic Plants Research Institute – Minstry of Higher Education and Scientific Research) and housed in a room (25 ± 2°C) with a 12/12 h light-dark cycle.

3.1.3. Peanut seeds

Red-skinned peanut seeds *(Arachis hypogaea*) (v. Ashford) were obtained from Samil Industrial Co in Kober- Khartoum North (Khartoum State, Sudan). The Aflatoxin content is less than 10 ppb the permissible level..

3.2. Methods

3.2.1. Feed Preparation for rats

The diets were weekly prepared, dried and stored at 6 °C , as follows:

Control diet (standard rat chow) was prepared from 1000g wheat flour (72% extraction), 5g NaCl, 40 ml sunflower oil, 250 g dried meat and 5g of vitamins and minerals .

Wheat bran diet was consisted of 100 g wheat bran and 900 g wheat flour (72% extraction), 5g NaCl, 40 ml sunflower oil, 250 g dried meat and 5g of vitamins and minerals .

Gum Arabic diet (*Acacia senegal*) was prepared from 100g *Acacia sengal* gum mixed with 900 g wheat flour (72% extraction), 5g NaCl, 40 ml sunflower oil, 250 g dried meat and 5g of vitamins and minerals .

Gum Arabic diet (*Acacia seyal*) was prepared from 100g *Acacia seyal* gum mixed with 900 g wheat flour (72% extraction), 5g NaCl, 40 ml sunflower oil, 250 g dried meat and 5g of vitamins and minerals .

Gum Arabic mixed diet [*Acacia (senegal + seyal*)] was consisted of 100 g (50g of *Acacia sengal* + 50g of *Acacia seyal*), 900 g wheat flour (72% extraction), 5g NaCl, 40 ml sunflower oil, 250 g dried meat and 5g of vitamins and minerals.

3.2.2. Experimental design

A total of 40 rats weighted (42-49 g) were randomly assigned to 5 feeding treatment groups throughout the study period (6 weeks). The control group (n = 8) was fed with standard rat chow, wheat bran diet group (n =8) was fed with wheat bran diet, *Acacia senegal* group (n =8) was fed with gum Arabic (*Acacia sengal*) diet, *Acacia seyal* group (n =8) was fed with gum Arabic (*Acacia seyal) diet,* and mix *Acacia (senegal+ seyal)* group (n =8)

was fed with mixed gum Arabic *Acacia(senegal+ seyal)* diet. Each group was placed in plastic cages protected with stainless steel. Racks were changed and cleaned 2 times a week. All rats had free access to water and standard normal rat feed for an acclimatization period of 2 weeks.

3.2.3. Evaluation of general health of rats

Observation was recorded regarding the general health of feeding rats. Feed intake, water consummation, weight gain, eyes character, difference in hair, abnormal behavior, and rat activity were considered. The experimental protocol was approved by the Animal Ethics Committee of Sudan University of Science and Technology and adhered to Guiding Principle in the Care and Use of Animals.

3.2.4. Body weight of rats during the study:

On the zero day before treatment, body weight of all the animals was measured which was then regularly followed by noting the changes in body weight on every 7 days interval throughout the study period using a digital weighing scale. All animals were observed a minimum twice per day.

3.2.5. Preparation and maintenance of starter culture

Bifidobacterium longum BB536 strain was obtained from the stored culture of the Department of Food Science and Technology, College of Agricultural Studies, Sudan University of Science and Technology. Cultures were transferred into 10% sterilized (121 ºC for 15 min) skim milk and incubated anaerobically at 37^{o}C for 24 h. The culture was further sub cultured twice in a similar sterilized skim milk for 48 h prior to use for fermentation.

3.2.6. Preparation of peanut milk

Peanut milk was prepared by a similar method to the one reported by Salunkhe and Kadam (1989) with slight modifications. Sorted peanut seeds

were roasted at 130°C for 20 min in an oven ((Baird and Tatlock (London) LTD. Chadwell – Heat. Essex. England). The roasting process was found to improve the nutrient component, facilitate the removal of the crust and decrease the peany flavor of peanut .The roasted peanut were then de-skinned and weighed before being soaked in water for at least 12 h. The de-skinned roasted peanut kernels were then washed with water. The roasted soaked kernels were then mixed with water in a ratio of 1:5w/w [peanuts (200g): water (1L)], transferred to a blender (Panasonic – MX – 101 SP2.Japan) and blended for 5 min at high-speed .The slurry formed was filtered using a double cloth to obtain the peanut milk, which was sterilized in autoclave at 121^{o}C for 15min.

3.2.7. Preparation of gum Arabic solution

Fifteen gram from different three samples of gum Arabic were weighted, dissolved in small amount of sterile water, completed to150 ml to obtain 10% w/v water three solutions and pasteurized in water bath at 60°C / 30min.

3.2.8. Fermentation medium

The growth media were formulated from peanut milk (control), peanut milk supplemented with 10% (v/v),different types of gums Arabic (*Acacia senegal, Acacia seyal* and mixed *Acacia (senegal + seyal*) .Formulated media were sterilized (121ºC for 15 min) and inoculated with 3% active culture of *B. longum* BB536 then incubated at 37º C for 24h.

3.2.9. The storage of the fermented products

The fermented beverages were held at refrigerator temperature 5-7 ºC for two weeks. Throughout the storage time, the viable counts of *Bifidobacterium longum* BB536, pH, titrable acidity, TSS, moisture and sugar content of the fermented beverages were determined. Analysis of samples were carried out at the initial time (0 days), after one week and two weeks intervals**.**

3.2.10. Enumeration of *Bifidobacterium longum* BB536

The enumerations of *B. longum* BB536 of different fermented beverages were attained using the plate count technique with MRS medium. The fermented samples were drawn at the initial time and weekly during the storage period. One ml of fermented beverage was used to make serial dilution in 9 ml peptone water, followed by plating on Rogosa agar (MRS) supplement with 0.05% L- cystiene. The plates were incubated anaerobically at 37˚C for 48 h. The growth was calculated as colony forming unit per ml (cfu/ml).

3.3. Clinical chemistry of blood of different treated rat groups

3.3.1. Method of blood collections:

At the end of the experiment, the rats were anesthetized with diethyl ether (using bell jar) and blood is obtained from the orbital sinus using heparinized capillary tubes. Blood samples from half (4 rats) of each treatment group were immediately placed in sterile tubes containing 40 EDTA and kept at 4 ºC for hematology analysis. The blood samples from the other half were collected in sterile tubes and centrifuged for 15.000 rpm for 20 min to obtain the serum for biochemical investigations.

3.3.2. Analysis of blood hematology:

Blood samples were analyzed for complete blood profile including: red blood cell (RBC), white blood cell (WBC), platelet (PL), hemoglobin concentration (HGB), and leukocyte differential count (NEU, LYM, etc.), platelet count (PLT), hematocrit (HCT), red blood cell indices: mean corpuscular volume (MCV),mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC). The measurements were performed by Hematology Analyzer (sys Mex kx-21N-USA).

3.3.3. Analysis of blood biochemistry:

For biochemical parameters, blood serum was subjected to evaluation of alanine amino transferase (ALT), aspartate amino transferase (AST), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP) activities, total protein, albumin, globulin, urea, creatinine Na, Ca, K, P, and blood glucose levels. The parameters were measured using Blood Clinical Analyzer (COBAS INTEGRA 400PLUS- Switzerland) .

3.3.4. Different bacterial ecology in ceacum and colon of treated rats

Ceacum and colon contents were collected under strict aseptic conditions in sterile Eppendorf tubes to avoid any cross contamination. A suspension of 10% (w/v) was made with buffered peptone water. The content was gently homogenized inside a cabinet and serially diluted prior to plating on different agar plates. Subsequent 10-fold serial dilutions of each sample were plated in triplicate. Media used for total aerobe, total anaerobe, enterobacteriaceae and enterocuccus was reported previously by Stanton *et al*. (2003). *Staphylococcus*, *coliform*, *lactobacillus* and bifidobacteria enumerated following Liong and Shah (2006) method. While for salmonella, Brilliant green agar was used. Incubation conditions of media used for enumerations are shown in Table (1). All samples were incubated at 37 ºC. Anaerobic condition was created in anaerobic jars using gas-generating kits.

Table 1: Media and incubation conditions used for enumeration of different microbiota communities in ceacum and colon of rats fed different types of supplement.

Type of media	Bacterial group	Incubation
Nutrient agar	Total aerobe**	Aerobic
Brain heart infusion agar	Total anaerobe**	Anaerobic
Eosin methylene blue	Enterobacteriacea*	Anaerobic
Macconky agar	Coliform**	Anaerobic
Esculin bile agar	Enterococcus***	Anaerobic
Brilliant green agar	Salmonella**	Aerobic
Mannitol salt agar	Staphylococcus**	Aerobic
De Man Rogosa Sharpe agar	Lactobacillus**	Anaerobic
De Man Rogosa Sharpe+ L. cytine	Bifidobacteria**	Anaerobic

All samples were incubated at 37 ºC. Anaerobic condition was created in anaerobic jars using gas-generating kits. * Incubation for one day. ** Incubation for two days.* ** Incubation for three days.

3.4. Physico-chemical analysis

3.4.1. Determination of titratable acidity

The titratable acidity (TA) of the Fermented beverages were determined according to the AOAC method (1990). Ten ml of the sample was drawn into a conical flask. Distilled water was added to the volume in the flask to complete 150 ml. The sample was then vigorously agitated and filtered. Twenty five milliliters of the filtrate were pipetted into a flask, five drops of phenolphthalein were added, and the sample was titrated against 0.1N NaOH till a faint pink color that lasted for at least 30 seconds was obtained. Then the acidity of different beverage samples were calculated as below.

Determination of titratable acidity**.**

$$\text{Titratable acidity} = \frac{(\text{N NaOH}) \times (\text{mls NaOH}) \times 0.9}{\text{Weight of sample}} \times 100$$

Were N = Normality of NaOH.

0.9 = Factor of lactic acid.

3.4.2. Determination of pH value

The pH value of the different fermented beverages was determined using a pH-meter (model HI 8521 microprocessor bench PH/MV/C meter, Romania). Two standard buffer solution of pH 4.00 and 7.00 were used for calibration of the pH meter at room temperature. The pH meter was allowed to stabilize for one minute and then the pH of the fermented samples were directly measured.

3.4.3. Determination of total soluble solids (TSS)

Total soluble solids (TSS) of the fermented beverages were determined at room temperature using a digital refractometer with degree Brix^{o} scale 0-100 according to AOAC (1990) method.

3.4.4. Total sugars

From the previous clear sample solution for determination of acidity, 50 ml were pipetted into a 250 ml conical flask and 5g citric acid and 50 ml distilled water were added slowly. Then, the mixture was gently boiled for 10 min to complete the inversion of sucrose and left to cool at room temperature. After that, the solution was transferred to 250 ml volumetric flask, neutralized with 20% NaOH solution in the presence of a few drops of phenolphthalein (NO. 6606 J. T Baker, Holland) until the colour of the mixture disappeared and the sample was made up to volume before titration.

Procedure:

A volume of 10 ml of the mixture of Fehling's (A) and (B) solutions was pipetted into 250 ml conical flask. Then, sufficient amount of the clarified sugar solution was added from a burette to reduce Fehling's solution in the conical flask. After that, the solution was boiled until a faint blue color is obtained. Then, a few drops of methylene blue indicator (S-d-FINE-CHEM LIMITED) were added to the Fehling's solution and titrated with sugar solution until brick-red color of the precipitate cuprous oxide was observed. Finally, the titer volume was recorded and the amount of inverted sugars was obtained from Lane and Eynon Table. The total sugars, reducing and non-reducing sugars were calculated by using the following formula:

Calculation

$$\text{Total sugars \{\% DM\}} = \frac{\text{(inverted sugar (mg) x dilution factor)}}{\text{Titer x sample weight (g) x (100\% - moisture \%) } \times 1000} \times 100$$

3.5. Proximate Analyses

3.5.1. Determination of moisture content

Moisture was determined according to the modified method of AOAC (1990). 5grams of the sample was weighted in sensitive balance, after weighting the dishes was transferred to an oven (Kat-NR. 2851, Electrohelios, Sweden) at

105 ± 0.1˚C for 6 hours. Afterwards, the dish with sample was transferred to desiccators and allowed to cool at room temperature before reweighting. The moisture content was calculated according to the following formula:

Moisture content (%) =

$$\frac{\mathrm{M2}-\mathrm{M3}}{\mathrm{M2}-\mathrm{M1}}\times 100$$

Where:

The M_1 = mass of dish + cover.

The M_2 = mass of dish + cover + sample before drying.

The M_3 = mass of dish + cover + sample after drying .

3.5.2. Determination of fat content

Fat content was determined according to the official method of AOAC (1990). A sample of 5g was weighed into an extraction thimble and covered with cotton, and then extracted with hexane. The thimble containing the sample and a pre-dried weight extraction flask containing about 100 ml hexane was attached to the extraction unit. The extraction process was conducted for 16h. At the end of the extraction period, the flask was disconnected from the unit and the solvent was evaporated. Later, the flask with the remaining crude hexane extracted was put in an oven (50 – 60ºC), cooled to room temperature reweighted and the dried extract was registered as fat content.

Fat content (%) =

$$\frac{\mathrm{W2}-\mathrm{W1}}{\mathrm{Sample\ \ weight}}\times 100$$

Where: W1= The weight of the empty extraction flask.

W2= The weight of the extraction flask after the extraction process.

3.5.3. Determination of protein content

Protein content of different fermented beverages was determined by Kjeldhal method according to the AOAC (1990) method as follows:

1. **Digestion**: two gram of the different fermented products were weighed in a crucible and transferred to a digestion flask with two tablets catalyst (mercury). 25 ml of concentrated sulphuric acid was added to the samples, the flask was placed on the digestion apparatus, heated until the mixture was colorless. Than the flasks were allowed to cool at room temperature.
2. **Distillation**: 25 ml of boric acid and three drops of bromocresol green+ methyl red indicator were added to each receiving flask. The digested samples were transferred from the digestion flask to volumetric flask and the volume was completed to 100 ml with distilled water. The receiving flask was placed on the distillation rack with the tip of the condenser extended below the surface of the acid. Immediately 5 ml of the diluted samples was added from the funnel of the distillation apparatus, then 10 ml NaOH (40%) was gently added. The distillation was continued until the volume in the receiving flasks was 7 ml, then the flask was removed from the distillatory.

