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M.Sc. in Biochemistry Science and Technology

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EFFECT OF FLAXSEED MUCILAGE AND GUM ARABIC ON KEFIR PROPERTIES

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ABSTRACT

EFFECT OF FLAXSEED MUCILAGE AND GUM ARABIC ON KEFIR PROPERTIES

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The aim of present study was to evaluate the new properties and activity of kefir after adding gum arabic and flaxseed mucilage in different concentration, also, to determine the symbiotic effect of this combination on *Lactobacillus acidophilus* and *Bifidobacterium lactis* counts during storage time. The effect of adding gum arabic at 0.2% (w/v) and flaxseed mucilage at 0.03, 0.05 and 0.1% (w/v) on the growth of probiotic bacteria were investigated at 0, 1,st 7,th 14,th 21st and 28th days of storage. Gum arabic and flaxseed mucilage had significantly ($P<0.05$) affected on the growth of *L. acidophilus* and *B. lactis* and on physicochemical variables of kefir. The addition of flaxseed mucilage and gum arabic and their mixtures significantly ($P<0.05$) increased the growth of *L. acidophilus* and *B. lactis* compared to control for all days of storage. Results showed that samples containing flaxseed mucilage and gum arabic had significantly ($P<0.05$) lower pH value compared to control and as a result higher titratable acidity. In addition, kefir enriched with flaxseed mucilage and gum arabic showed higher viscosity compared to control. For color measurements, samples having flaxseed mucilage and gum arabic showed significant ($P<0.05$) increase in L*, a* and b* value compared to control. However, as concentration of flaxseed mucilage increase the L* value decreased. Also, addition of flaxseed mucilage and gum arabic into kefir samples effect on the total solid and protein contents compared to control.

Key Words: Kefir, Flaxseed, Mucilage, Probiotic, Gum Arabic

ÖZET

KETEN TOHUMU MÜSİLAJI VE ARAP ZAMKININ KEFİR ÖZELLİKLERİ ÜZERİNE ETKİSİ

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77 Sayfa

Bu çalışmanın amacı, kefirin farklı konsantrasyonlarda arap zamkı ve keten tohumu müsilajı eklendikten sonra yeni özelliklerini ve aktivitesini değerlendirmek, ayrıca bu kombinasyonun *Lactobacillus acidophilus* ve *Bifidobacterium lactis* sayılarına depolama süresi boyunca simbiyotik etkisini belirlemektir. %0,2 (w/v) arap zamkı ve %0,03, 0,05 ve %0,1 (w/v) keten tohumu müsilajının probiyotik bakterilerin büyümesi üzerindeki etkisi 0, 1., 7., 14., 21. ve 28. depolama günlerinde incelenmiştir. Arap zamkı ve keten tohumu müsilajı, *L. acidophilus* ve *B. lactis'in* çoğalması ve kefirin fizikokimyasal değişkenlerini önemli ölçüde ($P<0,05$) etkiledi. Keten tohumu müsilajı ve arap zamkı ve bunların karışımlarının eklenmesi, tüm depolama günleri için kontrole kıyasla *L. acidophilus* ve *B. lactis'in* çoğalmasını önemli ölçüde artırmıştır ($P<0,05$). Sonuçlar, keten tohumu müsilajı ve arap zamkı içeren numunelerin kontrole kıyasla önemli ölçüde ($P<0,05$) daha düşük pH değerine ve bunun sonucunda daha yüksek titre edilebilir asitliğe sahip olduğunu göstermiştir. Buna ek olarak, keten tohumu müsilajı ve arap zamkı ile zenginleştirilmiş kefir kontrole göre daha yüksek viskozite göstermiştir. Renk ölçümleri için keten tohumu müsilajı ve arap zamkı içeren numuneler kontrole kıyasla L*, a* ve b* değerlerinde önemli artış göstermiştir. Ancak keten tohumu müsilaj konsantrasyonu arttıkça L* değeri azaltmıştır. Keten tohumu müsilajı ve arap zamkı ilavesi kontrole göre toplam katı madde ve protein içeriğini etkiledi.

Anahtar Kelimeler: Kefir, Keten Tohumu, Müsilaj, Probiyotik, Arap Zamkı

''Dedicated to my husband and my family''

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LIST OF ABBREVIATIONS

a*	Redness-greenness
ANOVA	Analysis of variance
b*	Yellowness- blueness
CFU	Colony Forming Unit
FM	Flaxseed mucilage
FAO	Food and Agriculture Organization
GA	Gum Arabic
KC	Kefir culture
L*	Lightness- darkness
LAB	Lactic acid bacteria
SPSS	Statistical Package for Social Sciences
WHO	World Health Organization
YC	Yogurt culture

CHAPTER I

INTRODUCTION

Healthy diet is one of the best choices for human to keep their body health and resistance to diseases. The probiotics has been known to human since centuries, due to its amazing health benefits. For this purpose, researchers pay attention for work in probiotics from fermented milk products. Also, consumers prefer to ingest such foods which are alternatives to conventional therapies for various chronic diseases.

There are some factors such as industrial growth, high consumer acceptance, improved quality of life, aging process that may lead to the development of new nutritional, therapeutic and healthy product by the inclusion of natural kefir grains into milk. Kefir is one of the foods that give us the nutrients the body need to maintain its health, feel good, and have energy. Also, kefir is one of the most favorite probiotics, consist of useful bacteria that are beneficial for health, so it is considered as a powerful supplement especially for digestive system. They keep human gut in a good balance by replacing bad bacteria with a new good one.

The bioactive compounds and probiotics complex from fermented milk are relatively effective and safe compared to other food components. The cultured fermented products such as kefir, yogurt, kimchee, sauerkraut, miso and natto, have been used for along term due to their effective prebiotics' properties (Zajsek and Gorsek, 2010). From all, kefir has received great interest from scientists due to its special and combination of probiotic activity.

1.1 Kefir

Kefir is a traditional natural acidic fermented probiotic milk product that originated thousands of years ago in the Caucasus Mountains. It represents an ancient tradition in human nutrition. Its name originates from the Slavic Keif, meaning “well-being”, owing to the beneficial effects associated with its consumption (Ebner et al., 2015; Rosa et al., 2017). Today, there is an increase demand in kefir consumption due to its beneficial effects and comfortable feeling for human body.

According to Wang et al. (2012) and Leite et al. (2013), kefir grains or starter culture are incubated with heat-treated milk under aerobic conditions to produce it. Kefir grain is like small clusters of off-white gelatinous nodules. The grains are gelatinous, irregular in size, insoluble in water and common solvents and varying from 0.3~3.5 cm in diameter. It is a complex of polysaccharides, proteins, symbiotic lactic acid bacteria (e.g., *Lactobacillus, Lactococcus, Leuconostoc* and *Streptococcus*) and yeast (e.g., *Saccharomyces, Candida, Kluyveromyces, Debaryomyces* and *Torulaspora*) (Figure 1.1).

Figure 1.1 Macroscopic structure of kefir grain (Mei et al., 2016).

The combination of these microorganism results in symbiotic effect which makes it a probiotic beverage and differentiates it from other fermented dairy product. It is produced using different types of milk by traditional process (Figure 1.2) or industrial process (Figure 1.3). It is easily digested and is the best-known source of potential probiotic (Irigoyen et al., 2005; Satir and Seydim, 2016).

Kefir has a smooth creamy consistency, somewhat acidic taste mostly due to the presence of lactic acid, mild effervescence due to carbon dioxide, and a low concentration of ethanol produced by yeast cells present in the grains. In addition, a variety of aromatic substances, including acetaldehyde, acetoin, and diacetyl, contribute to its distinctive flavor (Farnworth and Mainville, 2003).

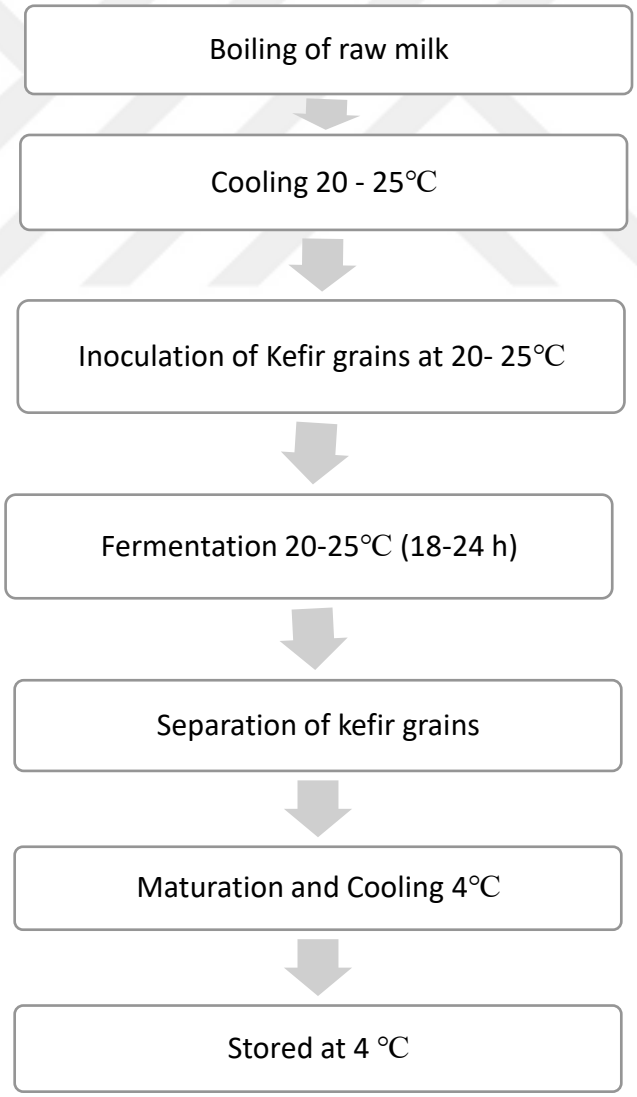

Figure 1.2 Traditional process of kefir (Shen et al., 2018).