3.5.4 Titration: The samples in the receiving flask were titrated against 0.1 N HCL, till color was changed from green to purple. The nitrogen content was calculated as follows:

$$N\% = \frac{\text{ml HCL} \times \text{Normality of HCL}(0.1) \times 0.014 \times 100}{\text{Sample weight}}$$

Protein (%) = (N %) × 6.25

Where N = Nitrogen content.

0.014=molecular weight of nitrogen/1000

3.5.5. Ash content

The ash content of the samples was determined according to the AOAC (1990) method. A 2g of the different fermented beverages were weighed into a clean, dry porcelain crucible and placed in muffle furnace (model Tipoforon Z A No 18203 Get Ran 1002) at 600˚C for 6 hours. The Crucible was transferred to desiccators, cooled to room temperature and weighed. The ash content was calculated as follows:

$$\text{Ash content (\%)} = \frac{W1-W2}{\text{Weight of sample}} \times 100$$

Where:

W1 = Weight of crucible with ash.

W2 = Weight of empty crucible.

3.5.6. Determination of crude fiber

Fiber was determined according to AOAC method (1990) .About 2g of a defatted sample was placed into a conical flask containing 200ml of H_2SO_4(0.26N). The flask was fitted with a condenser and allowed to boil for 30 minutes. At the end of the digestion period, the flask was removed and the digest was filtered through a proclaim filter crucible (No.3). After that, the precipitate was repeatedly rinsed with distilled boiling water, followed by boiling in 200ml NaOH (0.23N) solution for 30 min under reflux condenser and the precipitate was filtered rinsed with hot distilled water, 20 ml ethyl alcohol (96%) and 20ml diethyl ether.Finally, the crucible was dried at 105 ºC until a constant weight was obtained and the difference in weight was considered a crude fiber.

Crude fiber % =

$$\frac{[(\text{Dry residue + crucible(g)- (ignited residue +crucible (g))}]}{\text{Sample weight}} \times 100$$

3.6. Calculation of carbohydrates

Carbohydrates were calculated by difference, according to the following:

A available carbohydrates = 100% - [Moisture (%) + Protein (%) +Fat (%) + fiber (%) and Ash (%)].

3.7. Evaluation of sensory characteristics of the formulated beverage.

3.7.1. Panelist and recruitment

The data of 15 participants were used. They are semi trained aged (25-50years) male and female .Individuals who has a tree-nut allergy, and/or peanut allergy, and/or soy allergy were not eligible to participate.

3.7.2. Sample preparation for sensory panel

All four samples were produced on the same day and were stored at 4 °C. Each sample was distributed in to 20 g portions in plastic cups and was presented in a balanced random order to reduce order bias. four-digit codes were used to mask the identity of the samples.

3.7.3. Sensory evaluation

Each panelist was seated in individual area; All panelists received a tray of four coded samples at room temperature in balanced random order. Each tray also included a napkin, a glass of water, and the accompanying evaluation form before evaluating, panelists were instructed to evaluate each sample from left to right and to cleanse their mouth with water between each sample. They were also instructed to not speak to other panelists those who completed their evaluation.

Sensory attributes of peanut beverages were recorded in terms of appearance, color, flavor, texture, mouth feel and after taste overall acceptability (OAA). Fifteen panelists were used to rate the samples based on nine-point hedonic

scale (Ranganna, 2009). Four samples at a time were presented to the individual in cups containing approximately 100 g /wt cup at separate booth. The sensory scores included; Like extremely = 9, Like very much = 8, Like moderately = 7, Like slightly = 6,Neither like nor dislike = 5, Dislike slightly = 4, Dislike moderately = 3, Dislike very much = 2, Dislike extremely = 1. They were also asked to make comments about the acceptability ⁄ sensory quality of these amples.

3.7.4. Data collection

The panelists were asked to rate each sample based on the characteristics of appearance, color, flavor, texture, mouth feel and after taste overall acceptability using a nine-point hedonic scale. The hedonic scale ranged from one to nine, where one corresponded with “dislike extremely,” and nine corresponded with “like extremely.”

3.8. Statistical analysis

Analysis of data was carried out with one-way ANOVA using Minitab statistical soft were version 17. They were performed to determine significant differences between means of different groups. Probability levels of less than 0.05 were considered significant ($p < 0.05$).The Tukey’s-test was used to perform multiple comparisons between means.

CHAPTER FOUR

RESULTS AND DICUSSION

4.1. Proximate analysis of gum Arabic

Table (2) shows the proximate composition of gum Arabic. The moisture content of the *Acacia senegal* gum was found to be higher (8.5%) than *Acacia seyal* gum (7.3%) which is in agreement with the standard international specification (not more than 13-15 %) reported in the literature by Mona *et al*.2008; Siddig.*etal*.2005).The moisture content facilitates the solubility of gum Arabic carbohydrate hydrophilic and hydrophobic proteins.

The ash content for *Acacia senegal* gum is 3.45%, and *Acacia seyal* gum 2.6%. The ash content may be used to determine the amount of insoluble matter in acid, magnesium, potassium and calcium (Mocak *et al*.,1998). These results agree with the standard international specification (2-4 %) reported by Mona *etal*.(2008)and Siddig. *et al. (*2005).

The protein content of *Acacia senegal* is (2.60%) and *Acacia seyal* (1.8%) The physicochemical properties of the Acacia species gums fall within the range of the specifications of the gums reported by Karamalla *et al*. (1998), Al-Assaf *et al.* (2005), Hassan *et al*.(2005), Elmanan *et al.* (2008)

Table 2: Proximate composition of different type of gum Arabic.

Component	*Acacia senegal* (Hashab)	*Acacia seyal* (Taleh)
Dry matter	91.30 ± 1.00	92.70 ±1.0
Moisture	8.70 ± 0.10	7.30 ±0.10
Ash	3.52 ± 0.08	2.60 ±0.10
Protein	2.60 ± 0.10	1.80 ±0.10
Crude fat	0.03 ± 0.06	0.01 ±0.03
Crude fiber	3.20 ± 0.10	2.60 ±0.10
Carbohydrate	81.97±0.08	85.69±0.09

* Values are mean ± SD for triplicate independent runs.

4.2. General health and behavior of rats

Table 3 shows the results on behavior parameters for the rats fed with different supplemented diets. All rat groups survived during the experiment with normal eyes shape and lack any illness symptoms. All rat groups fed diet supplemented with different gums were slim with white smooth hair as compared to obese in control and rats received diet supplemented with wheat bran. Rat groups fed diet supplemented with different gums were more alert and fear response to catching, with normal sleeping and sensitive to temperature as compared to other groups namely the control and the group received diet supplemented with wheat bran.

As shown in table 3 the rat groups which fed different gum Arabic diets were characterized by alertness, unfriendly, normal sleep and not sensitive to hot weather temperature. They behave differently as compared with the control group. The sensitivity to the weather temperature may indicate that gum arabic makes an ideal regulation of the fluids in the body.

From the obtained observations the group fed with diet supplemented with the mix gum Arabic was characterized by perfect body composition, lightness of movement ,activity and beautiful appearance, followed by *Acacia senegal* group, which is characterized by slimness, then followed by Acacia seyal group, however, wheat bran group was obese compared to control group.

4.3. Actual weight gain of the rats

Table 4 shows the actual weight of rats during the experimental period of 6 weeks. The initial weight was measured before starting treatment and found no significant ($p<0.05$) difference between the five groups of rats.

All groups of rats have a normal growth pattern with differences in actual weight gain during the treatment period. Rats fed diet supplemented with gum Arabic (*Acacia senegal* (Hashab), *Acacia seyal* (Taleh), mix *Acacia* (senegal

+ seyal) gained lower weights as compared to control group and that wheat bran gained the highest weight, while the group fed diet supplemented with *Acacia senegal* (hashab) was the least actual weight gainer. Depletions in actual weight gain were not observed during the study. This preliminary results point toward the safe dose profiles of gum Arabic supplements fed to the rats.

The results showed that regular intake of gum Arabic(*Acacia senegal*) for six weeks resulted in less gain in body weight compared with the control group .Changes in body mass index were reported to occur with many other fiber intake, whether the fiber is obtained from naturally high-fiber diet or when it is ingested in a form of a supplement (Chandalia *et al*., 2000; Howarth *et al*.,2001; Lattimer and Haub, 2010). The group feed diet containing wheat bran did not show any reduction in actual weight gain. Supplementation with dietary fiber GA (*Acacia senegal*) resulted in less body weight gain , visceral adipose tissue and blood glucose. The results agree with previous studies that dietary fiber reduced body weight gain (Chandalia *et al.,* 2000; Lattimer and Haub, 2010), visceral adipose tissue and blood glucose (Islam *et al.,* ,2012; Babio *et al.*, 2010)). The reasults of body weight gain by GA (*Acacia senegal*) fed rats may be due to the fact that a high intake of dietary fiber, such as GA and wheat bran, could exert beneficial effects on fat metabolism (Ali *et al*., 2009; Slavin, 2003). Additionally, the dietary fiber was reported to promote satiety and satiation, improve glycemic control, affects gastric emptying and stomach hormone secretions, therefore, reduces weight gain (Chandalia *et al*., 2000). These results did not apply to the wheat bran group where there is more body weight gain,. Nasir (2014) stated that GA was found to affect the consequence of body weight gain during glucose and high fat diet and prevented glucose-induced obesity. Studies have reported that high ingestion of dietary fiber, including GA was associated with beneficial effects on fat

metabolism (Ali *et al.,* 2009; Slavin, 2003). The wheat bran fed groups was more obese, followed by the control group. In addition, the rats groups fed with Gum Arabic (*Acacia senegal*) diet were slimmer, than the rats groups fed in GA (*Acacia seyal*) and mixed types gum Arabic diets (Table3).

In humans GA treatment certainly modifies the body weight, decreased body mass index and body fat percentage among healthy adult females, which could be used in the treatment of obesity (Babiker *et al*.,2012), and fat deposition in both human and animal (Ushida *et al*., 2011), therefore, GA can help in management of body weight.

4.4. Feed intake and water consumption

The feed intake and water consumption of rats groups fed different types of diet is presented in Table 6 and 7 The results showed that there were significant ($p< 0.05$) differences between different groups of rats in feed intake and water consumption. Total feed intake was lower in the group fed *Acacia senegal* gum supplemented diet and high for the group received wheat bran supplemented diet. The results are inline with Nasir *et al*, (2012) who stated that GA significantly decreased food and fluid intake in mice. However El-kheir *et al*. (2009) found that birds fed high level of gum Arabic increased feed intake, suggesting that it enhances the palatability of the diet, thus it may be dependent on type of experimental animal. GA treatment decreases the caloric intake and increases the subjective ratings of feeling satiated (Calame, *et al.*,2011). Result in low feed intake could lead to reduced weight (Chandalia *et al*., 2000) of rats. Water is important to the health and makes up approximately two-thirds of the body by weight. In order to maintain physical balance in the body, it is necessary to coordinate and integrated link between the various organs. GA enhances the absorption of minerals depending on the body need and health. Thus control the content of intracellular and extracellular solutes by regulating the absorption. Potassium

and magnesium are the major intracellular solutes. Sodium is the major extracellular solutes. Serum protein is the additional "solute" of the intravascular component. For all practical purposes, water is in equilibrium among all of the fluid compartments. If the osmolality changes in any of the compartments, water will redistribute among compartments until osmolality equilibrates. This process controls water intake. Also It is noted that the reduction of the level of sugar in the blood reduces the consumption of water and vice versa.

These obtained results agree with Phillips (1998): Badreldin *et al*.,(2008). They have been reported that GA is not degraded in the stomach and small intestine, but undergo complete fermentation within the large intestine by microorganisms to short-chain fatty acids, particularly propionic acid (Phillips 1998 ; Badreldin *et al*., 2008).

4.5. Clinical chemistry of blood

4.5.1. Hematology and biochemistry

Hematology and biochemistry of blood were used to evaluate the toxicity status as presented in Table (8). The results did not indicate any health threatening symptoms to rats received the gum Arabic supplements. There are no significant differences ($p \geq 0.05$) in the results obtained for a complete blood count (CBC).No signs of any moderate and mild deficiencies of nutrients were revealed. These are considered signs of positive health effect. There were also significant ($P \geq 0.05$) increase in the HGB, MCHC, and platelet count as compared to control. All parameters were in the normal range, indicating absence of the malnutrition state in treating rats, no significant ($P \geq 0.05$) variations between gum Arabic treated groups and the other groups on RBC and WBC differential count; consistent with previous results on safety of gum Arabic on rats (Cerda *et al.*, (2003).

Table 3: General health and behavior of rats fed diet supplemented with gum Arabic and wheat bran

Characters and Manner	Treatment				
	Control group	**Wheat bran group**	***Acacia senegal* group**	***Acacia seyal* group**	**Mix *Acacia (senegal + seyal)* group**
Survival	100%	100%	100%	100%	100%
Illness symptoms	Nile	Nile	Nile	Nile	Nile
Activity	+	++	++++	++++	++++
Eyes shape	+	+	+	+	+
Body weight	+++	++++	+	++	++
Hair	+	++	+++	+++	++++
Behavior:					
Alertness	+	++	+++	+++	+++
Respond to catching	+	++	++++	++++	++++
Sleeping	+++	++	+	+	+
Temperature sensitivity	+++	++	+	+	+

Treatment groups received the followings: Control group fed on normal rats feed, wheat bran group fed on normal rats feed supplemented with wheat bran , senegal group fed on normal rats feed supplemented with *Acacia senegal* seyal group fed on normal rats feed supplemented with *Acacia seyal* mix group fed on normal rats feed supplemented with *Acacia senegal + seyal*

Table 4: Actual weight (g/ week) of different groups of rats fed on different feed.