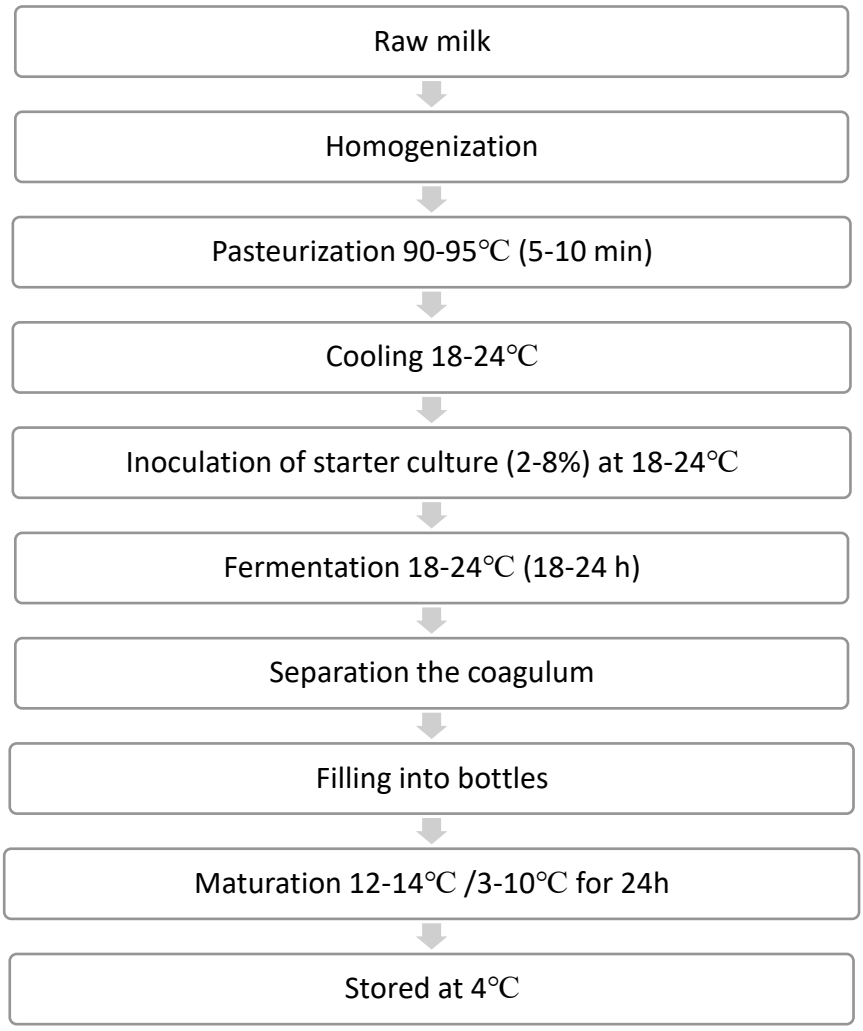

Figure 1.3 Industrial process of kefir (Shen et al., 2018).

1.2 Composition of kefir

The constituent of kefir is changeable (Zubillaga et al., 2001). This relies on different factors such as:

i. Source of milk.
ii. The fat and protein content.
iii. The fermentation of kefir.
iv. The combination of starter cultures or kefir grains.

The major products formed during fermentation process are lactic acid, CO_2 and alcohol. Zourari and Anifantakis, (1988) demonstrated that diacetyl and acetaldehyde, aromatic compounds, are also present in kefir. Many researchers found that in addition to beneficial bacteria and yeast, kefir contains vitamins (A, B_1, B_2,

B_6, B_{12}, C, D, E, niacin and carotene) and minerals (calcium, phosphor, magnesium, potassium, sodium and chloride) (Table 1.1). Also, kefir contains essential amino acids (Tryptophan, Phenylalanine, Tyrosine, Leucine, Isoleucine, Threonine, Methionine, Cystine, Lysine and Valine) which aids the human body in hemostasis, regulations and healing mechanism (Renner and Renz, 1986; Halle et al., 1994) (Table 1.2). Kefir is an excellent source of biotin and B vitamins which help the body's assimilation of other B vitamins, like B_1, B_2, B_6 and B_{12} (Halle et al., 1994). Kefir has the complete proteins (i.e., contains an adequate proportion of each of the nine essential amino acids) that are partially digested and as a result of the body easily utilizes them. Kefir is a good food for lactose malabsorption people which are unable to digest considerable amounts of predominant sugar of milk which is the lactose sugar (Otes and Cagindi, 2003). According to Odet (1995), the pH value of kefir is 4.2 to 4.6.

Table 1.1 Mineral, vitamins and energy composition of kefir

100g of kefir					
Mineral content		Vitamins (mg)		Other components	
Calcium (g)	0.12	A	0.1	Fat (%)	3.5
Phosphor (g)	0.1	B_{12}	0.5	Protein (%)	3.3
Magnesium (g)	12	Carotene	0.1	Lactose (%)	4
Potassium (g)	0.15	Niacin	0.1	Water (%)	88
Sodium (g)	0.05	B_1	0.1	Milk acid (g)	0.8
Chloride (g)	0.1	B_2	0.2	Ethyl alcohol (g)	0.9
Iron (mg)	0.05	B_6	0.1	Lactic acid (g)	1
Copper (µg)	12	C	1	Cholesterol (mg)	13
Molybdenum (µg)	5.5	D	0.1	Phosphatateds (mg)	40
Manganese (µg)	5	E	0.1	Energy (kcal)	65
Zinc (mg)	0.36				

Table 1.2 Amino acid and aromatic compound composition of kefir (Halle et al., 1994)

100g of kefir		
Essential amino acids (g)		Aromatic compounds
Tryptophan	0.05	Acetaldehyde
Phenylalanine Tyrosine	0.35	Diacetyl
Leucine	0.34	Acetoin
Isoleucine	0.21	-
Threonine	0.17	-
Methionine Cystine	0.12	-
Lysine	0.27	-
Valine	0.22	-

1.3 Microbial composition

The microorganism of kefir includes bacteria and yeast. Kefir bacteria is classified to homofermentative lactic acid bacteria (LAB) and heterofermentative lactic acid bacteria (LAB) as mentioned by Lopitz et al. (2006) and Leite et al. (2012). Homofermentative lactic acid bacteria (LAB) including:

- *Lactobacillus* species such as *L. delbrueckii* subsp. *bulgaricus*, *L. helveticus*, *L. kefiranofaciens* subsp. *kefiranofaciens*, *L. kefiranofaciens* subsp. *kefirgranum*, *L. acidophilus*.
- *Lactococcus* spp. such as *L. lactis* subsp. *lactis* and *L. lacti*s subsp. *cremoris.*
- *Streptococcus thermophilus* have been identified in kefir grains and in the fermented beverage.

Heterofermentative lactic acid bacteria (LAB), including:

- *L. kefiri*, *L.parakefiri*, *L. fermentum* and *L. brevis*,
- Citrate-positive strains of *L. lactis* (*L. lactis* subsp. *lactis biovar diacetylactis*), *Leuconostoc mesenteroides* subsp. *cremoris*.
- *Leuconostoc mesenteroides* subsp. *mesenteroides*.

The use of citrate by citrate-positive strains results in the production of key compounds that contribute to typical kefir flavor (Rattray and Connell, 2011). Acetic

acid bacteria types have been found in all kefir products isolated and in kefir culture. These species have an important role in both the bacterial population and the overall properties of kefir.

- Kefir Yeast: Although they contribute in production of metabolite that give kefir the favorite features and suitable sensory possessions. Kefir yeast is less studied than that of kefir bacteria. They are two types, lactose fermenters and non- lactose fermenters (Simova et al., 2002; Diosma et al., 2014).

The main yeast in kefir grains capable to fermenting lactose are:

- *Kluyveromyces marxianus.*
- *Kluyveromyces lactis.*
- *Debaryomyces.*

While the non-lactose fermenters that also found in kefir and kefir grains include:

- *Saccharomyces cerevisiae.*
- *Torulaspora delbrueckii.*
- *Saccharomyces turicensis.*
- *Issatchenkia orientalis.*
- *Kazachstania unispora.*
- *Kazachstania exigua.*
- *Saccharomyces martiniae.*
- *Debaryomyces occidentalis.*

1.4 Probiotic effect of kefir

Probiotic defined as a live microbial food supplement that beneficially affects the host animal by improving the microbial balance and they are used in fermented dairy products (Gorbach, 1996). Based on the definition of Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO), probiotics are “live microorganisms, conferring health benefit to the host when administered in adequate amounts (10^7 CFU/g)”. The term ‘probiotic’ dates back to 1965, when it referred to microorganisms that beneficially affects the host and work

on restore the balance of bacterial communities in the gut (Lilley and Stillwell, 1965).

As a kefir is the best choice for individual who prefer healthy diet, which consumed a lot nowadays, is considered a powerful supplement, which is rich in probiotics, it is made by the fermentation of any type of raw milk with starter culture or kefir grains after pasteurization process (Jalali et al., 2016; Baschali et al., 2017; Rosa et al., 2017).

Kefir grains are made up of many microflora that is gathering on matrix polysaccharide, protein, and microorganism as lactic acid bacteria and yeast (Gemechu, 2015). Bacteria and yeast work together to contribute in a probiotic effect of kefir, and protect the body from effect of other pathogenic microorganism by reduce lactose to lactic acid, which contribute in increasing acidity of milk and produce more ethanol and carbon dioxide by action of yeast as mentioned by Gemechu (2015) and Jalali et al. (2016).

Some studies according to experiments established that probiotics bacteria are affected by many different physicochemical stresses such as pH, acidity, temperature and preservatives (Otles and Cagindi, 2003; Terpou et al., 2017). Kefir contains the living organisms which are strong strains of probiotics that is consist of different type of bacteria and yeast which help to overtake pathogenic organisms, repopulate the digestive tract and help in digestion properly. It's a magic probiotic source.

1.5 Health effect of kefir

Many researchers have investigated the benefits of consuming kefir and it has a wide spectrum of important health benefits, including physiological, prophylactic and therapeutic properties (Figure 1.4). These effects are results of a wide variety of bioactive compounds produced during the fermentation process and the highly diverse microbiota, which act either independently or synergistically to influence these health benefits (Leite et al., 2013). But there is a little study direct on human body so in future we need more to investigate other beneficial effect in vivo.

Health benefit of kefir on human studies demonstrated kefir decreased the fasting blood glucose and hemoglobin A1c (HbA1C) levels and can be useful choice as a

complementary or adjuvant therapy for the prevention of diabetes (Ostadrahimi et al., 2015). Kefir is rich in protein which helps you feel full for long periods of time. So, the drink of kefir moderately led to a similar weight loss compared with low-fat milk (Fathi et al., 2016). Kefir has positive effects on the constipation symptoms because it improves bowel satisfaction scores and accelerates colonic transit (Turan et al., 2014).