Groups of Rats	Weight gain of groups / weeks							
	Initial body weight (g) Acclimatization period	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6 Final body weight (g)
Control	$44.13\pm11.46^{f}_{a}$	$69.38\pm7.74^{e}_{a}$	$83.50\pm9.65^{de}_{a}$	$89.13\pm14.50^{cd}_{ab}$	$94.88\pm10.38^{bcd}_{ab}$	$100.75\pm11.02^{abc}_{ab}$	$107.0\pm10.3^{ab}_{ab}$	$115.38\pm11.31^{a}_{ab}$
Wheat bran	$49.13\pm5.89^{f}_{a}$	$70.38\pm7.01^{e}_{a}$	$83.50\pm7.33^{de}_{a}$	$94.00\pm 9.49^{cd}_{a}$	$99.25 \pm 8.38^{c}_{a}$	$106.88\pm8.95^{bc}_{a}$	$117.0\pm 10.0^{ab}_{a}$	$121.38 \pm 9.55^{a}_{a}$
Acacia Senegal	$42.75\pm7.52^{e}_{a}$	$58.25\pm10.39^{de}_{b}$	$71.88\pm12.70^{cd}_{a}$	$76.50 \pm12.62^{bc}_{b}$	$81.63\pm11.78^{bc}_{b}$	$87.00 \pm 11.80^{abc}_{b}$	$93.88\pm11.43^{ab}_{b}$	$100.38\pm12.87^{a}_{b}$
Acacia Seyal	$42.75\pm7.52^{d}_{a}$	$69.13 \pm 3.93^{d}_{a}$	$76.38\pm11.36^{c}_{a}$	$85.75\pm12.60^{bc}_{ab}$	$90.00\pm13.07^{bc}_{ab}$	$95.88\pm12.60^{ab}_{ab}$	$102.13\pm12.9^{ab}_{ab}$	$107.75\pm12.83^{a}_{ab}$
Mix *Acacia*	$47.38\pm3.74^{e}_{a}$	$61.13\pm4.32^{de}_{ab}$	$72.00\pm6.99^{cd}_{a}$	$78.88 \pm 9.22^{bc}_{ab}$	$84.75\pm11.15^{bc}_{ab}$	$92.50\pm12.07^{ab}_{ab}$	$100.25\pm13.4^{a}_{ab}$	$105.75\pm12.26^{a}_{ab}$

Values are mean ± SD for replicate independently.

Values that carried different superscript letter in the same row are significantly different at $p<0.05$.

Values that carried same suberscript letter in the same column aren't significantly different at $p<0.05$.

Initial body weight = weight of rats after reception

Week0 = weight of rats after 2 weeks of accumulation

Control group = fed standard rats feed supplement

Wheat bran group = fed standard feed supplement added with 10% wheat bran

Acacia senegal group = fed standard rats feed supplement containing with 10% gum Arabic *Acacia senegal*

Acacia seyal group = fed standard rats feed supplement containing with 10% gum Arabic *Acacia seyal*

Mix group = fed standard rats feed supplement containing with 10% gum Arabic *Acacia senegal* 5% *Acacia seyal* 5%.

Table 5: Weight gain (g) of different groups of rats fed on different feed during experiment period.

Groups of Rats	Weight gain of groups
Control	46.00 ± 9.4[a]
Wheat bran	51.00 ± 6.4[a]
Acacia senegal	42.13 ± 8.5[a]
Acacia seyal	42.38 ± 7.2[a]
Mix *Acacia*	44.63 ± 8.9[a]

Values that carried the different superscript letter in the same column are significantly different at $p<0.05$.
Control group = normal rat diet
Wheat bran group = fed normal rat diet supplement added with 10% wheat bran
Acacia senegal group = fed normal rat diet supplement containing with 10% gum Arabic *Acacia senegal* .
Acacia seyal group = fed normal rat diet supplement containing with 10% gum Arabic *Acacia seyal* .
Mix group = fed normal rat diet supplement containing with 10% gum Arabic *Acacia senegal* 5% *Acacia seya*l 5%.
Weight gain of groups (Final body weight (g) (week 6) - initial body weight (week0) (g)

Table 6:Feeding intake g/day of different groups of rats fed different types of gum Arabic / weeks

Groups of Rats	Feeding consumption of groups / weeks						Total feed consumption
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	
Control	$67.19\pm14.02^{a}{}_{a}$	$56.80\pm0.00^{b}{}_{a}$	$52.57\pm9.72^{ab}{}_{a}$	$49.74\pm4.40^{b}{}_{a}$	$49.46\pm6.73^{b}{}_{ab}$	$54.13\pm15.43^{ab}{}_{ab}$	326.9 ± 30.4^{ab}
Wheat Bran	$59.46\pm12.37^{ab}{}_{a}$	$52.53\pm9.59^{b}{}_{a}$	$48.74\pm7.89^{b}{}_{a}$	$48.79\pm5.29^{b}{}_{a}$	$54.29\pm5.55^{b}{}_{a}$	$74.66\pm22.12^{a}{}_{a}$	338.5 ± 38.4^{a}
Acacia Senegal	$55.93\pm16.28^{a}{}_{a}$	$41.39\pm7.50^{ab}{}_{a}$	$43.53\pm10.56^{ab}{}_{a}$	$39.77\pm3.34^{b}{}_{b}$	$44.03\pm4.36^{ab}{}_{b}$	$46.59\pm5.93^{ab}{}_{b}$	270.06 ± 19.7^{8c}
Acacia Seyal	$55.14\pm20.53^{a}{}_{a}$	$48.16\pm11.78^{a}{}_{a}$	$48.13\pm11.99^{a}{}_{a}$	$43.24\pm4.33^{a}{}_{ab}$	$44.96\pm5.83^{a}{}_{ab}$	$42.60\pm8.77^{a}{}_{b}$	294.85 ± 22.32^{bc}
Mix	$53.52\pm16.03^{a}{}_{a}$	$44.87\pm14.08^{a}{}_{a}$	$49.64\pm12.70^{a}{}_{a}$	$45.27\pm5.57^{a}{}_{ab}$	$48.54\pm8.78^{a}{}_{ab}$	$50.27\pm11.41^{a}{}_{b}$	294.85 ± 22.32^{abc}

Values are mean ± SD for replicate independent runs.

Values that carried the different superscript letter in the same row are significantly different at $p<0.05$.

Values that carried the same suberscript letter in the same column aren't significantly different at $p<0.05$.

Control group = fed normal rat diet supplement

Wheat bran group = fed normal rat diet supplement containing with 10% wheat bran

Acacia senegal group = fed normal rat diet supplement containing with 10% Gum Arabic *Acacia senegal*.

Acacia seyal group = fed normal rat diet plement containing with 10% gum Arabic *Acacia seyal* .

Mix group = fed normal rat diet supplement containing with 10% gum Arabic *Acacia senegal* 5% *Acacia seyal* .

Table 7:Water consumption ml/day of different groups of rats fed on different types of Gum Arabic / weeks

Groups of Rats	Water consumption by groups / weeks						Total water consumption
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	
Control	$547.1\pm60.5^{ab}{}_{a}$	$611.4\pm78.8^{a}{}_{a}$	$600.0\pm43.2^{a}{}_{a}$	$624.3\pm50.9^{a}{}_{a}$	$602.9\pm45.0^{a}{}_{ab}$	$514.29\pm21.49^{b}{}_{a}$	3500.0 ± 145.7^{a}
Wheat Bran	$567.1\pm90.1^{a}{}_{a}$	$570.0\pm66.6^{a}{}_{ab}$	$612.9\pm62.6^{a}{}_{a}$	$565.0\pm69.0^{a}{}_{ab}$	$628.6\pm31.8^{a}{}_{a}$	$540.0\pm54.5^{a}{}_{a}$	3483.6 ± 90.5^{a}
Acacia Senegal	$490.7\pm109.6^{a}{}_{a}$	$514.3\pm66.1^{a}{}_{b}$	$515.7\pm47.6^{a}{}_{b}$	$460.0\pm46.2^{a}{}_{c}$	$470.6\pm66.8^{a}{}_{c}$	$528.6\pm95.6^{a}{}_{a}$	2979.9 ± 181.6^{b}
Acacia Seyal	$470.0\pm40.0^{b}{}_{a}$	$525.7\pm32.1^{ab}{}_{ab}$	$577.1\pm37.7^{a}{}_{ab}$	$482.9\pm33.5^{b}{}_{c}$	$520.0\pm73.0^{b}{}_{c}$	$535.7\pm67.3^{ab}{}_{a}$	3085.7 ± 68.8^{b}
Mix	$447.1\pm71.3^{b}{}_{a}$	$516.4\pm49.5^{ab}{}_{b}$	$574.3\pm34.9^{a}{}_{ab}$	$502.1\pm28.0^{ab}{}_{bc}$	$520.0\pm73.0^{ab}{}_{bc}$	$510.0\pm82.7^{ab}{}_{a}$	3070.0 ± 142.7^{b}

Values are mean ± SD for replicate independent runs.

Values that carried the different superscript letter in the same row are significantly different at $p<0.05$.

Values that carried the same suberscript letter in the same column aren't significantly different at $p<0.05$.

Control group = fed normal rat diet supplement

Wheat bran group = fed normal rat diet supplement added with 10% wheat bran

Acacia senegal group = fed normal rat diet supplement added with 10% gum Arabic *Acacia senegal*

Acacia seyal group = fed normal rat diet plement added with 10% gum Arabic *Acacia seyal*

Mix group = fed normal rat diet supplement added with 10% gum Arabic *Acacia senegal* 5% *Acacia seyal*.

Similarly Cerda *et al.* (2003) did not observe any significant difference ($p<0.05$) in blood parameters of rats fed gum Arabic all blood parameters which were within the normal range of rats . As reported by Petterino and Argentini-Storino (2006) (CBC) on overall health in case detected a wide range of disorders, including anemia, infection and leukemia. Abnormal increases or decreases in cell counts as revealed in complete blood count may indicate an underlying medical condition that required further evaluation (Anderson *et al.*,1999).

The variations in Hb and PCV have been recently included in metabolic profile tests (Kumar and Pashauri, 2000). The red cell count, erythrocyte indices and TLC have been associated with adaptability of animals to adverse environmental conditions (Koubkova *et al*., 2002).

Electrolytes play vital role in maintaining body fluid pH and the dynamic balance of water (Radostits *et al.*, 2007). The results in Table (9) explained that concentrations of Ca decreased in gum Arabic groups compared to control group, Na and K profile were within normal range among five rat groups. CL increased in wheat bran and acacia seyal groups compared to the control group, but still within the normal range. Gum Arabic enhances the absorption of sodium and potassium from the diet. (Tulung, 1997).

While phosphate is lower in gum Arabic and wheat bran groups of rats as compared to control group. The improvements in electrolyte balance could be due to supplementations with prebiotic that create acidic environment, which enhances nutrient absorption of some minerals (Yap, 2003, Kabeir *et al*., 2008). The previous study showed that GA affected the intestinal absorption of Na^+ and water in healthy mice while enhancing calcium and magnesium uptake (Nasir *et al*., 2008). In a rat model of chronic osmotic-diarrhea GA exerted pro-absorptive properties by increased sodium and water absorption (Nasir., 2012). Apparently, the effects of GA on intestinal Na^+ and water

absorption are dependent on the condition and the health of the intestine (Nasir, 2013).

Glucose is the primary metabolic fuel required for growth and survival (Herdt, 2000). Blood glucose decreased in the group received *acacia senegal* and seyal although its level was within the normal range of rats. Gum arabic decreased postprandial blood glucose in human and inhibited glucose movement in *invitro* (Edwards *et al*., 1987; Torsdottir *etal*, 1989). GA also found to inhibit absorption of glucose in the intestine via interaction with membrane abundance in mice (Nasir *et al*., 2010). Diet containing gum Arabic (*A.senegal*) has the lowest concentration of serum glucose compared with other groups. This result is in agreement with Wadood *et al*. (1989) who reported that gum Arabic significantly($p< 0.05$) reduced glucose concentration in rabbits. It is noted that the reduction of the level of sugar in the blood reduces the consumption of water and vice versa.

4.5.2. Blood biochemistry

With regards to serum biochemistry the result in table (10) shows significant ($p< 0.05$) decrease in liver function paramrters such as aspartate transferase (AST), gamma-glutamyltranfears (GGT), and total bilirubin concentration in blood. The result in table (10) shows a significant ($p< 0.05$) decrease in liver function test parameters such as serum ALT, AST, and ALP in *Acacia senegal* group were noted but still within a normal range. Also a slight decrease in the total protein, albumin, globulin showed almost similar value with control group and were within the normal range. The enzyme AST relates to metabolism: increased activity of the enzyme was related to increase of the body metabolism and hepatic activities (Seifi *et al.*,2007).AST is more sensitive than GGT for detecting liver lesions(Gonzalez *et al.,*2011). It is noted that there was decrease in AST enzyme in gum Arabic groups and wheat bran. These results indicated that dietary fiber in general and specific gum Arabic *Acacia sengal* affected the body metabolism and hepatic

activities, which induced body weight gain, feed intake and water consumption, mineral absorption and general health. As these parameters represent liver function, the increase in their levels will indicate liver damage. A decrease in the activities of these liver enzymes is not considered to give any toxicological significance. Kidney function of rats groups was not affected by the gum Arabic supplemented. The level of urea and creatinin were within the normal range of rats.

Blood biochemistry results in the present study did not show any abnormalities. Liver and kidney function tests and serum protein profile are important parameters in determining the safety of functional ingredient of final product (Farag *et al*., 2006; Patel *et al.*, (2008). The findings from present studies suggested that the administration of gum Arabic did not cause any toxicological effect since the values were within the normal range of rats. The original components of the metabolic profile were glucose, urea, inorganic. phosphorus, calcium, magniesium, sodum, potassium, albumin, globulin, haemoglobulin and coper . The plasma proteins, including albumin, are in a state of equilibrium with amino acids and tissue proteins. Albumin is synthesized by the liver and functions to maintain the osmotic pressure within the circulating system (Hankins, 2006). Decreased albumin level has been reported as characteristics of liver disease, kidney disease, inflammatory status and malnutrition (Lager and Jordan, 2012).

The blood urea level is used for measuring the adequacy of dietary protein level as well as nitrogen utilization efficiency (Lager and Jordan, 2012).Urea nitrogen concentrations are influenced by a wide variety of factors, including dietary protein intake and intestinal degradability, dietary amino acid composition, protein intake relative to requirements, liver and kidney functions, muscle tissue breakdown and dietary carbohydrate amount (Van Saun, 2004). Bobe and Behrensmeyer (2004) concluded that the serum levels, of glucose, proteins and urea are indications of liver function.

In general, blood metabolites were affected by diet composition and/or presentation, indicating that some metabolites could reflect the nutritional and/or physiological status of rats. Several studies have observed that gum Arabic (acacia Senegal)was effective in reduction of blood glucose level .It is interesting to note that the glucose level observed in senegal group were lower than the other groups. These result indicated that gum arabic affects carbohydrate metabolism and the action of hormones by slowing the digestion and absorption, These processes regulate insulin release. The decrease in glucose level decreased the consumption of water. The present result reveals that GA (Acacia senegal) decreases the weight gain as shown in the study by lowering feeding intake, affecting body metabolism, that acacia senegal inhibits intestinal glucose absorption by regulation of the intestinal glucose absorption. The fermentation of GA in the large intestine by microflora formed a new useful metabolic item for consumption besides short-chain fatty acids that affected the absorption and digestion . These metabolic items depend on the health of the intestine and subject. Apparently, shown that GA improves small intestinal absorption of sodium in normal rats (Codipilly and Wapnir, 2004; Wapnir *et al*.,1996; Nasir,2013) and of sodium and water in two animal models of diarrheal disease (Wapnir *et al*., 1997; Nasir,2013). A possible interpretation of this observation could be due to the alteration of the physiological condition of the gut with probiotics that may influence the metabolic activity, fluids balance and absorption of nutrients led to less feed and water consumptions as usual.

The proper functioning of the body is conditional on the maintenance of physical balance through the correlation and coordination between the processes that occur in different organs. The two organs nervous system and the hormonal system responsible for the organization and coordination in the body weight based on the previous result the gum arabic has a positive effect in the two organs. Also numerous studies yielded evidence against an

antioxidant effect of GA as well as protective effects in experimental hepatic, renal and cardiac toxicity (Ali *et al*., 2009). GA has been reported to exert a protective effect against gentamicin, cisplatin nephrotoxicity and doxorubicin cardiotoxicity used in rats (Al-Majed *et al.*, 2002; Abd-Allah *et al*., 2002). GA has been shown to decrease blood pressure (AlMosawi, 2002), to decrease plasma cholesterol concentrations in rats to foster dental remineralization , to display antimicrobial activity and to stimulate intestinal absorption cause counteracting of diarrhea (Ali *et al*, 2009). The mix group exerts more positive effect on general health of rat as compared to *Acacia senegal* or *Acacia seyal* alone.

4.6. Different microbial groups in ceacum and colon of rats

Selected microbial groups in ceacum and colon of rats fed with different types of gum Arabic and wheat bran preparations are reported in Tables(11) and (12). Based on feeding trials, alterations in ceacum and colonic microbial groups of the rats are ensured.

4.6.1 The microbial effect of rats fed diets containing gum Arabic on the useful population of cecum and colon in rats

Feeding GA mix *Acacia (senegal + seyal)* induced the highest Bifidobacteria increase in ceacum by more than two fold (2.69 log CFU/g) than in colon (1.58 log CFU/g) of the same group. The increases was also high in Acacia senegal group recording population of 2.03 log CFU/g in ceacum and 0.85 log CFU/g in colon (Table 11). Lactobacillus exhibited the highest increase among all microbiota communities in ceacum . However, the increases were significant ($P < 0.05$) in groups received wheat bran and mix Acacia supplements (Table11). Colonic increases were 0.74 and 0.93 log CFU/ g; and ceacum of the same groups recorded rises of 1.74 and 1.38 log CFU/g, respectively. There was a significant increase in the total anaerobes of

Table 8: Heamatology parameters of rats fed different prebiotic supplements for a period of 6 weeks

Parameter[b]	Treatments[c]					Stander range
	Control	Wheat bran	Senegal	Seyal	Mix	
RBC (10^6u/L	6.88±1.73[a]	7.94 ±0.28[a]	7.68±0.10[a]	7.54±0.37[a]	7.70±0.24[a]	7.27-9.65
HGB (g/dL)	12.25±3.14[a]	13.60±0.62[a]	13.10±0.35[a]	12.98±0.41[a]	12.45±0.83[a]	13.7-17.6
WBC(10^3u/L)	6.95± 3.11[a]	8.57±2.32[a]	7.62±2.41[a]	6.95±1.39[a]	9.25 ±1.04[a]	1.96-8.25
Lymphocytes(%L)	50.1 ±31.6[a]	56.05 ±6.90[a]	47.3±36.1[a]	44.0 ±29.6[a]	55.55±3.86[a]	66.6-90.3
HCT (%)	37.00±9.27[b]	42.40±1.69[a]	40.60 ±1.14[a]	40.05±1.57[a]	39.80±1.31[a]	39.6 – 52.5
MCV fL (um^3)	53.47±1.49[a]	53.40±0.40[a]	52.82±1.08[a]	53.10±1.06[a]	53.42± 0.37[a]	48.9- 57.9
MCHC (g/L)	33.27 ± 0.76[a]	33.27 ±0.76[a]	32.25 ± 0.19[a]	32.40± 0.48[a]	32.92± 0.53[a]	32.9- 37.5
Platelet count (10^3u/L)	599500 ± 327337[ab]	309090 ± 305133[ab]	753750 ± 92622[a]	641250 ± 212448[ab]	190940 ± 235129[b]	638 - 1177
MCH (pg)	17.77± 0.31[a]	17.15± 0.26[a]	17.05± 0.37[a]	17.17± 0.35[a]	17.12± 0.48[a]	17.1- 20.4
P- LCR/%	4.70± 0.90[a]	5.425± 1.14[a]	5.05 ± 0.42[a]	5.80±1.230[a]	5.25 ± 0.73[a]	
MPV Fl(um^3)	5.83± 1.23[a]	6.5750± 0.15[a]	6.60±0.18[a]	6.75 ± 0.265[a]	6.55±0.13[a]	6.2-9.4
PDW%	7.83 ± 0.45[a]	7.825 ± 0.45[a]	7.85 ± 0.37[a]	8.00 ± 0.00[a]	8.25 ± 0.21[a]	43.2 -64.3
RWD Cv/%	17.03±0.64[a]	17.650± 1.12[a]	17.525 ± 0.29[a]	16.73± 1.02[a]	16.08 ± 1.06[a]	

* Values are mean ± SD for replicate independent runs.

**Values that carry different superscript letter in the same row are significantly different at $p<0.05$.

***Values that carry same suberscript letter in the same column aren't significantly different at $p<0.05$.

WBC = white blood cell, RBC = red blood cell, HGB = hemoglobin, HCT = hematocrit, MCV= mean corpuscular volume, MCHC= mean corpuscular hemoglobin concentration.

Treatments groups received the followings: Control group fed on normal rats feed, wheat bran group fed on normal rats feed supplemented with wheat bran , Senegal group fed on normal rats feed supplemented with *Acacia senegal* and seyal group fed on normal rats feed supplemented with *Acacia seya*l mix group fed on normal rats feed supplemented with *Acacia senegal + seya*l))

Table 9: Blood biochemistry profile of rats fed different *types of gum arabic* supplements for a period of 6 weeks [a]

Parameters	Treatments					Stander range
	Control	Wheat bran	Senegal	Seyal	Mix	
Glucose (mg/L)	109.5± 12.4[a]	114.5± 4.6[a]	58.0±12.9[b]	86.0± 20.7[ab]	104.0±17.9 [a]	70 – 208
Urea (mg/L)	28.25± 8.81[a]	19.25±5.44[ab]	14.25±2.22[b]	14.50±1.92 [b]	23.00±4.90 [ab]	12.3 – 24.6
Total globulin(g/dL)	1.95± 0.31[a]	2.10± 0.42[a]	1.98± 0.44[a]	2.23± 0.26[a]	1.96± 0.44[a]	1.5 – 2.5
Albumin g/Ll	3.30±0.57[a]	2.18± 0.57 [a]	2.500±0.816[a]	2.28± 1.35[a]	2.050±0.311 [a]	3.4 – 4.8
Total protein(g/dL)	4.75±0.75[a]	4.28±0.83[a]	4.47± 0.62[a]	3.93±0.52[a]	4.58±1.00[a]	5.2 – 7.1
Phosphorus (mg/L)	6.03 ± 0.79[a]	4.60 ± 0.49[a]	4.75 ± 1.21[a]	5.82± 0.95[a]	5.97 ± 1.59[a]	5.58 –10.41
Na (mmol/L)	119.0±13.83[a]	124.0 ±12.68[a]	124.2±18.01[a]	127.0± 2.94[a]	127.2±5.25[a]	142 - 151
K (mmol/L)	4.40±0.34[a]	4.50±0.082[a]	4.0±0.33[a]	4.40 ±0.79 [a]	4.47±0.49 [a]	3.82 – 5.55
Cl (mmol/L)	101.5±9.75[ab]	108.0±5.60 [a]	89.8 ±10.21[b]	107.5 ± 3.70 [a]	91.7 ±3.59 [b]	100 – 106
Uric acid (umol/L)	1.90 ± 0.66[a]	2.70 ± 0.92[a]	2.60 ± 0.93[a]	2.72 ± 1.03[a]	1.77 ± 0.26[a]	
Ca (mg/L)	8.8±1.30[a]	6.8±1.53[ab]	5.3±0.34[b]	5.5±0.40[b]	4.9±0.22[b]	9.5 -11.5

Mean±StDev of four rats

Treatments groups received the followings: Control group fed on normal rats feed, wheat bran group fed on normal rats feed supplemented with wheat bran , Senegal group fed on normal rats feed supplemented with Acacias seyal group fed on normal rats feed supplemented with *Acacia seyal*(. mix group fed on normal rats feed supplemented with *Acacia senegal + seyal*)).* P≤ 0.05 significantly different (control vs. supplements recipients).

Table 10: liver enzyme of rats fed different *samples of gum Arabic* supplements for a period of 6 weeks

Parameter	Treatments					Stander range
	Control	Wheat bran	Senegal	Seyal	Mix	
ALT (U/L)	82.3 ± 28.8[a]	93.0 ±21.2[a]	69.75 ±10.66[a]	86.8 ± 25.8[a]	92.8 ±27.5[a]	18- 45
CK (U/L)	730.0 ±102.1[a]	384.3 ±134.9[b]	445±203[ab]	508.0 ±146.7[ab]	523.0±136.7[ab]	162 - 1184
AST (U/L)	74.5 ± 20.1[a]	57.9 ± 31.1[a]	36.7 ± 21.1[a]	46.8 ± 6.47[a]	65.2 ± 24.4 [a]	74 – 143
ALP (U/L)	69.5±19.3[a]	60.3±20.5[a]	49.7±9.9[a]	62.0±17.3[a]	59.0±19.5[a]	62. 230
Creat (mg/dL)	0.19 ± 0.65[a]	0.27± 0.92[a]	0.26 ± 0.93[a]	0.27±1.03[a]	0.18± 0.26[a]	0.2 – 0.5
D.Bill(mg/dL)	0.46± 0.08 [a]	0.39± 0.09 [a]	0.36± 0.08 [a]	0.39± 0.1215 [a]	0.43± 0.04 [a]	0.03- 0.05
LDH (U/L)	81.4 ±10.2[a]	68.8±13.2[a]	87.2±12.1[a]	80.7±15.3[a]	74.5±4.6[a]	

Mean ± SD of four rats * P≤ 0.05 significantly different (control vs. supplements recipients).

Treatments groups received the followings: Control group fed on normal rats feed, wheat bran group fed on normal rats feed supplemented with wheat bran , Senegal group fed on normal rats feed supplemented with *Acacia senegal* (Hashab), seyal group fed on normal rats feed supplemented with *Acacia seyal(* Taleh), mix group fed on normal rats feed supplemented with *Acacia senegal + seyal*)) (Hashab + Taleh).

[c]ALT= alanine aminotransferases, ALP = alkaline phosphatase, AST= aspartate aminotransferase, CK = creatine kinase, Creat = Creatinine, LDH = lactate dehydrogenase.

ceacum. While in colon the increases was not significant as compared to the control in all groups of rats received different types of *gum Arabic* supplements. The supplemented group showed the reduction in total aerobes in colon and ceacum, but the reduction was not significant as compared to the control group. However, mix Acacia group recorded reduction of 0.96 log CFU/ g in ceacum and1.28 log CFU/ g in colon that might be due to the two types of the mix gum Arabic supplements.

Gum Arabic has a positive influence on the microbiological flora in the ceacum. It is a source of fermentable carbon for the bacteria living in the large gut, thus promoting an increased number of bacteria cells, especially Bifidobacterium and Bacteroides, in the ceacum (Hill, 1983). These bacteria are also used as Probiotics. Their amount is increased at the expenses of potential pathogenic strains, such as coliform and Salmonella, thus preventing the growth of pathogens in the gut. . A 15% gum Arabic solution fed to rats resulted in an increase in weight of the ceacum (Tulung, 1997). The increase of cells is positive, since bacteriological translocation is avoided and the immune system of people suffering from indigestion is strengthened.

Human studies reported that metabolic end-products resulting from carbohydrate fermentation (lactate and acetate) inhibited growth of pathogenic bacteria, both gram-positive and gram-negative (Gibson and Manning, 2004). The other benefits of Bifidobacterium spp. to the host include B vitamin synthesis, immune modulation, and blood ammonia reduction (Gibson and Roberfroid, 1995).

Table 11: Total aerobe and potential helpful population from ceacum and colon of rats received different samples of gum Arabic.