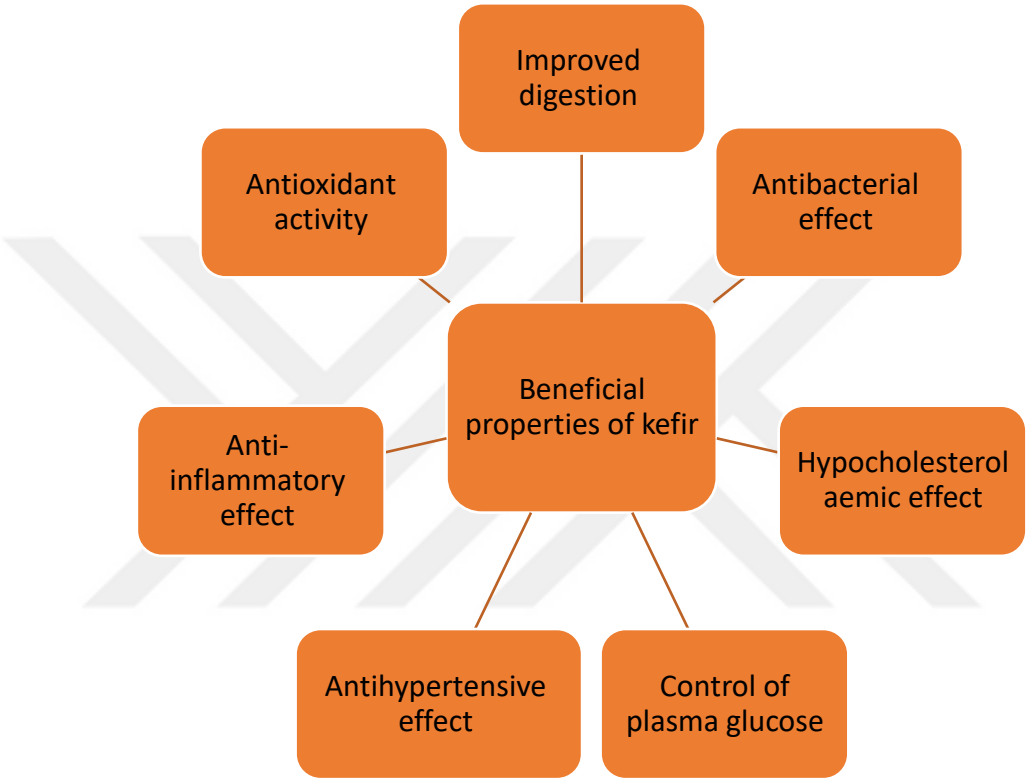

Figure 1.4 Beneficial properties of kefir.

The kefir could inhibit salivary mutans *Streptococci* as well as the sodium fluoride rinse. So, it may be used in caries control strategies adjunctively (Ghasempour et al., 2014). Kefir was able to control the inflammatory response as it contain anti-inflammatory compounds present in symbiotic cultures of kefir (Adiloglu et al., 2013). It is increasing the efficacy and tolerability of triple therapy in eradicating *Helicobacter pylori* (Bekar et al., 2011). Also, it is found that kefir do not prevent antibiotic-associated diarrhea (Merenstein et al., 2009).

Benefits of kefir on animal model: there are many studies in vitro demonstrated the benefits of kefir. Seo et al. (2018) evaluated those extracellular vesicles (EV)

produced from kefir can ease the tumor necrosis factor (TNF) induced inflammation in intestinal cells by inhibiting inflammatory cytokine production. This explains its effectiveness in preventing inflammation. In addition, using the inflammatory bowel disease (IBD) animal experimental model showed that the mixture of *L. kefir*, *L. kefiranofaciens*, and *L. kefirgranum* EV was effective in preventing metrorrhagia and diarrhea, and reducing myeloperoxidase (MPO) activity.

Amorim et al. (2019) investigated the bovine milk fermented by the probiotic culture has role in hypertensive effects, and is capable of inhibiting angiotensin-converting enzyme (ACE) activity in vivo. Andreia et al. (2015) estimated that kefir treatment for 60 days was able to improve the endothelial function in spontaneously hypertensive rats (SHR) by moderately restoring the reactive oxygen species (ROS) / nitric oxide (NO), (ROS/NO) imbalance and the endothelial architecture due to endothelial progenitor cells induction.

Kefir has a protective effect against irradiation induced hepatic damage due to its antioxidant and anti-inflammatory activities. These effects are mediated by enhanced antioxidant enzyme activities such as, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) and reduced lipid peroxide contents and inflammatory biomarkers, leading to hepatoprotection against the deleterious effects of γ-irradiation (Ali et al., 2020).

Oryan et al. (2018) and Yildiz et al. (2019) demonstrated that kefir has therapeutic choice in wound healing without causing cytotoxicity and unusual inflammatory response. By increasing rate of healing and improving the remodeling stage by reducing content of interleukin-1β (IL-1β) which is a potent pro-inflammatory cytokine and basic fibroblast growth factor (b FGF) which is potent angiogenic factor. Also, increasing the transforming growth factor-β1 (TGF-β1) and hydroxyproline contents. Kefir in this regard enhanced re-epithelialization and decreased scar tissue formation.

As Kim et al. (2015) founds in their study that consumption of kefir suppressed proliferation of opportunistic pathogens of *Enterobacteriaceae*; which produce infections following a perturbation to their host, and increased numbers of probiotic *Lactobacillus* and *Lactococcus* in the total intestinal bacteria. Jalali et al. (2016)

suggested that kefir may have potential to be an effective for treatment of erythroleukemia, as it induced aggressive disease course as apoptosis and necrosis in KG-1 cell line.

According to Chen et al. (2014) kefir has role in postmenopausal osteoporosis, and that may due to increase in calcium absorption through the transient receptor potential vanilloid subfamily member 6 (TRPV6) calcium channel. Therefore, kefir may have the potential to prevent or treat osteoporosis in humans resulting from estrogen deficiency which leads to more bone resorption than formation. Vinderola et al. (2006) identified that the different components of kefir are capable of stimulating immune cells of the innate (natural) immune system such as natural killer (NK) cells that attack foreign cells in the body. In this way, kefir may be able to down-regulate the T helper cell type 2 (Th2) immune phenotype or to promote cell mediated immune responses against different types of tumors and also against intracellular pathogenic infections.

Cutinia et al. (2019) evaluated in his study the kefir improved cardiac hemodynamic parameters in spontaneously hypertensive rats (SHR)-treated animals completely. The data show that kefir treatment for a long term reduced blood pressure by mechanisms including reduction of cardiac hypertrophy, improvement of calcium-handling proteins and cardiac contractility, and reduction in the central nervous system (CNS) regulation of its sympathetic activity.

Kefir has main role in the digestive system; in the stomach, it has a gastroprotective effect on ulcers (Cogulu et al., 2010; Fahmy and Ismail, 2015). Also, it contributes to the eradication of *Helicobacter pylori* when used in therapy and increases immunity in the gut as it increases enzyme activity and the absorption of nutrients and it play role in protects against *Giardia intestinalis* infection caused by a tiny parasite called *Giardia lamblia* (Thoreux and Schmucker, 2001; Urdaneta et al., 2007; Bekar et al., 2011; Franco et al., 2013). So, these findings of beneficial effect of kefir, makes it a magic probiotic beverage.

1.6 Flax

Flax is the seed from the flax plant, an annual flowering herb, which is a member of the *Linaceae* family. It thrives in deep moist soils rich in sand, silt, and clay. It is

native to the regions of the world with temperate climate extending from the Eastern Mediterranean, through Western Asia and the Middle East, to India. The Latin name of flaxseed, *Linum usitatissimum L.* means “very useful”, and it has two basic varieties: brown and yellow or golden (also known as golden linseeds) (Figure 1.5) (Duan et al., 2003).

The whole flaxseed is flat and oval with pointed tips and contains a seed coat or true hull (also called testa), a thin endosperm, two embryos and an embryo axis (Morris, 2007). Every part of the flax or linseed plant is utilized commercially, either directly or after processing. The shell yields good quality fiber having high mechanical properties and low density instead the seed provides oil rich in omega-3, lignans and digestible proteins; it is also use to manufacture paints, linoleum, oilcloths, printing inks, varnishes, soaps, rope, paper and numerous other products (Pengilly, 2003).

Figure 1.5 (a) Brown flaxseed, (b) Yellow od Golden flaxseed.

1.7 Composition of flaxseed

Flaxseed is considered rich in nutrients according to findings by many researchers. El-Beltagi et al. (2007) mentioned in his study that flaxseed contains 20% proteins, 7.7% moisture, 3.4% ash and 30-40% oil, with alpha-linolenic acid (ALA) making a precursor of omega-3 fatty acids. It is polyunsaturated fatty acid that makes flaxseed the leading source of plant-derived omega-3 and have role in lowering bad cholesterol. Also, contain flavonoids, minerals, vitamins, and carbohydrate that contribute to many potential health benefits (Tables 1.3-1.5). Oomah and Mazza

(1993) and Morris (2007) reported that flaxseed contains 35–45% of fiber and about two-third is insoluble such as cellulose, hemicellulose and lignin and one third is soluble fiber which is mucilage. Flaxseed contains large number of phenolic compounds as ferulic acid, chlorogenic acid and gallic acid (Table 1.4). Mazza and Biliaderis (1989) evaluated the soluble fiber of flaxseed is mucilage that is located in the outer layers of seed coat. It makes up about 7–10% of seed weight. Fodje et al. (2009) demonstrated that high amounts of acetate and propionate which is short chain fatty acids (SCFAs) resulted when flaxseed was fermented in vitro. Daun et al. (2003) found the chemical and nutritional composition of flaxseed can vary with:

i. Heredity and variations.
ii. Environment of cultivating.
iii. Seeds processing and treatments.

However, the chronic consumption of flaxseed is not useful and reflect the adverse actions of lignans in both male and pregnant female, due to change of estrogen metabolism and cause to produce fewer active forms of it (Carraro et al., 2012). As well as the existence of Adverse Health Compounds such as other phytochemicals and toxic factors in the seed that contribute to adverse health effects (Carraro et al., 2012), (Table1.6).

Table 1.3 Minerals, vitamins and carbohydrates composition of flaxseed

mg/100g of flaxseed					
Minerals		Vitamins		Carbohydrates	
Calcium	236	γ-tocopherol	522	Neutral arabinoxylan	1.2
Magnesium	431	α-tocopherol	7	Acidic Rhamnogalacturonan	0.4
Phosphorus	622	δ-tocopherol	10		
Potassium	831	Ascorbic acid/ C	0.5		
Sodium	27	Thiamin/ B_1	0.5		
Zinc	4	Riboflavin/ B_2	0.2		
Copper	1	Niacin	3.2		
Iron	5	Pyridoxine/ B_6	0.6		
Manganese	3	Pantothenic acid	0.6		

Table 1.4 Phenolic compound in 100g of flaxseed

Phenolic Compositions	
Ferulic acid glucoside	10.9 mg/g
Chlorogenic acid	7.5 mg/g
Gallic acid	2.8 mg/g
p-Coumaric acid	9.5 mg/g
SDG	1.65 mg/g
Laricinesol	0.017 mg/g
Pinoresinol	0.008 mg/g
Total Flavonoids	(0.35-0.7) mg/g

Table 1.5 Fatty acid, amino acid and dietary fibers Composition of flaxseed

g/100g of flaxseed					
Fatty acid		Amino acid		Dietary fibers	
α-linolenic acid	22.8	Glutamic acid	19.6	Soluble Fibers	4.3-8.6
Linoleic acid	5.9	Aspartic acid	9.3	Insoluble Fibers	12.8-17.1
Oleic acid	7.3	Arginine	9.2		
Stearic acid	1.3	Glycine	5.8		
Palmitic acid	2.1	Cysteine	1.1		
		Histidine	2.2		
		Isoleucine	4		
		Leucine	5.8		
		Lysine	4		
		Methionine	1.5		
		Proline	3.5		
		Serine	4.5		
		Threonine	3.6		
		Tryptophan	1.8		
		Tyrosine	2.3		
		Valine	4.6		

Table 1.6 Adverse health compounds

Adverse Healthy Components	
Cadmium	0.52 μg/kg of flaxseed
Protease inhibitors	13.3 mg/ g crude protein
Myo-inositol phosphate phytic acid	
Cyanogenic glycosides compounds:	264-354 mg/100g of flaxseed
Limamarin	10-11.8
Linustatin	136-162
Neolinustatin	105-183

1.8 Health benefit of flaxseed

Flaxseed is the new wonder food that have many potential health benefits as reported by many researchers. Dugani et al. (2014) mentioned that oil and mucilage obtained from flaxseed had anti-ulcer activity in a rat model of ethanol-induced gastric ulcer. Dupasquier et al. (2007) showed that flaxseed has anti-atherogenic effect in animal model which confer the human atherosclerotic condition. Oomah (2001) investigated that flaxseed had antioxidant and antihypertensive properties and this achieved by mixture of high levels of branched-chain amino acids (BCAAs) and low levels of Aromatic Amino Acids (AAAs).

Flaxseed contains viscous dietary fiber which can easily excreted fat and dropped low-density lipoprotein level in the body without affecting on appetite (Kristensen et al., 2012). As Bernacchia et al. (2014) mentioned that flax has low in carbohydrates. For this reason, flax contributes little to total carbohydrate intake; it's recommended for people with specific diseases. Winter (2013) investigated in his study the vitamin E might also block the formation of carcinogenic nitrosamines formed in the stomach from nitrites in foods and protect against cancer by enhancing immune function also helps in lower the risk of heart disease, some types of Alzheimer disease.

According to Prased et al. (1998), the flaxseed lignan secoisolariciresinol (SECO) and its diglucoside secoisolariciresinol diglucoside (SDG) are found to be a healthycompounds due to its many benefits that partially attributed to their antioxidant properties. Data had suggested that SDG was a potent ACE inhibitor since it had reduced arterial pressures by reducing the angiotensin I. Flaxseed have

small amount of adverse healthy compounds such as cadmium, cyanogenic glycosides, inhibitors of trypsin which are removed through thermal and mechanical processes, including cooking, autoclaving and boiling (Bernacchia et al., 2014).

1.9 Properties of flaxseed mucilage

Mucilage is jelly-like substance and responsible for storing water in plants, making them drought-resistant. FM extracted by placing seeds into water and then filtrated it (Ziolkovska, 2012). According to Mazza and Biliaderis (1989) the mucilage in aqueous solutions exhibited high solubility and good foam stability properties. The viscosity was maximum at a pH range 6-8 and it was reduced in solutions containing NaCl. The moisture sorption characteristics of the mucilage were similar to those of other plant gums and were dependent on temperature.

Liu et al. (2016) was evaluated that flaxseed mucilage is used as food gum according to its rheological properties such as; thickening, emulsification and gelling, and it has implementations that show mucilage as food additives in food manufactures. It is possessing an excellent water holding ability like guar gum (Fedeniuk and Biliaderis, 1994) forms viscous aqueous solutions (Chen et al., 2006), have ability to work as a foaming agent in solutions and also stabilizes oil-in water emulsions (Khalloufi et al., 2009). As it mentioned by Kaewmanee et al. (2014) the utilization of flaxseed mucilage relies on their special functional characteristics, like emulsifying, viscosity, foaming properties, gelation and water binding as well as on their bioactive compounds' activity in the protections and dealing with many health disorders.

1.9.1 Health benefit of mucilage

Mucilage is viscous compound and has many practical uses in the modern world include treating burns, wounds, ulcers, irritation, diarrhea, and constipation and many beneficial effects. Singer et al. (2011) demonstrated the consumption of FM has been found to be effective in many health disorders such as diabetes and cardiovascular disease corrections, protection against colon cancer and treatment of obesity. Cui and Ding (2013) investigated that the incorporation of soluble dietary fibers into beverages, dairy products, and processed foods are easier in compared to insoluble dietary fibers, and its role as thickeners, emulsifiers, stabilizers, and fat replacers. According to study conducted by Rebole et al. (2002) the implying of flaxseed

mucilage into diet of broiler chicks cause increasing in intestinal viscosity and as a result decreased fecal digestibility of fatty acid and fat, but it had no effect on protein digestion. As it contains soluble fiber, so it increases the lubrication of bowel contents and retards stomach emptying process and absorption of food.

As mentioned by Jenkins et al. (1987) and Cunnane et al. (1994) soluble gum of the flaxseed powerful in the treatment of cardiovascular diseases by display low blood cholesterol effect. Kristensen et al. (2012) demonstrated that the flaxseed fiber is effective in fat excretion and energy balance. Studies were conducted by Du et al. (2010) showed the ingestion of dietary fibers are highly effective in the prevention of obesity in both women and men. Gutierrez et al. (2010) demonstrated that flaxseed mucilage as food additives have antitumor and antioxidant properties; that work by capturing free radicals and preventing cancers due to the oxidation of macromolecules such as proteins, lipid or DNA**.** According to Gibson et al. (2004) non-digestible polysaccharides such as galacto-oligosaccharides, fructo oligo-saccharides and cyclodextrins are known to be prebiotic substances which selectively stimulate the growth and/or activity of the gastrointestinal micro-flora.

1.10 Gum

The term “gum” is defined as a group of naturally occurring polysaccharides owing to their aptitude to form either ‘gel’ or the ‘viscous solution’. Natural gums including acacia, ghatti, karaya, locust bean, albizia, khaya, guar, tragacanth and xanthan, are obtained as exudates or extractives from the bark of stems, branches and roots of various plants. Plant family’s notable for the production of gums is *Anacardiaceae, Combritaceae, Meliaceae, Rosaceae* and *Rutaceae.* Various reasons have been advanced for the production of gums by plants, including: as products of normal plant metabolism; as a protective mechanism against a pathological condition afflicting the plant; and as a consequence of infection of the plant by microorganisms (Smith and Montogomery 1959). Natural gums used for various purposes are prominent due to its properties such as:

i. Considers as a biocompatible plant.
ii. Has the ability to make chemical modifications.
iii. Economic.

iv. Safe and non-toxic.

v. Have a degradation ability in a biological system.

Gum arabic is one of special gums that when diffuse in water able to make a transparent colloidal solution.

Gum Arabic

Gum arabic (GA, E-Number 414) is an edible, dried, gummy exudate from the stems and branches of *Acacia senegal* and *A. seyal* that is rich in non-viscous soluble fiber (Williams and Phillips, 2000) (Figure 1.6). It is defined by the FAO/WHO Joint Expert Committee for Food Additives (JECFA) as 'a dried exudation obtained from the stems of *Acacia senegal (Leguminosae)* Willdenow or closely related species of *Acacia (family Leguminosae)*' (FAO, 1999). It is a highly branched, high-molecular-weight, water-soluble polysaccharide. The gum has multiple uses in a food technology as thickeners, stabilizer, gelling agents, syneresis control, emulsifiers or suspension stability and also has role as prebiotic source (Lucy, 2002; Nikoofar et al., 2013). It is consisting of arabinose and galactose in a 1:1 ratio, complex polysaccharide obtained as a mixed calcium, magnesium, and potassium salt, that 1,3-linked b-D-galactopyranosyl units form the backbone (Champaign, 1999) (Table 1.7).

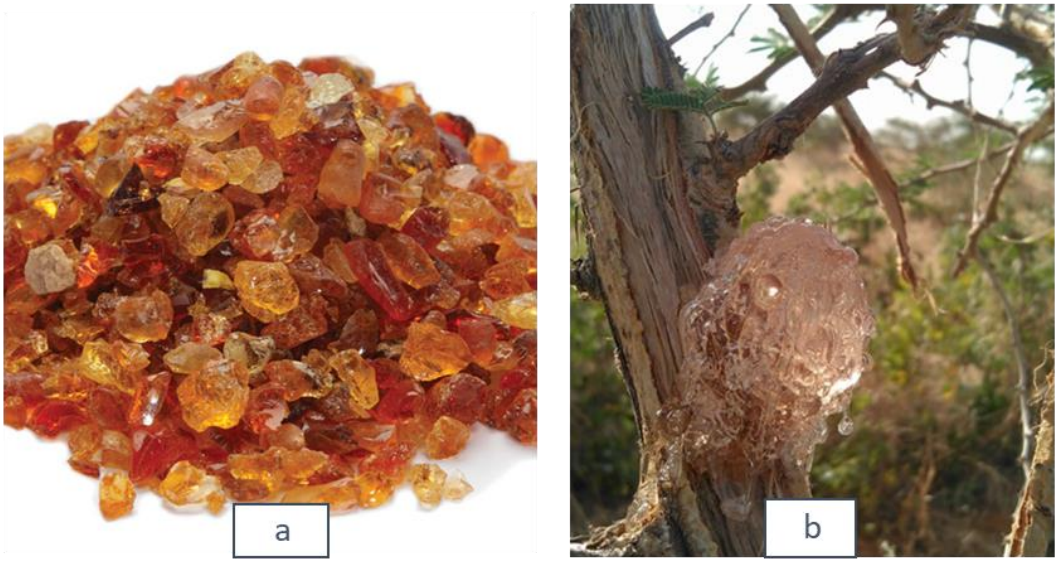

Figure 1.6 (a)Tree with gum arabic exudates; (b) granules of gum arabic.

Table 1.7 Physico-Chemical composition of gum arabic taken from *Acacia senegal*

Parameter	***Acacia senegal***
Rhamnose (%)	14
Arabinose (%)	29
Galactose (%)	36
Glucuronic acid (%)	14.5
Nitrogen (%)	0.365
Protein (%)	2.41
Volatile matter (%)	51- 65
Ash (%)	3.6
pH	4.4
Crude fiber (%)	0
Lactose (%)	14
Ca (g/100g)	0.7
Mg (g/100g)	0.201
K (g/100g)	0.95
Na (g/100g)	0.014
Fe (g/100g)	0.001
P (g/100g)	0.6

Many researchers have investigated that the variety of the chemical composition of gum arabic is due to:

i. The source of gum arabic.
ii. The age of the trees.
iii. The climatic change.
iv. The environment of soil (Al-Assaf et al., 2005).