Bacterial groups	Location	Treatment				
		Control	Wheat bran	Senegal	Seyal	Mix
Total anaerobe	Colon	$3.87\pm0.59^{a}_{b}$	$4.52\pm.24^{a}_{abcd}$	$4.72\pm0.76^{b}_{abc}$	$3.94\pm0.94^{b}_{ab}$	$4.37\pm0.50^{b}_{abc}$
	Ceacum	$3.81\pm0.70^{a}_{b}$	$3.25\pm0.65^{a}_{abc}$	$4.61\pm1.47^{a}_{abc}$	$4.08\pm0.82^{a}_{a}$	$4.09\pm0.80^{a}_{abc}$
Total aerobe	Colon	$6.30\pm0.59^{a}_{a}$	$5.16\pm0.66^{a}_{a}$	$5.34\pm0.52^{a}_{ab}$	$5.60\pm0.91^{a}_{a}$	$5.02\pm0.37^{a}_{ab}$
	Ceacum	$5.70\pm0.72^{a}_{a}$	$4.77\pm 0.25^{a}_{a}$	$5.40\pm0.98^{a}_{a}$	$5.23\pm0.60^{a}_{a}$	$4.74\pm0.60^{a}_{b}$
Lactobacillus	Colon	$3.89\pm0.63^{a}_{b}$	$4.63\pm0.82^{a}_{ab}$	$4.04\pm0.61^{a}_{ab}$	$4.64\pm0.74^{a}_{ab}$	$4.82\pm 0.67^{a}_{ab}$
	Ceacum	$3.86\pm0.57^{a}_{b}$	$5.60\pm0.79^{a}_{a}$	$4.23\pm0.89^{a}_{a}$	$4.65\pm0.74^{a}_{a}$	$5.24\pm0.58^{a}_{a}$
Bifidobacteria	Colon	$3.75\pm0.677^{b}_{b}$	$4.90\pm0.53^{ab}_{ab}$	$4.58\pm0.73^{ab}_{a}$	$5.04\pm0.87^{ab}_{a}$	$5.33\pm 0.58^{a}_{a}$
	Ceacum	$3.09\pm0.16^{c}_{b}$	$4.26\pm0.57^{b}_{ab}$	$5.12\pm0.473^{a}_{ab}$	$3.84\pm0.54^{ab}_{ab}$	$5.78\pm0.44^{a}_{ab}$

Values are mean ± SD for replicate independent runs.

Values that carried the different superscript letter in the same row are significantly different at $p<0.05$.

Values that carried the same suberscript letter in the same column aren't significantly different at $p<0.05$.

Treatments groups received the followings: Control group fed on normal rats feed, wheat bran group fed on normal rats feed supplemented with wheat bran , senegal group fed on normal rats feed supplemented with *Acacia senegal* (Hashab), seyal group fed on normal rats feed supplemented with *Acacia seyal*(Taleh), mix group fed on normal rats feed supplemented with *Acacia senegal + seyal*)) (Hashab + Taleh).

4.6.2. Effect of feeding rats different types of gum Arabic on the harmful bacteriapopulation of cecum and colon in rats

The harmful pathogens of gut microbiota include species of *staphylococcus, Enterococcus*, *Enterobacteriacea, Salmonella* and *Coliform*. Table (12) shows decrease in the pathogens bacteria in all groups of rats received the different types of gum Arabic supplements, but the decreased was not significant as compared to the control. The *Salmonella* in the ceacum and colon of rats decreased by feeding *Acacia seyal*, mix Acacia gum and *Acacia senegal* as compared with the control group. While the *Coliform* remained insignificantly in ceacum and colon. *Enterocuccus* was reduced in comparison with the control and *Staphylococcus* was slightly reduced in wheat bran and *gum Arabic* recipient groups as compared with the control one. In the wheat bran group there was a decrease in *Coliform* and *Salmonella* and an increase in Enterobacteriaceae population. On another hand in colon of the *Acacia senegal* group of rats there was a decrease in the *Salmonella* and *Enterocuccus* and increase in Enterobacteriaceae .While there was an increased in *Coliform* and *Staphylococcus* in the colon of the *Acacia seyal* and mix gum recived groups of rats.

These results indicated that a dietary fiber GA (*Acacia senegal*) has a different effect on the growth of the harmful pathogen like *Salmonella* .Dietary fiber GA fermented in the large intestine by microorganisms to short -chain fatty acids, particularly propionic acid (Phillips, 1998). These organisms play significant role in lowering the pH of the large intestine through the release of lactic and acetic acid (Asahara *et al*., 2004). These fermentation process has appositive regulation in the growth and survival of useful microorganism, and prevents the growth of pathogenic bacteria in intestinal tract (Campbell *et al*.,1997).

Venturi *et al*. (1999) reported the impact of probiotic preparations on the composition of human intestinal microbiota, facal concentrations of *Streptococcus* salivarius ssp. thermopiles, lactobacilli and Bifidobacteria increased significantly ($p<0.05$) in all treatment recipient individuals compared to those at a basal level from the 20th day and remained stable throughout the study. It was also reported in significant ($p<0.05$) increases in bactericides, clostridia, coliform, total aerobic and anaerobic bacteria the overall observation of microbiota distribution showed a higher population of total anaerobe, Lactobacillus, bifidobacteria, Enterobacteriaceae in colon. While total aerobes, *Coliform, Enterocuccus, Staphylococcus* were higher in ceacum and *Salmonella* decreased in the colon.

4.7. Chemical composition of the raw peanut

Referring to results in Table (13), raw peanut contains lower levels of carbohydrates, fiber, Ash, moisture and higher levels of fat and protein as compared to reference values of raw peanut in food composition table. In general, these variations might be due to the variety of peanut species, production, storage and harvesting phase.

The results of the proximate composition of roasted peanut are presented in the same table (Table 13). It shows that the moisture content of raw peanut was 5.75 which decreased to 3.04 after roasting processes. It can be noticed that moisture content decreased significantly. These results are in agreement with those found by Damame *et al*. (1990), Abayomi *et al*. (2002 and Adegoke *et al*. (2004). Roasting of peanut also increased fat, proteins, fiber and ash in a ratio of 0.71, 1.4, 0.32, 0.2 and 0.62% respectively. While decrease carbohydrate (1.73%).These results on the composition of roasted peanut are in agreement with those reported by Abayomi *et al.* (2002) and Adegoke *et al.* (2004).

Table 12: Potential pathogens (Log CFU/g) from ceacum and colon of rats received different gum Arabic supplements

Bacterial group	Location	Treatment				
		Control	Wheat bran	Senegal	Seyal	mix
Enterocuccus	Colon	$4.17\pm0.54^{a}_{b}$	$3.34\pm0.51^{a}_{cde}$	$1.96\pm2.2^{a}_{abc}$	$3.26\pm0.54^{a}_{b}$	$3.61\pm0.89^{a}_{abc}$
	Ceacum	$3.45\pm0.56^{a}_{b}$	$3.52\pm0.78^{a}_{abc}$	$2.98\pm2.19^{a}_{abc}$	$2.77\pm0.30^{a}_{a}$	$3.48\pm0.10^{a}_{abc}$
Coliform	Colon	$3.29\pm0.95^{a}_{b}$	$2.70\pm0.12^{a}_{e}$	$3.76\pm0.72^{a}_{bc}$	$3.75\pm0.74^{a}_{ab}$	$3.75\pm0.28^{a}_{bc}$
	Ceacum	$3.59\pm1.01^{a}_{b}$	$2.99\pm0.52^{a}_{bc}$	$3.90\pm0.43^{a}_{bc}$	$3.46\pm0.88^{a}_{a}$	$3.61\pm0.98^{a}_{bc}$
Enterobacteriacea	Colon	$4.05\pm0.66^{a}_{b}$	$4.12\pm0.66^{a}_{abcde}$	$3.95\pm0.86^{a}_{cd}$	$3.22\pm0.54^{a}_{ab}$	$2.87\pm0.43^{a}_{cd}$ *
	Ceacum	$3.24\pm0.47^{a}_{b}$	$3.12\pm0.64^{a}_{abc}$	$3.12\pm0.61^{a}_{abc}$	$3.27\pm0.39^{a}_{a}$	$3.06\pm0.67^{a}_{abc}$
Staphylococcus	Colon	$3.31\pm0.62^{a}_{b}$	$3.09\pm0.58^{a}_{de}$	$3.01\pm0.57^{a}_{bc}$	$3.04\pm0.43^{a}_{ab}$	$3.07\pm0.48^{a}_{bc}$
	Ceacum	$3.33\pm0.58^{a}_{b}$	$3.36\pm0.44^{a}_{abc}$	$4.16\pm1.14^{a}_{abc}$	$2.80\pm0.57^{a}_{a}$	$3.55\pm0.80^{a}_{abc}$
Salmonella	Colon	$3.16\pm0.87^{a}_{b}$	$2.05\pm1.40^{a}_{c}$	$0.61\pm1.23^{a}_{c}$	$1.80\pm2.08^{a}_{c}$	$1.34\pm1.55^{a}_{d}$ *
	Ceacum	$3.22\pm1.04^{a}_{b}$	$3.59\pm1.04^{a}_{bcde}$	$2.42\pm2.8^{a}_{d}$	$2.85\pm0.66^{a}_{ab}$	$2.26\pm1.59^{a}_{a}$

Values are mean ± SD for replicate independent runs.

Values that carried the different superscript letter in the same row are significantly different at $p<0.05$.

Values that carried the same suberscript letter in the same column aren't significantly different at $p<0.05$.

Treatments groups received the followings: Control group fed on normal rats feed, wheat bran group fed on normal rats feed supplemented with wheat bran , senegal group fed on normal rats feed supplemented with *Acacia senegal* (Hashab), seyal group fed on normal rats feed supplemented with *Acacia seyal*(Taleh), mix group fed on normal rats feed supplemented with *Acacia senegal + seyal*)) (Hashab + Taleh).

Table 13: Proximate composition (%) of raw and roasted peanut

Components	Raw peanut	Roasted peanut	Peanut reference values**
Moisture	6.10 ± 0.21	3.40 ± 0.19	6.00±1.7
Fat	45.14 ±0.25	45.85 ± 0.29	45.90±3.0
Proteins	28.83 ±0.31	29.48 ± 0.39	22.40±1.6
Fiber	6.22 ± 0.15	6.54 ± 0.20	8.50±7.7
Ash	2.17 ± 0.13	2.26 ± 0.05	2.30±0.1
Carbohydrates	11.54±0.047	12.47 ± 0.04	14.60±0.1

**Values from food composition table (Barbara, *et al.*, 2012)

*Values are mean ± SD for replicate independent runs.

4.8. *Bifidobacterium longum* BB536 growth in peanut milk supplemented with different types of gum Arabic

In Table 14 Comparison of *Bifidobacterium longum* BB536 growth in different beverages (peanut milk, peanut milk supplemented with *Acacia senegal*, peanut milk supplemented with *Acacia seyal* and peanut milk with a mix of *Acacia enegal seyal*), and is shown in Table 14 . All beverages supplemented with Gum Arabic showed a high growth of *Bifidobacterium longum* BB536 compared to the control. *Bifidobacterium longum* BB536 viable count significantly ($p<0.05$) increased by extending the fermentation period in all types of fermented beverages, as compared to strain in the initial level in the starting of the fermentation. Different maximum growth of strain *BB536* was detected. The maximum growth for control, peanut milk supplemented with *Acacia senegal* and peanut milk supplemented with mix *Acacia (senegal+ seyal*) was attained at 18h of incubation, while the maximum growth of strain BB536 supplemented with *Acacia seyal* was attained at 24h of fermentation .The growth rate of *B. longum* BB536 in different fermented beverages were 5.36, 6.79, 6.3 and 6.95 log CFU/ml for control, peanut milk supplemented with *Acacia senegal*, peanut milk supplemented with *Acacia seyal* and peanut milk mix *Acacia (senegal+ seyal*) respectively, as compared to strain at initial growth of fermentation. These variations in the growth rate of strain BB536 could be attributed to the types of gum Arabic used as supplements. Peanut contains almost the essential nutrient needed for bacterial growth. The growth of strain BB536 improved by supplementation with different GA as compared to the peanut milk without GA (Table14). Gum Arabic contain about 78-88% solid materials and essential amino acids (Montengro *et al*., 2012) . After the maximum point, there was a reduction in the viable number of strain BB536, that could be due to lack of availability of nutrient required for the growth as stated by Kabeir *et al*., (2005). Also increase of acidity and cold storage of

beverages decreases the viability of *bifidobacteria* cells (Tamime *et al.*, 2005). Inspite of decline in viable count of strain BB536 in all types of fermented beverages at fermentation time, it s still above the number required to presence in probiotic food which is at least 6 log cfu/ml fermented product at the time of consumption (Viderola and Reinheimer, 2000 IDF, 1992; Lourens-Hattingh and Viljoen, 2001).

The highest growth of the strain BB536 was detected in peanut supplemented with mixed *Acacia (senegal+ seyal*). The mix of gums Arabic show better promoting effect as compared to the control in the growth and survival of probiotic bacteria. It was found to be affected by the chemical and microbiological composition of the medium and availability of nutrients (Shah, 2000b). These indicate the importance of using prebiotic gum Arabic for the fermentation with bifidobacteria. The mixture of gum Arabic with peanut milk could be considered as an excellent medium for *B. longum* BB 536 growth.

4.9. pH and titrable acidity during fermentation of peanut milk with *Bifidobacterium longum* BB536

The result in Table (15) showed the pH values during the fermentation of different peanut milk samples. There was significant ($P<0.05$) decrease in pH for all types of beverages with extending the fermentation period. The decrease in pH is due to increased acid production as a result of fermentation of sugars by *B.longum BB536* which produces acetic and lactic acid this is in agreement with Elghali *et al.* (2014) who stated that, highest increase in acid production was associated with the maximum increase in the viable population of bacteria. Moreover, the accumulated acids produced by strain *BB536* reported to have antibacterial activity such as prevention of the growth of pathogens (Bullen *et al*.,1976; Goderska *et al.* 2002; Samona *et al.* 1996).