1.10.1 Benefits and uses of gum arabic

Gum arabic has a lot of industrial uses such as a stabilizer, thickening agent and emulsifier, especially in the food industry (e.g., in soft drinks syrup, gummy candies and marshmallows), it has also used in the pharmaceutical industries, textile, pottery, lithography, and cosmetics industries (Verbeken et al., 2003). As Karlton and Ibrahim (2013) reported that gum arabic is considering slightly acidic gum and as neutral origin. So, it has multiple uses in different field as binder and thickener in the making of confectionaries, soft drinks, food sweeteners, and drugs. This is due to be

as hydrocolloid and uses as a gelling agent and as food emulsion due to its excellent emulsifying properties.

As in Buffo et al. (2001) investigation, gum arabic when added to oil in water system it can adsorb and lower the interfacial tension by producing small droplets during homogenization. Many researchers studied the effect of gum arabic and other types on the growth and activity of probiotic bacteria as it has prebiotic substances (Sabooni et al., 2018; Yilmaz et al., 2017; Sadek et al., 2004; Ghasempour et al., 2012). According to the results, the incorporation of 0.5% of GA had significantly increased the count of *Bifidobacterium bifidum*.

Some researcher has been suggested to use gum arabic to envelope probiotic bacteria such as (*Bifidobacterial* species and *Lactobacillus paracasei*) to prevent their disruptions from chemical effect during drying and to increase the growth and firmness of probiotics from actions of acid in gastrointestinal tract (Lian et al., 2002, 2003; Desmond et al., 2002). It has also been found that the intake of gum arabic by humans, in approximately of 10 grams per day elevate the *Bifidobacterium* and *Bacteroides* counts in stools. Also, other similar study has proposed the intake of 10-15g of GA per day by humans increase the number of probiotic bacteria, especially *B. lactis* and *L. acidophilus* (Wyatt et al., 1986).

Gamal el-din et al. (2003) reported gum arabic has been used internally for the treatment of inflammation of the intestinal mucosa, and externally to cover inflamed surfaces. Many recent studies have found that gum arabic possesses anti-oxidant, nephroprotectant and other effects (Rehman et al., 2001; Gamal el-din et al., 2003). Clinically, as it was evaluated by Suliman et al. (2000) it has been tried in patients with chronic renal failure, and it was claimed that it helps reduce urea and creatinine plasma concentrations and reduces the need for dialysis from 3 to 2 times per week. Bliss et al. (1996) proposed that the supplementation of the diet with gum arabic has been shown to increase fecal nitrogen excretion and lower serum urea nitrogen concentration in patients with chronic renal failure.

Consumer interests regarding healthy diets and wellness have led to increase the consumption of foods containing probiotics and prebiotics. Probiotics when administered in adequate amounts confer a benefit to the consumer's health.

Prebiotics are non-digestible carbohydrates that reach the colon, where they are selectively fermented stimulating the growth and/or activity of one or a limited number of beneficial bacteria (Gibson and Roberfroid, 1995). Probiotics and prebiotics, or their combinations, is a choice in new food developments due to its ability to improve gut health and as a result body comfortable.

1.11 Aim of the study

So, according to previous researches and studies one probable method to enhance the growth and firmness of probiotic bacteria are to fortify dairy products with prebiotics source. The combination of a prebiotic with probiotic result in a symbiotic effect. Mucilage and gum arabic as prebiotic when it had combined with probiotic it will improve its survival rate and provide the more beneficial effect to the host.

Therefore, the aim of this study was to evaluate the new properties and efficacy of kefir after adding gum arabic (0.2% w/v) and flaxseed mucilage in different concentration (0.03%, 0.05%, 0.1% w/v); to determine the symbiotic effect of this combination at 0, 1,st 7,th 14,th 21st and 28th days of storage on *Lactobacillus acidophilus* and *Bifidobacterium lactis* counts; and to show how flaxseed mucilage and gum arabic can affect on pH, viscosity, titratable acidity, color, protein and total solid values of kefir during storage time.

CHAPTER II

MATERIALS AND METHODS

2.1 Materials

Flaxseed (*Linum usitatissimum* L.) used in the recent study was obtained from local market in Gaziantep (Turkey). Fresh cow milk was taken from local market in Gaziantep (Turkey). Kefir starter culture (SEVDANEM) used in this study manufactured under Republic of Turkey in the Department of Food Engineering Laboratories, Suleymen Demirel University (Isparta, Turkey) registered under TR-32-K-000120. Probiotic yogurt culture (*Lactobacillus delbrueckii* subsp. *bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus* obtained from DANEM SÜT VE SÜT ÜRÜNLERİ AMB.GIDA EĞT.DAN.SAN.VE, Turkey). Gum Arabic (E414) was obtained from GEMİCİ GIDA TİCARET LİMİTED ŞİRKETİ, origin (*France*). *Bifidobacterium animalis* ssp. *lactis* B94 contain ($5*10^9$ CFU- 5 billion active probiotic in 60 mg, *France*). MRS agar (DeMan, Rogosa and Sharpe), (*Germany*).

2.2 Extraction of flaxseed mucilage

About 100g Flaxseed was taken and made up to 2L with distilled water in a glass beaker. The water soluble compounds were obtained by extraction on a constant magnetic stirrer (Ms300Hs, Mtops, China) for 3 h (At 55℃ and 60 rpm). Then the flaxseed mucilage was filtrated from seeds through Sieve Fine Mesh and then by muslin cloth. The resulted liquid mucilage (1600 mL) was distributed in a small quantity (about 150 mL) into small pyrex glass dishes (Borcam) and put in a forced convection oven (JSOF-100, JS Research Inc. Korea) at 55℃ overnight. The dried mucilage was collected and measured for every 100g of flaxseed as seen in (Table 2.1). Approximately 3.3g dried mucilage was obtained from each 100g flaxseed (Figure 2.1).

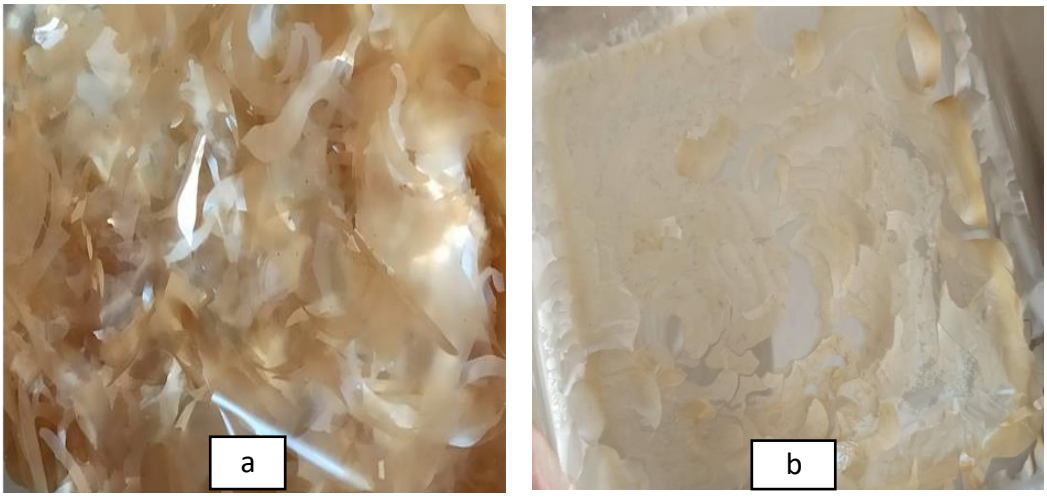

Figure 2.1 a: Collected form of mucilage, b: dried form in glass pyrex

Table 2.1 Mucilage yield extractions

Run	Flaxseed amounts(g)	Mucilage yield(g)
1	100	3.9
2	100	3.1
3	100	2.2
4	100	3.1
5	100	3.3
6	100	3.3
7	100	3.2
8	100	4.9
Average	100	3.3

2.3 Experimental design

Raw cow milk was pasteurized at 90℃ for 10 min and cooled down to 25℃, at room temperature (pH of milk was 6.22). Then kefir starter culture was inoculated 0.02% (w/v), yoghurt starter culture was 0.03% (w/v) and *Bifidobacterium lactis* was 0.06% (w/v) after activation at 30℃ for 7h. Also flaxseed mucilage was added in concentration 0.03, 0.05, 0.1% (w/v) and gum arabic at 0.2% (w/v) as following (Table 2.2):

1.control (kefir culture (KC)).

2. control (kefir culture + yoghurt culture (YC) + *Bifidobacterium lactis*).

3. gum arabic with (kefir culture + yoghurt culture + *Bifidobacterium lactis*).

4. mucilage 0.03% with (kefir culture + yoghurt culture + *Bifidobacterium lactis*).

5.mucilage 0.05% with (kefir culture + yoghurt culture + *Bifidobacterium lactis).*

6. mucilage 0.1% with (kefir culture + yoghurt culture + *Bifidobacterium lactis*).

7.mucilage 0.03% + gum arabic with (kefir culture + yoghurt culture + *Bifidobacterium lactis*).

8.mucilage 0.05% + gum arabic with (kefir culture + yoghurt culture + *Bifidobacterium lactis*).

9.mucilage 0.1% + gum arabic with (kefir culture + yoghurt culture + *Bifidobacterium lactis*).

Table 2.2 Kefir product and contents

Product	100 mL milk
1	0.02g KC
2	0.02g KC+ 0.03g YC+ 0.06g *B. lactis*
3	0.2g GA+ 0.02g KC+ 0.03g YC+ 0.06g *B. lactis*
4	0.03g FM+ 0.02g KC+ 0.03g YC+ 0.06g *B. lactis*
5	0.05g FM+ 0.02g KC+ 0.03g YC+ 0.06g *B. lactis*
6	0.1g FM+ 0.02g KC+ 0.03g YC+ 0.06g *B. lactis*
7	0.03g FM+ 0.2g GA+ 0.02g KC+ 0.03g YC+ 0.06g *B. lactis*
8	0.05g FM+ 0.2g GA+ 0.02g KC+ 0.03g YC+ 0.06g *B. lactis*
9	0.1g FM+ 0.2g GA+ 0.02g KC+ 0.03g YC+ 0.06g *B. lactis*

Milk (250 mL each) was divided into 9 parts, each part was stirred with magnetic stirrer (Ms300Hs, Mtops, China) for 20 min to dissolve mucilage and gum. All samples were put in an incubator (incubator ES 500) at 25℃ for 24h. Samples were stored at refrigerator at 4℃ for 28 days. All parameters were measured at all days of storage (0, 1,st 7,th 14,th 21st and 28th) (Figure 2.2).