Table 14: Viable count of *Bifidobacterium longum* BB536 log(cfu/ml) during fermentation period of different beverages

Beverages fermented Time (h)	Total growth of *B. longum* BB536 (log cfu/ml)			
	Peanut milk without GA (Control)	**Peanut milk supplemented with *Acacia senegal gum***	**Peanut milk supplemented with *Acacia seyal gum***	**Peanut milk supplemented with mix*Acacia* (*senegal+ seyal)***
0	$2.52\pm 0.07^{h}_{c}$	$2.77\pm.07^{h}_{b}$	$2.63 \pm 0.06^{h}_{bc}$	$2.90 \pm 0.01^{h}_{a}$
6 hrs	$3.68\pm 0.02^{f}_{c}$	$4.73\pm0.03^{f}_{b}$	$4.52\pm0.04^{f}_{c}$	$5.83\pm0.01^{f}_{a}$
12 hrs	$6.81 \pm 0.03^{b}_{d}$	$8.72\pm.017^{c}_{b}$	$7.97 \pm0.01^{c}_{c}$	$8.92\pm0.01^{c}_{a}$
18 hrs	$7.88 \pm 0.03^{a}_{d}$	$9.56\pm0.03^{a}_{b}$	$8.88\pm0.01^{c}_{c}$	$9.86\pm0.01^{a}_{a}$
24 hrs	$7.83\pm 0.02^{a}_{d}$	$8.74\pm0.02^{b}_{b}$	$8.93 \pm 0.02^{b}_{c}$	$9.75\pm0.02^{b}_{a}$
30 hrs	$6.66 \pm 0.04^{c}_{d}$	$7.51\pm0.03^{d}_{b}$	$7.83\pm0.02^{d}_{c}$	$8.71\pm0.02^{d}_{a}$
36 hrs	$5.87\pm 0.03^{d}_{d}$	$7.66\pm0.02^{d}_{b}$	$7.54\pm0.04^{d}_{c}$	$8.73\pm0.03^{d}_{a}$
42 hrs	$4.71\pm 0.04^{e}_{d}$	$6.87\pm0.02^{e}_{b}$	$5.92\pm0.02^{e}_{c}$	$7.93\pm0.01^{e}_{a}$

Values are mean ± SD for replicate independent runs.

Values that carry different superscript letters in the same row are significantly different at $p<0.05$.

Values that carry same suberscript letters in the same column aren't significantly different at $p<0.05$.

Peanut milk (mix) prepared using peanut milk and 1% gums of *Acacia senegal* and *Acacia seyal* in 1:1.

The rate of pH decreases at maximum growth of strain BB536 were 1.16, 1. 01 ,1 .21 and 0.9 in fermented peanut milk, peanut milk supplemented with *Acacia senegal* (Hashab)peanut milk supplemented with *Acacia seyal* (Taleh) peanut milk supplemented with a mix *Acacia (senegal + seyal)* respectively. Peanut milk supplemented with gum Arabic may offer some better properties to the fermentation medium, indicated by lowering the pH .The decrease of pH values in beverages supplemented with Gum Arabic could be due to the variety of carbohydrates, which the strain BB536 can ferment (Osman *etal.*,1993).

4.10. Titratable acidity during fermentation of peanut milk with *Bifidobacterium longum* BB536

Table 16 showed the titratable acidity of different peanut fermented milk samples. Titratable acidity increased by extending the fermentation period. High acidity in supplemented beverages with GA was due to high growth of the strain BB536 and carbohydrates breaks down. The increase in acidity is correlated well with pH reduction mainly due to growth of *Bifidobacterium longum BB536* The rate of pH decreases at maximum growth of strain BB536 were 1.16, 1. 01 ,1 .21 and 0.9 in fermented peanut milk, peanut milk supplemented with *Acacia senegal* peanut milk supplemented with *Acacia seyal* peanut milk supplemented with a mix *Acacia (senegal + seyal)* respectively. The maximum growth rate of *B. longum* BB536 in different fermented beverages were 5.36, 6.79, 6.3 and 6.95 log CFU/ml for control, peanut milk supplemented with *Acacia senegal*, peanut milk supplemented with *Acacia seyal* and peanut milk mix *Acacia (senegal+ seyal*) respectively, they produce acids, which causes an increase in acidity and a decrease in pH (Abou-Dobara *et al.*,2016). The fermentation profile for increase in cell numbers and production of acids resulted in a reduction in the growth rate after maximum growth attained, at the end of these fermentations suggests that the growth stopped because of lack of carbohydrate substrate. The level of organic acids at the end of the fermentation might be responsible for the

reduction in cell numbers (Gupta *et al*., 2010). Studies carried out under controlled pH conditions have indicated that the accumulation of acids during the fermentation is responsible for decrease in growth rate (Desjardins *et al*., 1990).

There is a relation between microbial growth and acidity production the strong of the relationship as demonstrated by percent correlation (60 – 96) dependent on types of Gum Arabic supplement.

4.11.Total soluble solid (TSS) changes during fermentation of different beverages with *Bifidobacterium longum BB536*

Table 17 shows changes in total soluble solids (TSS) during fermentation of different formulated beverages with *Bifidobacterium longum* BB536.

There were significant ($P<0.05$) decrease in TSS levels in all types of fermented beverages by extending the fermentation period. The rates of TSS decrease at maximum growth were 0.08, 0.33, 0.03, and 0.27 in fermented peanut milk, peanut milk supplemented with *Acacia senegal* peanut milk supplemented with *Acacia seyal* and peanut milk supplemented with mix *Acacia (senegal + seyal).* A similar decreases in TSS during traditional fermentation of malwa was reported (Muyanja *et al*., 2010). Gum Arabic containts about 78-88% of solid substances and amino acids (Montengro *etal.*,2012). The GA supplementation in peanut milk led to a high concentration of solid substances during the fermentation time in the beverages. The main metabolic products of carbohydrate fermentation by probiotic activity are organic acids substantiated by a drop in pH of the surrounding environment. This result agreed with the study of McMaster *et al*. (2005), who noted a great loss in viability of Bifidobacterium due to increased acidity, which lowers the survivability in fermented milk than in control without fermentation (Ouwehand *etal*, 2002)

Table 15: pH changes during the growth of *Bifidobacterium longum BB536* in fermented beverages

Fermented Beverages Time (h)	pH			
	Peanut milk without GA (Control)	**Peanut milk supplemented with gum *Acacia senegal gum***	**Peanut milk supplemented with gum*Acacia seyal gum***	**Peanut milk supplemented with gum mix*Acacia (senegal+ seyal)***
Initial growth time 0 h	6.67± 0.04^{a}	6.25 ± 0.05^{a}	6.35±0.02^{a}	6.41 ± 0.04^{a}
6h	6.46 ±0.03^{a}	6.11± 0.01^{ab}	6.21± 0.01^{b}	6.39 ± 0.02^{b}
12h	6.21± 0.02^{b}	6.03 ± 0.07^{bc}	6.15 ± 0.01^{bc}	6.20 ± 0.02^{c}
18 h	5.51 ± 0.02^{c}	5.24 ± 0.03^{c}	5.45 ± 0.04^{c}	5.51 ± 0.01^{d}
24h	5.26 ± 0.01^{d}	5.0 ± 0.01^{d}	5.15± 0.02^{d}	5.08 ± 0.02^{e}
30h	4.69 ± 0.03^{e}	4.55 ± 0.02^{e}	4.87 ± 0.10^{e}	4.98 ± 0.03^{f}
36h	4.51 ± 0.02^{f}	4.24 ± 0.01^{h}	4.45 ± 0.02^{f}	4.51 ± 0.01^{h}
42 h	4.10±0.01^{h}	4.07 ±0.00^{i}	4.25 ±0.01^{h}	4.48±0.01^{i}

Values are mean ± SD for replicate independent runs.

Values that carry different superscript letters in the same row are significantly different at $p<0.05$.

Values that carry same superscript letters in the same column aren't significantly different at $p<0.05$.

Peanut milk (mix) prepared using peanut milk and 1% gums of *Acacia senegal* and *Acacia seyal* in 1:1.

Table 16: Titratable acidity (%) during the initial and maximum growth of fermentation the strain of *Bifidobacterium longum BB536* in different beverages

Fermented Beverages Time (h)	Types of beverages			
	Titratable acidity (%)			
	Peanut milk without GA (Control)	**Peanut milk supplemented with *Acacia senegal gum***	**Peanut milk supplemented with *Acacia seyal gum***	**Peanut milk supplemented with mix*Acacia* (*senegal+ seyal)***
Initial growth time 0 h	0.17 ± 0.00^{c}	0.19 ± 0.00^{e}	0.19 ± 0.00^{c}	0.18 ± 0.00^{f}
6h	0.19 ± 0.00^{c}	0.21 ± 0.02^{d}	0.21 ± 0.02^{d}	0.20 ± 0.01^{e}
12h	0.26 ± 0.02^{b}	0.23 ± 0.03^{cd}	0.22 ± 0.02^{c}	0.21 ± 0.01^{d}
18h Maximum growth time	0.28 ± 0.00^{b}	0.28 ± 0.00^{c}	0.24 ± 0.00^{d}	0.29 ± 0.00^{cd}
24h	0.29 ± 0.00^{b}	0.30 ± 0.01^{b}	0.26 ± 0.01^{c}	0.30 ± 0.00^{c}
30h	0.30 ± 0.02^{ab}	0.31 ± 0.02^{b}	0.29 ± 0.03^{b}	0.33 ± 0.01^{b}
36 h	0.31 ± 0.00^{ab}	0.33 ± 0.00^{ab}	0.31 ± 0.00^{ab}	0.34 ± 0.00^{a}
42 h	0.33 ± 0.00^{a}	0.34 ± 0.01^{a}	0.32 ± 0.01^{a}	0.34 ± 0.00^{a}

Values are mean ± SD for replicate independent runs.

Values that carry different superscript letters in the same row are significantly different at $p<0.05$.

Values that carry same superscript letters in the same column aren't significantly different at $p<0.05$.

Peanut milk (mix) prepared using peanut milk and 1% gums of *Acacia senegal* and *Acacia seyal* in 1:1.

Table 17: TSS (%) changes during the growth of the strain *Bifidobacterium longumBB536* in different beverages

Fermented beverages time (h)	TSS			
	Peanut milk without GA (Control)	**Peanut milk supplemented with *Acacia senegal gum***	**Peanut milk supplemented with *Acacia seyal gum***	**Peanut milk supplemented with mix*Acacia* (*senegal+ seyal)***
0h	2.37 ± 0.05^{a}	2.82 ± 0.02^{abc}	2.96 ± 0.01^{b}	2.89 ± 0.01^{a}
6h	2.23 ± 0.03^{b}	2.68 ± 0.01^{abc}	2.77 ± 0.02^{c}	2.43 ± 0.02^{d}
12h	2.07 ± 0.06^{c}	3.03 ± 0.07^{ab}	3.06 ± 0.12^{ab}	2.31 ± 0.01^{e}
18h maximum growth	2.29 ± 0.01^{b}	3.15 ± 0.14^{a}	3.11 ± 0.01^{a}	$2.62\ \pm0.03^{b}$
24h	1.81 ± 0.01^{d}	2.98 ± 0.02^{ab}	2.99 ± 0.03^{ab}	2.52 ± 0.01^{c}
30h	1.63 ± 0.03^{e}	2.47 ± 0.03^{bc}	2.33 ± 0.03^{d}	2.22 ± 0.03^{f}
36h	1.39 ± 0.01^{f}	2.36 ± 0.06^{c}	2.22 ± 0.02^{d}	1.12 ± 0.03^{g}
42h	1.17 ± 0.02^{g}	1.32 ± 0.05^{d}	1.99 ± 0.01^{e}	0.80 ± 0.12^{h}

Values are mean ± SD for replicate independent runs.

Values that carry different superscript letters in the same row are significantly different at $p<0.05$.

Values that carry same superscript letters in the same column aren't significantly different at $p<0.05$.

Peanut milk (mix) prepared using peanut milk and 1% gums of *Acacia senegal* and *Acacia seyal* in 1:1.

4.12. Total sugars during fermentation of different beverages with *Bifidobacterium longum BB536*

The result in table (18) shows significant ($P<0.05$) decrease in sugar levels of all fermented beverages with an extended fermentation period. The strain BB536 ferment Gum Arabic and produces sugar and organic acids, mainly acetic, lactic, propunic, butyric and other organic acids(Sefa-Dedeh *et al.*,2003).

The rates of sugar decreased at maximum growth of strain *Bifidobacterium longum* BB536 and they were 0.06, 0.92, 1.47 ,and 0.63 in fermented peanut milk, the peanut milk supplemented with *Acacia senegal*(Hashab), peanut milk supplemented with *Acacia seyal* taleh, and peanut milk mix , respectively . Gum Arabic is a branched-chain, complex polysaccharide, (Badreldin *et al.,* 2008; Abdul-Hadi *et al*., 2010), and these variations in total sugar refer to the strain activity, which break down complex polysaccharide during the fermentation time in different fermented beverages containing gums Arabic, and correlated well with the decrease in TSS.

4.13. Changes in Water content during the fermentation of different fermented beverages

The results in Table (19) show the Water content of different fermented beverages. There were a light increases in moisture of different fermented beverages by extending the fermentation period. The amount of moisture in fermented peanut milk, peanut milk supplemented with *Acacia senegal* peanut milk supplemented with *Acacia seyal* peanut milk supplemented with a mix (senegal + seyal), was 0.58, 0.73 ,0.71, and 0.98 % respectively. Gum Arabic is utilized in food products as an emulsifier and stabilizer material(Montengro *et al*.,2012).The stabilizer reduces water in fermented medium production. Therefore, during the fermentation process, increase in water level might indicate a high enzymatic activity that breaks down the macro component into simpler ones and to the release of water.