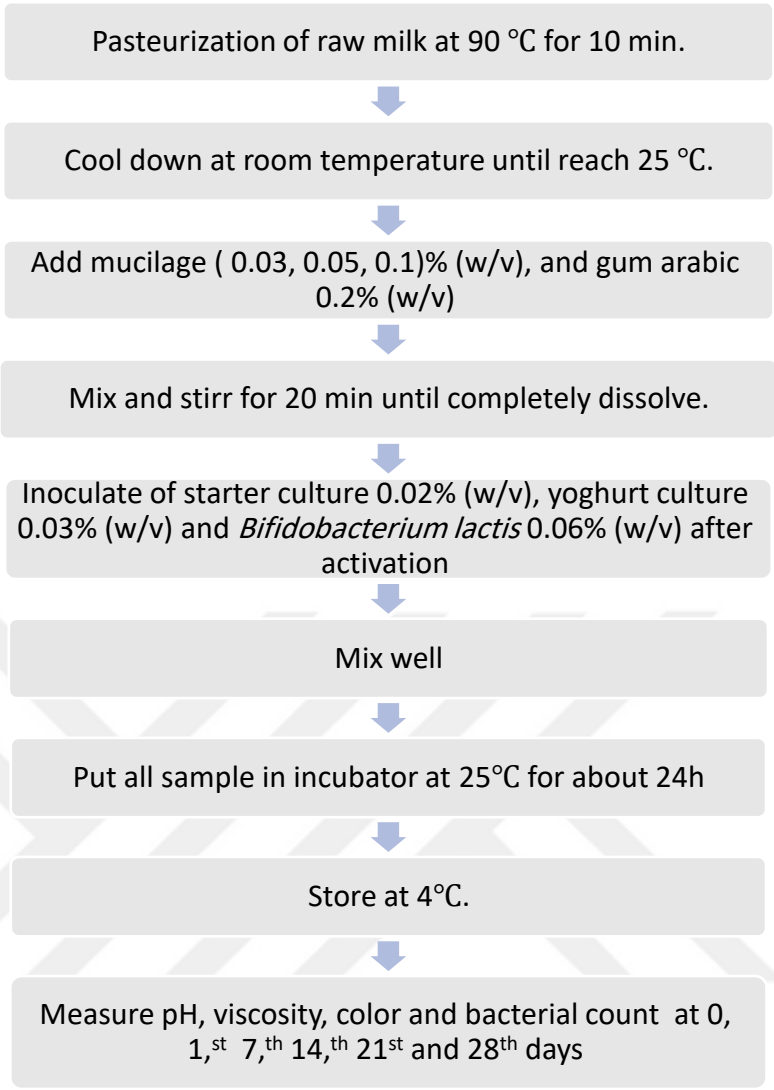

Figure 2.2 Production flow chart of kefir.

2.4 pH and titratable acidity analysis

The pH values were recorded by using a pH meter (pH/mV/Cond./TDS/Temp. meter 86505), at 0, 1,st 7,th 14,th 21st and 28th days, at temperature 25℃.

Titratable acidity was measured by adding 3 drops of phenolphthalein to kefir and titrate it with 0.1 NAOH. The % acidity was measured and calculated as lactic acid according to following equation (Çelekli et al., 2019):

$$\%\ lactic\ acid = \frac{\mathrm{v} * (0.009) * 100}{\mathrm{m}}$$

Where v is the volume of titrant and m is the weight of the sample.

2.5 Viscosity

The viscosity of kefir was measured by a viscometer (Brookfield, DV3T™ viscometer, USA). Viscosity measurement was done at 30 rpm with V-72(72) spindle during 25 sec and 250 mL sample was used for each run (Çelekli et al., 2019).

2.6 Color

The colors (L*, a*and b*) of all kefir samples were measured by using Hunter lab ColorFlex (A60-1010-615 Model colorimeter, Hunter lab, Reston, Virginia, USA) at 0, 1,st 7,th 14,th 21st and 28th days of storage (Çelekli et al., 2019).

2.7 Total solid

Approximately 3 g of kefir was placed in a pre weighed, pre dried small glass dish and transferred to a hot air oven at 105℃ up to constant weight reached. Samples were cooled in a desiccator before final weights recorded (Turkish Standard Method TS1330).

The total solid content of the milk as follows:

Total solid = 100- moisture%

$$\text{Moisture\%} = \frac{ws(w2-w1)}{ws} * 100$$

Where; w1: weight of empty dish

w2: weight of dish after drying

ws: weight of sample

2.8 Microbiological analysis

2.8.1 Preparing MRS with maltose

MRS agar was prepared for counting of *Lactobacillus acidophilus* by adding 68.2g up to 1 liter of sterilized water at room temperature and 1% of maltose then stirred and dissolved by laboratory heating mantle (Fibroman-C series, J.P.SELECTA,s.a. Spain) then autoclaved at 121℃ for 15 min (Dave and shah 1997).

2.8.2 Preparing MRS with raffinose

MRS agar was prepared for counting of *Bifidobacterium lactis* by adding 68.2g up to 1 liter of sterilized water at room temperature and 1% of raffinose then stirred and dissolved by laboratory heating mantle (Fibroman-C series, J.P.SELECTA,s.a. Spain) then autoclaved at 121℃ for 15 min (Dave and shah 1997).

2.8.3 Serial dilution

Sterilized peptone water (0.1%) was used as the diluents in this microbiological analysis. About 25 g of each sample was aseptically taken, followed by dilution in 225 mL sterilised peptone water (0.1%), mix and stir on a continuous magnetic stirrer (Ms300Hs, Mtops, China) for 10 min and serial dilution up to 10^{-6}. Next, 0.2 mL of each dilution was transferred to a sterilized petri dish and suitable media was used to determine the viable cell counts using the spread plate method. All tests were done in duplicates.

2.8.4 Microbial analysis

The enumerations of specific lactic acid bacteria were specified as log CFU/mL. The counts of bacteria in kefir fortifying with 0.2% (w/v) gum arabic, 0.03, 0.05, 0.1% (w/v) of flaxseed mucilage, 0.03% (w/v) yoghurt starter culture and 0.06% (w/v) *Bifidobacterium lactis* were recorded at 0, 1,st 7,th 14,th 21,st 28th days by use of MRS agar. All kefir samples were diluted tenfold by using of peptone water 0.2% v/v, after that spread on MRS agar then were incubated at CO_2 incubator at 30℃ and 5% for 72 hours (3 days). Lastly, the numbers of colonies were calculated. Enumerations of bacteria were done in duplicate on petri dishes. The counts of *Lactobacillus acidophilus* and *Bifidobacterium lactis* bacteria were recorded_according to the following method Dave and shah, (1997). The counts of *Lactobacillus acidophilus* (Figure 2.3) was done by using spread plate technique on MRS agar with adding 1% of maltose. After that, all petries were put in anaerobic incubator at 30 ˚C for 72 hours after that the colonies were counted.

Bifidobacterium lactis (Figure 2.3) enumeration was done by spreading on MRS agar with adding 1% of raffinose after that petries were put in anaerobic incubator at 30℃ for 72 hours after that the colonies were counted.

Figure 2.3 Colonies of *Bifidobacterium lactis* (a, and a1) and *Lactobacillus acidophilus* (b, and b1)

2.9 Protein analysis

To 10 mL of milk add 0.5 mL of 0.5% phenolphthalein indicator and 0.4 mL of neutral saturated potassium oxalate in conical flask then neutralize with 0.1 M NaOH in the burette to the standard pink color. After that, adding exactly 2 mL of formalin (37% formaldehyde). Then titrate the new acidity produced with 0.1 M NaOH to the same pink color (a). Titrate separately 2 mL of formalin and 10 mL of water with the same alkali (b) as blank. The protein content of the milk as follows (Turkish Standard Method TS1330):

$$\% \text{ Protein} = 1.7 * (a-b)$$

2.10 Sensory analysis

The 7 trained assessors (graduate students in Gaziantep University Food Engineering Department) were employed to estimate the kefir samples according to the following Characteristics: overall appearance (texture), color, taste/flavour, smell/odour, thickness. According to score value listed as: liked-3, normal-2, dislike-1 according to the standard TS EN ISO 8589.

2.11 Statistical analysis

The differences between samples were assessing by using ANOVA to compare probiotic bacteria, pH, titratable acidity, viscosity and colors at different conditions. Statistical analyses were done by using the SPSS statistical package version 26.0 (IBM Corporation, USA). Duncan multiple range test was performed to compare more than two values. Statistical analysis was done to show how samples of kefir fortified with flaxseed mucilage and gum arabic and the storage period affect the probiotic and all parameters.

CHAPTER III

RESULTS AND DISCUSSION

3.1 Viability of *Lactobacillus acidophilus*

The variations in the viability of *L. acidophilus* in all kefir samples had recorded at all days of storage. Their results are shown in Tables 3.1 and 3.2. Statistical analysis indicated that addition of flaxseed mucilage, gum arabic and probiotic significantly ($P<0.05$) increased the growth of *L. acidophilus*.

Enriched kefir with flaxseed mucilage had a significant count ($P<0.05$) on the growth of *L. acidophilus*, at 0, 1,st 7,th 14th and 21st days of storage in comparison with control (Table 3.1). Use of 0.03, 0.05 and 0.1% of flaxseed mucilage caused to significant ($P<0.05$) increase in counts of *L. acidophilus* compared to control samples. Increasing count of *L. acidophilus* initiated from 0 day up to 21st days compared to control (Table 3.1 and 3.2). Moreover, the viable counts of *L. acidophilus* at 28th days of storage slightly increase in control compared to other samples. Flaxseed mucilage is considered as prebiotic and have a role in improving the probiotic growth as mentioned by HadiNezhad et al. (2013) non-digestible polysaccharides are known to have a prebiotic effect and additionally, flaxseed soluble fiber, often called mucilage, acts as an effective prebiotic source and have a role in improved lactic acid bacteria survival and expansion in kefir system.

Tuorila and Martello (2002) reported that plant extracts consist of many bioactive compounds such as: phenolic substances, carotenoids, nutrients, minerals, vitamins and the dietary fiber which make it a favorable material to enhance bacterial growth.

The viable count of *L. acidophilus* significantly ($P<0.05$) increased as the amount increased from 0.03 to 0.05% of flaxseed mucilage; but at 0.1% of flaxseed mucilage, it slightly dropped but it still more than 0.03% of flaxseed mucilage. This deceased may be due to some substances produced by other probiotic bacteria that

Table 3.1 Effect of addition of flaxseed mucilage, gum arabic and yogurt culture and *B. lactis* at different concentration on *L. acidophilus* count (log CFU/ mL) in kefir samples

	Time (day)					
Product	0 day	1st day	7th day	14th day	21st day	28th day
1	2.00±0.01 [a]	3.17±0.00 [a]	3.18±0.00 [a]	3.92±0.04 [a]	4.95±0.06 [a]	6.16±0.01 [h]
2	2.17±0.00 [a]	3.21±0.00 [b]	3.35±0.00 [a,b]	4.52±0.07 [c]	5.26±0.05 [b]	5.82±0.01 [e]
3	2.79±0.30 [b]	3.26±0.00 [c]	4.10±1.09 [b,c]	4.66±0.15 [c]	5.49±0.00 [c]	5.56±0.01 [a]
4	3.05±0.17 [b,c]	3.43±0.01 [d]	4.64±0.00 [c]	4.67±0.01 [c]	5.62±0.01 [d]	5.59±0.00 [b]
5	3.34±0.45 [c,d]	3.61±0.01 [f,g]	4.65±0.00 [c]	5.72±0.00 [f]	5.82±0.00 [e]	6.11±0.00 [g]
6	3.48±0.01[c,d]	3.51±0.01 [e]	4.61±0.01 [c]	5.34±0.03 [d]	5.62±0.01 [d]	6.11±0.00 [g]
7	3.60±0.14[d]	3.62±0.01 [g]	4.76±0.00 [c]	5.51±0.00 [e]	5.95±0.00 [f]	6.01±0.00 [f]
8	3.54±0.00[d]	3.60±0.00 [f,g]	4.20±0.02 [b,c]	5.37±0.02 [d]	5.22±0.04 [b]	5.63±0.01 [c]
9	3.30±0.01 [c,d]	3.59±0.00 [f]	4.58±0.00 [c]	4.38±0.09 [b]	4.96±0.01 [a]	5.65±0.01 [d]

Different small letters show statistical difference among samples at each time at α=0.05 level.

found in yoghurt culture as mentioned by Gilliland and Speck (1977a, b). This study indicated that the hydrogen peroxide produced by yogurt cultures especially *L. delbrueckii* ssp. *bulgaricus* is the main agent that is responsible for the loss in counts of *L. acidophilus* when added to yogurt.

Mihoubi et. al. (2017) recorded that enrichment of yogurt with flaxseed increase number of *L. delbrueckii* ssp. *bulgaricus*. The result conducted by Mihoubi et. al. (2017) that the loss in viability of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* at the end of storage could be attributed to *L. delbrueckii ssp. bulgaricus* which produces lactic acid during refrigerated storage. This mechanism is known as post-acidification. The loss of viability and as a result countability of probiotic bacteria is a result of acid produced by post-acidification during refrigerated storage (Mishra, et. al., 2005, Madureira, et. al., 2011).

Gum arabic added into kefir samples at 0.2% had a significant ($P<0.05$) increase in number of *L. acidophilus* in comparison with control from 0 day up to 21st days of

Table 3.2 Effect of fermentation and storage time on *L. acidophilus* count (log CFU/ mL) in kefir samples

	Product								
Time	1	2	3	4	5	6	7	8	9
0 day	2.00±0.01^{A}	2.17±0.00 ^{A}	2.79±0.30 ^{A}	3.05±0.17 ^{A}	3.34±0.45 ^{A}	3.48±0.00 ^{A}	3.60±0.14 ^{A}	3.54±0.00 ^{A}	3.30±0.00 ^{A}
1st day	3.17±0.00 ^{B}	3.21±0.01 ^{B}	3.26±0.01 ^{A,B}	3.43±0.01 ^{B}	3.61±0.01 ^{A}	3.51±0.01 ^{A}	3.62±0.01 ^{A}	3.60±0.00 ^{B}	3.59±0.00 ^{B}
7th day	3.18±0.00 ^{B}	3.35±0.01 ^{C}	4.10±1.09 ^{B,C}	4.64±0.00 ^{C}	4.65±0.00 ^{B}	4.61±0.01 ^{B}	4.76±0.00 ^{B}	4.20±0.02 ^{C}	4.58±0.00 ^{D}
14th day	3.92±0.04 ^{C}	4.52±0.07 ^{D}	4.66±0.15 ^{C,D}	4.67±0.00 ^{C}	5.72±0.00 ^{C}	5.34±0.03 ^{C}	5.51±0.00 ^{C}	5.37±0.02 ^{E}	4.38±0.09 ^{C}
21st day	4.95±0.06 ^{D}	5.26±0.05 ^{E}	5.49±0.00 ^{D}	5.62±0.01 ^{D}	5.82±0.00 ^{C}	5.62±0.01 ^{D}	5.95±0.00 ^{D}	5.22±0.04 ^{D}	4.96±0.01 ^{E}
28th day	6.16±0.01 ^{E}	5.82±0.01 ^{F}	5.56±0.01 ^{D}	5.59±0.00 ^{D}	6.11±0.00 ^{C}	6.11±0.00 ^{E}	6.01±0.00 ^{D}	5.63±0.01 ^{F}	5.65±0.01 ^{F}

Different capital letters show difference among time at each product at α=0.05 level. (1: C, 2: YC+B.L, 3: 0.2GA, 4: 0.03FM, 5: 0.05FM, 6: 0.1FM, 7: 0.03FM+0.2GA, 8: 0.05FM+0.2GA, 9:0.1FM+0.2GA)

storage. Many studied have proven the effect of some gums, as source of prebiotic, on the viability and expansion of live bacteria (Sabooni et al., 2018; Yilmaz et al., 2017; Sadek et al., 2004; Ghasempour et al., 2012). These substances enhance the growth of probiotic by supplying nutrients to it and protect it from effect of high acidity in the gut (El-Abd et al, 2018). Also, other similar study has described an increase in lactic acid bacteria, and especially of *L. acidophilus*, after ingestion of (10-15g) of gum arabic (Wyatt et al., 1986). However, the count of *L. acidophilus* at 0.2% of gum arabic significantly ($P<0.05$) decreased compared to samples with flaxseed mucilage at all days of storage time (Figure 3.1).

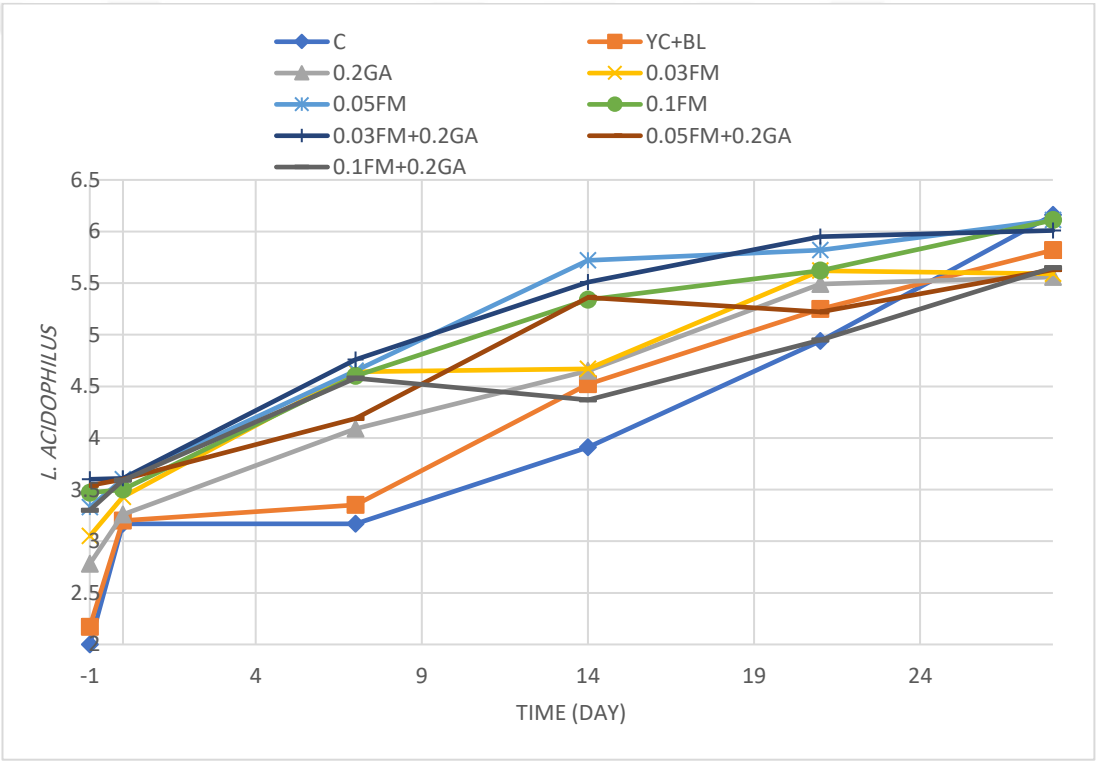

Figure 3.1 Effect of FM, GA and *B.lactis* + YC on the growth of *L. acidophilus* at different concentrations and through fermentation and storage time (FM; flaxseed mucilage, GA; gum arabic, YC; yogurt culture, BL; *B. Lactis*)

Fortified kefir samples with 0.03% yoghurt culture and 0.06% *Bifidobacterium* with 0.02% of kefir culture for all samples except the control had significantly ($P<0.05$) support the expansion of *L. acidophilus* at all days of storage except 28th days (Tables 3.1 and 3.2). Enriched milk with lactic acid bacteria caused to lipolysis of

milk fat which increase production of free fatty acid that enhance the growth of *L. acidophilus* (Coskun and Ondul, 2004; Yadav et al., 2007). However, the count of *L. acidophilus* at 0.03% yoghurt culture and 0.06% *Bifidobacterium* significantly decreased ($P<0.05$) compared to samples with flaxseed mucilage and samples with gum arabic up to 21st days of storage. In contrast, at 28th days, it slightly increased ($P<0.05$) over samples with gum arabic (Figure 3.1).

The mixing of flaxseed mucilage and gum arabic significantly ($P<0.05$) affected the count of *L. acidophilus* from 0 day and up to 21st of storage days compared to control (Tables 3.1 and 3.2). Use of 0.03, 0.05 and 0.1% flaxseed mucilage and 0.2% of gum arabic significantly increased the count of *L. acidophilus* in comparison to control, from 0 day and up to 21st days of storage (Table 3.1). Fodje et al. (2009) demonstrated that high level of short-chain fatty acids such as acetate and propionate which activate the growth of *L. acidophilus* resulted during fermentation of flaxseed in vitro. Also, FM as rich in dietary fiber can form a new source in food manufactures as prebiotic compounds (Roberfroid and Slavin, 2000; Fodje et al. 2009). As mentioned by Niamah et al. (2016) gum arabic added to yoghurt maintain viability of *L. acidophilus* and *S. thermophilus* while the survivability of *B.bifidum* decreased during storage time. Prebiotic substances such as transgalactooligosaccharides, polydextrose, galacto oligosaccharides, banana psyllium, wheat dextrin, whole grain wheat, acacia gum and whole grain corn enhanced the cells viability of probiotic bacteria in dairy products (Slavin, 2013). GA is considered as one of prebiotic source when mix with probiotic its result as symbiotic effect (Clark et al. 1993). In another study, Niamah et al. (2016) found the count of *L. acidophilus* bacteria increased as concentrations of GA increased after 21 days of storage time.