Table 18: total sugars (%) during the initial and maximum growth of *Bifidobacterium longum BB536* in different beverages

Fermented beverages Time(h)	Types of beverages			
	Total sugars			
	Peanut milk without GA (Control)	**Peanut milk supplemented with *Acacia senegal gum***	**Peanut milk supplemented with *Acacia seyal gum***	**Peanut milk supplemented with mix*Acacia* (*senegal+ seyal)***
Initial growth 0h	0.41±0.00^{a}	0.29 ± 0.09^{e}	0.38 ± 0.00^{de}	0.39 ± 0.00^{de}
6h	0.39±0.01^{a}	0.32±0.01^{e}	0.63 ± 0.03^{e}	0.51±0.02^{c}
12h	0.31±0.01^{c}	0.89±0.01^{c}	0.98±0.00^{d}	0.88±0.01^{b}
18h	0.35±0.02^{b}	1.21 ± 0.00^{a}	1.85 ± 0.00^{a}	1.02 ± 0.02^{a}
Maximum growth				
24h	0.28±0.01^{d}	1.19±0.01^{a}	1.81±0.01^{a}	1.01±0.01^{a}
30h	0.22±0.01^{e}	1.03±0.05^{b}	1.43±0.09^{b}	0.95±0.09^{ab}
36 h	0.21±0.00^{e}	0.95±0.04^{c}	1.22±0.03^{c}	0.93±0.04^{ab}
42h	0.12±0.01^{f}	0.65±0.01^{d}	0.95±0.08^{d}	0.85±0.015^{b}

Values are mean ± SD for replicate independent runs.

Values that carry different superscript letters in the same row are significantly different at $p<0.05$.

Values that carry same superscript letters in the same column aren't significantly different at $p<0.05$.

Peanut milk (mix) prepared using peanut milk and 1% gums of *Acacia senegal* and *Acacia seyal* in 1:1.

Table 19: Water content %of the different fermented beverages during growth and refrigeration storage

Fermented beverages Time (h)	Types of beverages			
	Water content			
	Peanut milk without GA (Control)	**Peanut milk supplemented with *Acacia senegal gum***	**Peanut milk supplemented with *Acacia seyal gum***	**Peanut milk supplemented with mix*Acacia* (*senegal+ seyal)***
Initial growth time 0 h	87.30±0.01[b]	86.26±0.01[b]	86.59 ±0.00[b]	86.12±0.02[b]
Maximum growth time 18 h	87.88±0.06[a]	86.99±0.06[a]	87.30 ±0.01[a]	87.10± 0.01[a]

Values are mean ± SD for replicate independent runs.

Values that carry different superscript letters in the same row are significantly different at $p<0.05$.

Values that carry same superscript letters in the same column aren't significantly different at $p<0.05$.

Peanut milk (mix) prepared using peanut milk and 1% gums of *Acacia senegal* (Hashab) and *Acacia seyal* (Taleh) in 1:1.

4.14. The survival of *Bifidobacterium longum* BB536 (log cfu/ ml) during the storage of different fermented beverages

Table (20) shows the survival rate of *Bifidobacterium longum* BB536 over 14 days during refrigeration storage of different fermented beverages to assess the shelf life. There was significant reduction in viable counts between beverages during the storage as compared to the initial storage level.

The rate of reduction in the first week of the refrigerated storage were 2.04, 2.75, 2.25 , and 2.06 log CFU /ml in fermented peanut milk, fermented peanut milk supplemented with *Acacia senegal*, fermented peanut milk supplemented with *Acacia seyal* and fermented peanut milk supplemented mix *Acacia (senegal + seyal*), respectively. It is clear that the rates of reduction were differed among different fermented beverages. Moreover *Bifidobacterium longum* BB536 reductions recorded in the second week of the refrigerated storage, which were 1.19, 1.0, 0.95, 2.06 and 1.08 log cfu/ml of fermented peanut milk, fermented peanut milk supplemented with *Acacia senegal* fermented peanut milk supplemented with *Acacia seyal* , and fermented peanut milk supplemented with gum mix *Acacia (seyal + seyal*), respectively. The final viable count of *B. longum* BB536 in fermented peanut milk supplemented with mix *Acacia (seyal + seyal*), after two weeks refrigeration storage was above the minimum number required to presence in probiotic food to exert health benefits upon consumption, which is 6 log cfu/ml Viderola and Reinheimer, 2000 ; IDF, 1992), while the strain counts in the other fermented beverages were below the number required to fulfill probiotic effect.

In this respect Kabeir *et al.,* (2005) stated that the *B. longum* BB536 viability and survival under refrigerated storage of Sudanese fermented Medida beverages was not affected for a period of two weeks. Although Alkalin *et al*. (2004) reported that there was a significant decrease in the viable count of

B.longum BB46 in yogurt after just one week refrigeration. This signify that the viability and survival of *Bifidobacterium* in fermented products was dependent on the carrier medium type and pH levels of the fermented foodstuffs during the storage.The supplementation of beverages with gum Arabic improved survival as compared to control beverages without gum Arabic. The possibility of growth ability may change the chemical composition of the fermentation medium to better survival environment. Overall, most strains of *bifidobacterium* are sensitive to pH values below 4.6. Therefore, for practical application, a pH value of the final product must be maintained above 4.6 to prevent the decline of *bifidobacterium* populations (Tamime and Robinson, 1985; Modeler *et al*., 1990; Laroia and Martin, 1991). The survival of probiotics bacteria in fermented dairy bio-products depends on such varied factors as the strains used, interactions between species present, culture conditions, chemical composition of the fermentation medium (e.g. carbohydrate source), final acidity, milk solids content, availability of nutrients, growth promoters and inhibitors, concentration of sugars (osmotic pressure), dissolved oxygen (especially for *Bifidobacterium sp*.), level of inoculation, incubation temperature, fermentation time and storage temperature. The variances in survival were interpreted by the metabolic activity of *Bifidobacterium* in different fermented products, which may be influenced by the nitrogen and carbon source activity and availability in the growth media as stated by Chou and Hou, (2000).

Lactobacillus acidophilus and bifidobacteria survival may be affected by the fat composition of the fermentation medium , in addition it is found that full fat yogurt reduced the property for *Bifidobacterium.bifidum* as compared with the low-fat yogurt (Vinderola *et al*., 2000). The growth and survival of probiotics in dairy products affected by the fermentation time, incubation temperature, storage temperature, and the addition of casein. while the level of dissolved oxygen in the product have also been the main

factor that influence the survival of probiotic bacteria in dairy products (Klaver *et al*., 1993). The survivability of Probiotics in fermented products should be fixed in the shelf life of the fermented products (Dinakar and Mistry, 1994),while vastly reduced in the viable number of the growth of the probiotic bacteria during the shelf life of others fermented products (Stanton *et al.,* 2003) has been reported. The survival of *B. longum* BB536 in fermented beverages refrigerated storages for 2 weeks decreased to a level of < 7log CFU/ml due to pH reduction, which were explained by increases in acidity, (Stanton *et al.*, 2003).

4.15. Changes in pH during storage of different fermented beverages

The reduction in pH during refrigeration storage of different fermented beverages was presented in Table (21). There was significant ($p<0.05$) reduction in pH of all types of fermented beverage during the two weeks of refrigeration. The rate of pH reductions in the first week were 0.15, 0.06, 0.43, and 0.69 pH in fermented peanut milk (control), fermented peanut milk supplemented with *Acacia senegal* , fermented peanut milk supplemented with *Acacia seyal* , and fermented peanut milk supplemented with mix *Acacia (senegal + seyal*), respectively . The reduction in pH is mainly due to the relatively acidic pH in the large intestine, thus preventing the growth of pathogens. However, fermentation of sugars and accumulation of acid. That is why *Bifidobacterium* maintains a low pH and storage temperature were the most important factors in *Bifidobacterium* mortality (Kabeir. *et al*., 2015: Sakai *et al*.1987).

Table 20: *Bifidobacterium longum* BB 536 log (cfu/ ml) survival throughout the storage of different fermented beverages

Fermented Beverages period	The viable count of *Bifidobacterium longum* BB536			
	Peanut milk without GA (Control)	**Peanut milk supplemented with *Acacia senegal gum***	**Peanut milk supplemented with *Acacia seyal gum***	**Peanut milk supplemented with mix*Acacia* (*senegal+ seyal)***
Initial 0 week	$7.88 \pm 0.030^{d}a$	$9.56\pm0.03^{b}_{a}$	$8.88\pm0.01^{c}_{a}$	$9.87\pm0.01^{a}_{a}$
Week1	$5.84 \pm 0.03^{d}_{b}$	$6.81\pm0.10^{b}_{b}$	$6.63\pm 0.03^{c}_{b}$	$7.81\pm0.01^{a}_{b}$
Week2	$4.65 \pm 0.04^{d}_{c}$	$5.81\pm0.03^{b}_{c}$	$5.68 \pm 0.04^{c}_{c}$	$6.73 \pm 0.03^{a}_{c}$

Values are mean ± SD for replicate independent runs.

Values that carry different superscript letters in the same row are significantly different at $p<0.05$.

Values that carry same suberscript letters in the same column aren't significantly different at $p<0.05$.

Peanut milk (mix) prepared using peanut milk and 1% gums of *Acacia senegal* and *Acacia seyal* in 1:1.

The pH decreases recorded in the second week of refrigeration were 0.41, 0.26, 0.21and 0.04 in fermented peanut milk, the fermented peanut milk supplemented with *Acacia senegal* , fermented peanut milk supplemented with *Acacia seyal* , and fermented peanut milk supplemented with mix *Acacia (senegal + seyal*), respectively .These results can indicate that the use of gum Arabic may control pH reduction, and can increase the shelf life of fermented beverages. Thus, Bifidobacterium maintains production of lactic and acetic acid, hydrogen peroxide, and the bactericides which are identified as inhibitor factors of the development of pathogenic bacteria. Also lactic acid and acetic acid in fermented dairy product have an antibacterial effect reported by Bullen *et al.*,(1976), Therefore, pH is a major factor that restricts growth and stability of probiotic bacteria.

Lankaputhra *et al.* (1996) reported survival of three out of nine bifidobacteria strains in the pH range of 4·3 – 3·7. Kabeir *et.al* (2005) found the decrease of *B. longum* BB 536 in cold storage of the fermented madida was less than that justified for bifidobacteria. Followed by 2 weeks refrigerated storage the count of *B. longum* BB536 in the fermented *madida* reduced by 0·9 log CFU ml−1. Thus, the shelf life of this strain on the fermented peanut milk supplemented with gum Arabic is better.

4.16. Titratable acidity of the different fermented beverages during refrigeration storage

Table (22) shows the titratable acidity of different fermented beverages. Titratable acidity of the fermented beverages increased by extending the storage period for two weeks .The rate of titratable acidity increases were 0.03, 0.05, 0.07, and 0.1in fermented peanut milk, fermented peanut milk supplemented with *Acacia senegal* , fermented peanut milk supplemented with *Acacia seyal* , and fermented peanut milk supplemented with mix *Acacia (senegal + seyal*), respectively. The increasing rates recorded in the second week were 0.68, 0.55,0.51, and 0.48 in fermented peanut milk, fermented

Table 21: The pH of the different fermented beverages during refrigerated storage

Fermented Beverages Time (h)	Types of beverages			
	pH			
	Peanut milk without GA (Control)	**Peanut milk supplemented with *Acacia senegal gum***	**Peanut milk supplemented with *Acacia seyal gum***	**Peanut milk supplemented with mix*Acacia* (*senegal+ seyal)***
Initial storage	5.51 ± 0.02^{c}	5.24 ± 0.03^{c}	5.45 ± 0.04^{c}	5.51 ± 0.01^{d}
After Week 1	5.36 ± 0.08^{b}	5.30 ± 0.01^{b}	5.02 ± 0.01^{b}	4.82 ± 0.03^{b}
After Week 2	4.95 ± 0.04^{c}	5.04 ± 0.01^{c}	4.81 ± 0.04^{c}	4.78 ± 0.03^{c}

Values are mean ± SD for replicate independent runs.

Values that carry different superscript letters in the same row are significantly different at $p<0.05$.

Values that carry same superscript letters in the same column aren't significantly different at $p<0.05$.

Peanut milk (mix) prepared using peanut milk and 1% gums of *Acacia senegal* and *Acacia seyal* in 1:1.

peanut milk supplemented with *Acacia senegal*, fermented peanut milk supplemented with *Acacia seyal*, and fermented peanut milk supplemented with mix *Acacia (senegal + seyal*), respectively. The increased acidity could be explained by the accumulation of lactic acid and other organic acids produced during fermentation of the formulated beverages by *B.longum* BB536 (Sefa *et al.*,2003). Increasing the acidity of foods, both through fermentation or the addition of weak acids, has been used as a preservation method since early times.

Desjardins *et al.* (1990)stated that the short shelf life of numerous fermented milk products during refrigerated and frozen storage was correlated to increased acidity, and to the low tolerance of bifidobacteria strain to lactate and acetate acids (Rasic and Kurman 1983). Increased acidity, could cause the decrease of bifidobacteria growth by 1–2 log cfu ml^{-1} in the fermented milk after cold storage at 4°C for 15 days (Hughes and Hoover 1995). Rasic and Kurman (1983) clarified that increased acidity affects the growth of Bifidobacteria and can most likely reduce 2 log in milks medium (pH 4·7–4·3) through 1–2 weeks refrigerated storage. Chou and Hou.(2000) reported the decrease in *B.longum* counts by 3.3 log cfu ml−1 fermented soy milk beverage in two weeks storage 5°C.

4.17. Changes in total soluble solids (TSS) during refrigeration storage of different fermented beverages

The presented results in Table (23) showed the TSS of different fermented beverages**.** There was a significant ($p<0.05$) decrease in the total soluble solids in all types of fermented beverages under refrigeration storage for two weeks. The decrease in the first week of refrigeration storage of formulated fermented peanut milk, fermented peanut milk supplemented with *Acacia senegal* (Hashab), fermented peanut milk supplemented with *Acacia seyal* (Taleh), and fermented peanut milk supplemented with mix *Acacia (senegal + seyal*), respectively, were 1.1, 0.30, 0. 43, and 0.58 %, respectively.

Table 22: Titratable acidity of the different fermented beverages during refrigeration storage

Fermented Beverages Time(h)	Types of beverages			
	Titratable acidity			
	Peanut milk without GA (Control)	**Peanut milk supplemented with *Acacia senegal gum***	**Peanut milk supplemented with *Acacia seyal gum***	**Peanut milk supplemented with mix*Acacia* (*senegal+ seyal)***
Initial storage	0.28 ± 0.00^{c}	0.28 ± 0.00^{c}	0.28 ± 0.00^{c}	0.29 ± 0.00^{c}
After Week 1	0.31 ± 0.02^{b}	0.33 ± 0.02^{b}	0.35 ± 0.01^{b}	0.39 ± 0.03^{b}
After Week 2	0.99 ± 0.01^{a}	0.88 ± 0.00^{a}	0.86 ± 0.01^{a}	0.87 ± 0.01^{a}

Values are mean ± SD for replicate independent runs.

Values that carry different superscript letters in the same row are significantly different at $p<0.05$.

Values that carry same superscript letters in the same column aren't significantly different at $p<0.05$.

Peanut milk (mix) prepared using peanut milk and 1% gums of *Acacia senegal* and *Acacia seyal* in 1:1.

The decrease in the second week was lower as compared to that of the first week recording values of 1.4, 0.51, 0.6 and 0.39 respectively. The decrease of total soluble solids could be due to slight degradation of carbohydrates , protein and enzyme activity utilizing these nutrients and other organic solid component produced during fermentation of the formulated beverages.

4.18. Total sugars during refrigerated storage period of different fermented beverages

Results in Table (24) presented the total sugars during the refrigerated storage period of different fermented beverage. There was significant ($p<0.05$) decrease in the total sugars of different fermented beverages .The percentages of sugar decrease in the first week were 0.01., 0.04,0.01 and 0.02 in fermented blend peanut milk, fermented peanut milk supplemented with *Acacia senegal* (Hashab), fermented peanut milk supplemented with *Acacia seyal* (Taleh), and fermented peanut milk supplemented with mix *Acacia* (*senegal* + *seyal*), respectively. The percentages of sugar reduction in the second week were higher as compared to that of the first week recording values of 0.17, 0.55,0.91, and 0.19 in the fermented peanut milk, peanut milk (*senegal*) peanut milk (*seyal*) peanut milk mix *Acacia* (*senegal* + *seyal*) gum respectively.

These results could have an important nutritional significance, because they indicate that the prebiotic addition improves bifidobacterial enzyme activity during the peanut milk fermentation (Cumchuere and Robinson1999; Wang *et al.* 2003; Donkor *et al.,* 2007).

Table 23: Changes in TSS % of the different fermented beverages during refrigeration storage

Fermented Beverages Time (h)	Types of beverages			
	TSS			
	Peanut milk without GA (Control)	**Peanut milk supplemented with gum *Acacia senegal gum***	**Peanut milk supplemented with gum*Acacia seyal gum***	**Peanut milk supplemented with gum mix*Acacia* (*senegal+ seyal)***
Initial storage	2.29±0.01[a]	3.15±0.14[a]	3.11±0.01[a]	2.62 ±0.03[a]
Week 1	2.20 ± 0.02[b]	3.13 ± 0.01[a]	3.11 ± 0.01[a]	2.60 ± 0.01b
Week 2	0.80 ± 0.01[c]	2.62 ± 0.02[b]	2.51 ± 0.0[b]	2.21 ± 0.01[c]

Values are mean ± SD for replicate independent runs.

Values that carry different superscript letters in the same row are significantly different at $p<0.05$.

Values that carry same superscript letters in the same column aren't significantly different at $p<0.05$.

Peanut milk (mix) prepared using peanut milk and 1% gums of *Acacia senegal* and *Acacia seyal* in 1:1.

Table 24: Total sugar of the different fermented beverages during refrigeration storage

Beverages Fermented Time(h)	Types of beverages			
	Total sugars			
	Peanut milk without GA (Control)	**Peanut milk supplemented with *Acacia senegal gum***	**Peanut milk supplemented with *Acacia seyal gum***	**Peanut milk supplemented with mix*Acacia* (*senegal+ seyal)***
Initial storage 0h	0.31 ± 0.01^{a}	1.21 ± 0.00^{a}	1.85 ± 0.00^{a}	1.02 ± 0.02^{a}
Week 1	0.30 ± 0.00^{b}	1.17 ± 0.00^{b}	1.84 ± 0.00^{b}	1.00 ± 0.0^{b}
Week 2	0.13 ± 0.00^{c}	0.62 ± 0.00^{c}	0.93 ± 0.00^{c}	0.81 ± 0.00^{c}

Values are mean ± SD for replicate independent runs.

Values that carry different superscript letters in the same row are significantly different at $p<0.05$.

Values that carry same superscript letters in the same column aren't significantly different at $p<0.05$.

Peanut milk (mix) prepared using peanut milk and 1% gums of *Acacia senegal* and *Acacia seyal* in 1:1.

4.19. Changes in water content throughout the storage period of different fermented beverages

In food, water content is essential for shelf life, it is used to predict microbiological and chemical stability of food products (Gabriele. 2001). As presented in Table (25), water content of different fermented beverages significantly ($p<0.05$) increased during the storage period for two weeks due to release of water from the breakdown of macro components . The water content increased during the first week of refrigerated storage of different formulated fermented peanut milks, peanut milk supplemented with *Acacia senegal* , peanut milk supplemented with *Acacia seyal*, and peanut milk supplemented with a mix (*senegal* + *seyal*) gum , to 1.1, 1.12, 2.27, and 2.38%, respectively. While the increase in the second week of refrigerated storage of peanut milk, peanut milk supplemented with *Acacia senegal* , peanut milk supplemented with *Acacia seyal* , and peanut milk supplemented with a mix *Acacia* (*senega* + *seyal*) gum, was 1.35, 1.92, 3.24, and 3.1, respectively . That rate of increase was high in the second week of storage as compared to the first week. In the fermentation process, increase in water content might indicate a high enzymatic activity that breaks down the macro components into simpler ones and to the release of water.

4.20 Chemical composition of different beverages fermented by *Bifidobacterium longum BB536*

Table (26) shows the chemical composition of peanut milk and peanut milk supplemented with acacia gums beverages fermented with *B. Longum BB536* at initial (0h) and maximum growth time (18h). The result presented in table 3, revealed that there were no significant ($p>0.05$) changes in composition of beverages. In fermented peanut milk there was increase in moisture, protein ,ash and fiber; while fat, carbohydrate and total soluble

Table 25: Water content of the different fermented beverages during refrigeration storage*

Fermented Beverages Time (h)	Types of beverages			
	Water content			
	Peanut milk without GA (Control)	**Peanut milk supplemented with *Acacia senegal gum***	**Peanut milk supplemented with *Acacia seyal gum***	**Peanut milk supplemented with (*senegal+ seyal)***
Initial storage time 0 h	87.26 ±0.01^{c}	86.06±0.01^{c}	86.59 ±0.00^{c}	86.12±0.02^{c}
Week 1	88.66 ± 0.00^{b}	87.18 ± 0.01^{b}	88.86 ± 0.00^{b}	88.20 ± 0.00^{b}
Week 2	89.91 ± 0.00^{a}	89.10± 0.00^{a}	92.10 ± 0.00^{a}	91.30 ± 0.00^{a}

Values are mean ± SD for replicate independent runs.

Values that carry different superscript letters in the same row are significantly different at $p<0.05$.

Values that carry same superscript letters in the same column aren't significantly different at $p<0.05$.

Peanut milk (mix) prepared using peanut milk and 1% gums of *Acacia senegal* and *Acacia seyal* in 1:1.

solids decreased by fermentation in peanut milk beverages .Moreover, In fermented peanut mik supplemented with Acacia gums there were increases in protein, ash, fiber, total soluble solid, and moisture. While fat decreased by fermentation.

4.21 The energy value of fermented beverages

The energy value of different fermented beverages is presented in Table (27).The calculated energy ranged from 66.42 kcal/100ml in peanut milk supplemented with mix acacia gums (*acacia sengal+ acacia seyal*) to 71.31 kcal/100ml in peanut supplemented with *Acacia seyal*. Adult men would need to ingest at least 4.5 L (3200 kcal) of all four fermented beverages to meal. While adult women need to ingest at least 3.5 L (2300 kcal), of fermented beverages .The amount to be taken daily to meet the daily energy requirements for adult men and for adult women) as recommended by FAO/WHO/BULL (1979).

4.22. Sensory evaluation of beverages

The effect of mixing gum Arabic with peanut milk on sensory evaluation scores was stated in Table(28). The average scores of different sensory attributes of fermented peanut milk, peanut milk supplemented with *Acacia senegal*, peanut milk supplemented with *Acacia seyal*, peanut milk supplemented with mix *Acacia (senegal + seyal*) were calculated. There were clear differences in the appearance and color, flavor, consistency, mouth feel, after taste and overall acceptability scores of different treatments. The most obvious differences were found in the beverages supplemented with Acacia seyal gum Arabic, which were liked slightly by the panelists. However, color and appearance scores of peanut with *Acacia senegal* beverage were higher than those of *Acacia seyal* and mixed Acacia .The white color of *Acacia senegal* beverage is preferred for the consumers so it gained the highest scores for colour and appearance as compared with the others bevarages.

Table 26: Chemical composition of peanut milk beverages fermented with *Bifidobacterium longum* BB536

Component	Peanut milk		Peanut milk *A.senegal*		Peanut milk *A.seyal*		Peanut milk mix A.senegal + seyal	
	Initial growth	Maximum growth	Initial growth	Maximum growth	Initial growth	Maximum growth	Initial growth	Maximum growth
Moisture (%)	87.30±0.06	87.88 ±0.02	86.25±0.01	86.99 ±0.06	87.59 ±0.00	88.29±0.01	86.81±0.02	87.10±0.00
Fat content (%)	4.82 ±0.01	4.70 ± 0.02	4.90 ± 0.00	4.25 ± 0.0	4.49 ± 0.00	4.10 ± 0.01	4.74 ±0.0	4.11 ±0.01
Protein content (%)	2.70 ±.01	2.82 ±0.01	2.18± 0.02	2.97 ± 0.01	2.17 ±0.06	2.70 ±0.01	2.04± 0.00	2.17 ±0.01
Ash content (%)	0.19±0.013	0.20±0.01	0.14 ±0.01	0.23 ±0.017	0.18±0.003	0.23±0.016	0.19±0.03	0.21±0.014
Total solid (%)	12.40±0.00	12.13±0.00	13.75±0.00	13.01± 0.00	13.41± 0.00	12.71± 0.00	13.19± 0.00	12.87 ±0.00
Carbohydrates (%)	4.99 ± 0.05	4.40 ±0.01	6.53 ±0.02	5.56 ±0.01	5.57 ±0.02	4.68±0.00	6.22 ±0.02	6.41±0.02
Fiber (%)	0.18 ±0.00	0.34±0.00	0.13 ± 0.01	0.24 ± 0.01	0.10 ± 0.01	0.19 ± 0.00	0.15 ± 0.00	0.20 ± 0.01

* Values are mean ± SD for replicate independent runs.

****** Values that bear different superscript letters in the same raw of each specific beverage are significantly different at $p<0.05$.

*Peanut milk was prepared using peanut milk without adding gum Arabic

Table 27: The energy value of beverages (100 ml) at maximum growth of *Bifidobacterium longum BB 536*

Total energy (kcal/100ml)*	**Peanut milk**	**Peanut milk *Acacia senegal***	**Peanut milk *Acacia seyal***	**Peanut milk mix *Acacia senegal* + *seyal***
Total energy (kcal/100ml)	71.18	67.41.16	66.42	71.31
Energy from fat	42.30	38.25	36.90	36.99
Energy from protein	11.28	11.88	10.8	8.68
Energy from carbohydrates	17.6	17.28	18.72	25.64

* The energy value was calculated using factors of 4.00 kcal/g for protein, 9.00 kcal/g for fat and 4.00 kcal/g for total carbohydrate. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (2002/2005).

Scores of color and appearance of peanut milk supplemented with *Acacia seyal* was similar to the color without gum. But mixed were lower than that for peanut milk .With this lower score of color and appearance peanut milk with *Acacia seyal* might be attributed to the brown color of the beverages, flavor, consistency and mouth feel grades of prefer product. Peanut milk with *Acacia senegal* was the best and most preferred product by panelists. The product score value highest in consistency and mouth feel and over all acceptability. consistency of peanut milk control was slightly lower as compared with those of gum supplemented milk. This incorporation of *Acacia senegal* gum with peanut milk improved its sensory evaluation scores more than the *Acacia seyal* and mixed gum.

Table 28: Sensory score of synbiotic beverage samples

Treatments	Characteristic				
	Appearance and colour	**Flavor**	**consistency**	**Mouth feel**	**Overall Acceptability**
Peanut milk supplemented with *Acacia senegal*	7.9±2.7	7.5±2.0	7.8±2.2	7.9±2.17	7.2±2.4
Peanut milk without GA Control	6.8±1.6	7.7 ±1.7	7.6± 1.8	6.1±1.9	6.7±2.5
Peanut milk supplemented with mix Acacia *senegal*+ *seyal*	7.5±2.7	5.7±1.6	6.2±2.04	5.87±1.8	6.0±2.7
Peanut milk supplemented with *Acacia seyal*	6.8±1.5	6.9 ±1.7	6.7±2.02	6.7±2.02	5.9±2.3

Scoring results of the four non-dairy probiotic samples based on nine-point hedonic scale ratings, where 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like or dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, and 9 = like extremely (hedonic scale).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

1- Feeding gum Arabic supplemented to rats exerted a positive health effect the weight gian of fed rats were not retarded, no sings of toxicity or pathogenicity revealed on blood hematology and biochemistry analysis.

2- Gum Arabic has a positive effect on glucose metabolism and reduction of body weight which could be used in turn as prophylactic or treatment of obesity and the development of metabolic syndrom.

3- Gum Arabic effectively inhibited development of Sallmonela in colon via a exchanging effect on beneficial microorganisms such as Bifidobacterium and lactobacillus levels in ceacum and colon .

4- Gum Arabic was found to promote growth of the probiotic bacteria Bifidobacterium at both invitro and in in vivo levels.

5.2. Recommendation

1- Encourage the introduction of gum Arabic and probiotic strains into non-dairy based products for health benefit purposes instead of using conventional yoghurt culture.

2- More research should be conducted on gum Arabic effects on growth and survival of probiotic strains.

3- Improve the acceptability and sensory characteristics of different probiotic fermented beverages.

4- More research to be conducted on nutritional values and functional properties of the developed probiotic fermented product to explore it is health benefits.

5- More research should be conducted on Prebiotication with Gum Arabic on growth of *Bifidobacterium longum BB536* during fermentation of peanut milk

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