Use of 0.03, 0.05 and 0.1% flaxseed mucilage mixed with 0.2% of gum arabic shows similar effect ($P<0.05$) on the number of *L. acidophilus* from 0 day until 14th days (Table 3.1). The highest bacterial counts were observed at 0.03% of flaxseed mucilage mixed with 0.2% of gum arabic at 0, 1,st 7,th and 21st days during storage. Moreover, the viable count decreased at 0.1% and 0.05% of flaxseed mucilage with 0.2% of gum arabic compared to 0.03% of flaxseed mucilage with 0.2% of gum arabic at 14th and 21st during storage period (Figure 3.1).

3.2 Growth of *Bifidobacterium lactis*

Changes in growth of *Bifidobacterium lactis* in all samples were followed at all days of storage and their results are shown in Tables 3.3 and 3.4. Statistical analysis indicated that addition of flaxseed mucilage and gum arabic lead to significant ($P<0.05$) increase in the count of *B. lactis* (Table 3.3).

The fortified kefir with flaxseed mucilage caused to significant effect ($P<0.05$) on the viability of *Bifidobacterium* at all days of storage in comparison with control (Table 3.4). The addition of 0.03, 0.05 and 0.1% of flaxseed

Table 3.3 Effect of addition of flaxseed mucilage, gum arabic and yogurt culture and *Bifidobacterium lactis* at different concentration on *B. lactis* count (log CFU/ mL) in kefir samples

	Time (day)					
Product	0 day	1st day	7th day	14th day	21st day	28th day
1	5.60±0.01 ^{b}	6.17±0.00 ^{c}	6.43±0.01 ^{b}	6.70±0.00 ^{b}	6.50±0.00 ^{b}	6.45±0.03 ^{b}
2	5.53±0.00 ^{a}	5.65±0.00 ^{a}	5.91±0.00 ^{a}	5.99±0.00 ^{a}	5.54±0.00 ^{a}	5.54±0.00 ^{a}
3	5.82±0.00 ^{c}	5.87±0.00^{b}	5.88±0.01 ^{a}	6.05±0.07 ^{a}	5.47±0.01 ^{a}	5.43±0.01 ^{a}
4	6.20±0.01 ^{d}	6.48±0.01 ^{d}	6.58±0.00 ^{b,c}	6.71±0.00 ^{b}	6.50±0.00 ^{b}	6.59±0.02 ^{b,c}
5	6.21±0.00 ^{e}	6.58±0.02 ^{d,e}	6.70±0.01 ^{c}	6.92±0.00 ^{c}	6.51±0.01 ^{b,c}	6.61±0.05 ^{b,c}
6	6.30±0.01 ^{f}	6.62±0.01 ^{e}	7.19±0.06 ^{d}	7.24±0.08 ^{d}	6.52±0.01 ^{b,c}	6.99±0.16 ^{e}
7	6.31±0.00 ^{f}	6.76±0.01 ^{f}	6.78±0.00 ^{c}	6.91±0.02 ^{c}	6.67±0.19 ^{c}	6.89±0.08 ^{d,e}
8	6.39±0.00 ^{g}	6.80±0.02 ^{f}	7.03±0.21 ^{d}	7.04±0.12 ^{c}	6.57±0.00 ^{b,c}	6.76±0.12 ^{c,d}
9	6.39±0.00 ^{g}	6.81±0.09 ^{f}	7.07±0.17 ^{d}	7.06±0.09 ^{c}	6.64±0.00 ^{b,c}	6.94±0.08 ^{e}

Different small letters show statistical difference among samples at each time at $\alpha=0.05$ level.

Table 3.4 Effect of fermentation and storage time on *B. lactis* value in kefir samples

	Product								
Time	1	2	3	4	5	6	7	8	9
0 day	5.60±0.01 ^{A}	5.53±0.00 ^{A}	5.82±0.00 ^{B}	6.20±0.01 ^{A}	6.21±0.00 ^{A}	6.30±0.01 ^{A}	6.31±0.00 ^{A}	6.39±0.00 ^{A}	6.39±0.00 ^{A}
1st day	6.17±0.00 ^{B}	5.65±0.00 ^{C}	5.87±0.00 ^{B}	6.48±0.02 ^{B}	6.58±0.03 ^{C}	6.62±0.01 ^{B}	6.76±0.01 ^{B,C}	6.80±0.02 ^{B,C}	6.81±0.09 ^{B,C}
7th day	6.43±0.01 ^{C}	5.91±0.00 ^{D}	5.88±0.01 ^{B}	6.58±0.00 ^{C}	6.70±0.01 ^{D}	7.19±0.06 ^{D}	6.78±0.00 ^{B,C}	7.03±0.21 ^{C}	7.07±0.17 ^{D}
14th day	6.70±0.00 ^{E}	5.99±0.00 ^{E}	6.05±0.07 ^{C}	6.71±0.00 ^{D}	6.92±0.00 ^{E}	7.24±0.08 ^{D}	6.91±0.02 ^{C}	7.04±0.13 ^{C}	7.06±0.09 ^{D}
21st day	6.50±0.00 ^{D}	5.54±0.00 ^{B}	5.47±0.01 ^{A}	6.50±0.01 ^{B}	6.51±0.00 ^{B}	6.52±0.01 ^{B}	6.67±0.19 ^{B}	6.57±0.00 ^{A,B}	6.64±0.00 ^{B}
28th day	6.45±0.03 ^{C}	5.54±0.00 ^{B}	5.43±0.01 ^{A}	6.59±0.02 ^{C}	6.61±0.05 ^{C}	6.99±0.16 ^{C}	6.89±0.08 ^{B,C}	6.76±0.12 ^{B,C}	6.94±0.08 ^{C,D}

Different capital letters show difference among time at each product at α=0.05 level. (1: C, 2: YC+B.L, 3: 0.2GA, 4: 0.03FM, 5: 0.05FM, 6: 0.1FM, 7: 0.03FM+0.2GA, 8: 0.05FM+0.2GA, 9:0.1FM+0.2GA)

mucilage caused to increase in the growth of *Bifidobacterium lactis* in comparison with control samples. Increasing count of *B. lactis* started from 0 day up to 28th days (Table 3.3). Flaxseed mucilage is considered as prebiotic and have a role for improving the probiotic growth, Naran et al. (2008) defined flaxseed mucilage as prebiotic source which contains a mixture of rhamnogalacturonan I and arabinoxylan which are neutral polysaccharides. Smolová et al. (2017) indicated that flaxseed oil with high level of α-linolenic acid (ALA) has a positive effect on the growth of *Bifidobacterium*. Plant extracts have many bioactive constituents which make it a good source for bacterial growth and survivability (Tuorila and Martello, 2002).

The viable count of *B. lactis* significantly ($P<0.05$) increased as the amounts increased at 0.03, 0.05 and 0.1% of flaxseed mucilage for all days of storage except at 21st it had shown the same increase for all concentrations. However, the highest bacterial counts of *B. lactis* were observed at 14th day of storage for kefir samples enriched with flaxseed mucilage (Figure 3.2).

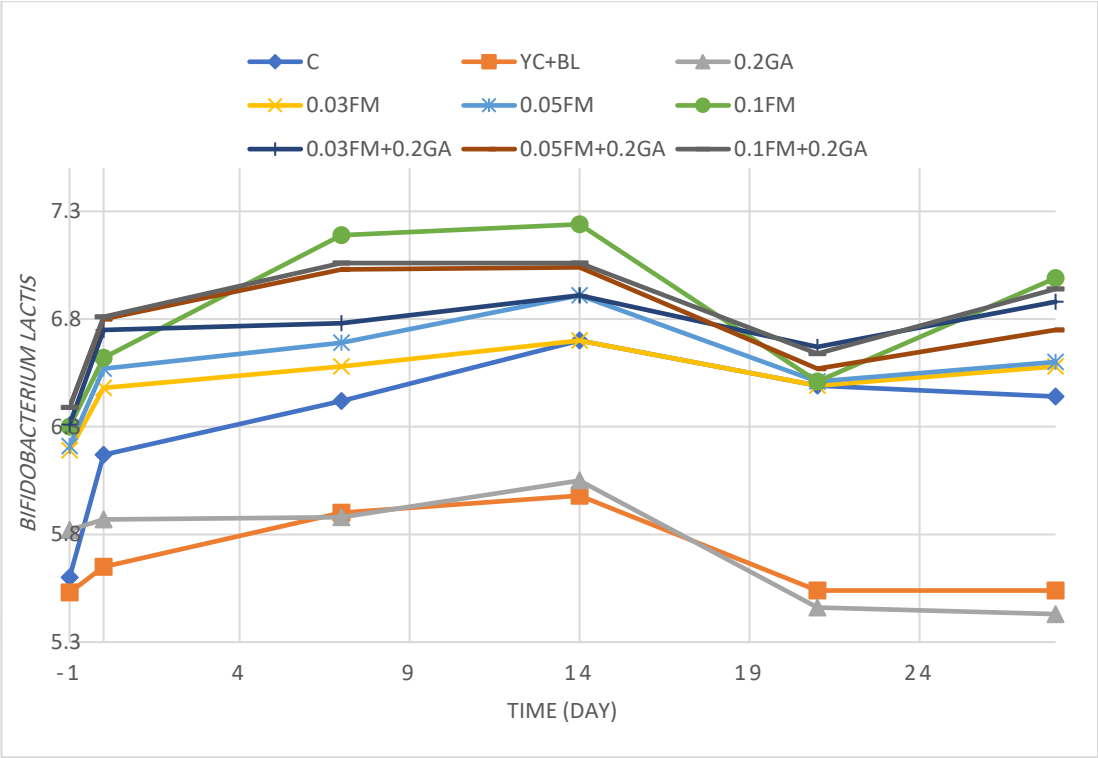

Figure 3.2 Effect of FM, GA and *B. lactis* + YC on the growth of *B. lactis* at different concentrations and through fermentation and storage time (FM; flaxseed mucilage, GA; gum arabic, YC; yogurt culture, BL; *B. Lactis*)

In contrast, in our samples the addition of gum arabic into kefir samples at 0.2% had a significant ($P<0.05$) decrease in the number of *B. lactis* in comparison with the control from 0 day up to 28th days of storage (Figure 3.2). It may be due to the effect of other probiotic bacteria found in yogurt culture that we added to kefir samples.

Use of 0.03% yoghurt culture and 0.06% *Bifidobacterium* with 0.02% of kefir culture significantly decreased the number of *B. lactis* in comparison with control at all days of storage. Moreover, the effect of yoghurt culture with *Bifidobacterium* was the same as 0.2% of gum arabic from 7th day up to final storage time at 28th day.

The mixing of flaxseed mucilage and gum arabic affected the growth of *B. lactis* at all days of storage in comparison with control sample (Table 3.4). Enriched kefir samples with 0.03, 0.05 and 0.1% flaxseed mucilage and 0.2% of gum arabic increased the counts of *B. lactis* in comparison at all days during storage (Table 3.4).

GA is considered as one of prebiotic source when mix with probiotic its result as symbiotic effect (Clark et al., 1993). Ziaolhagh and Jalali (2017) studied that enriched with xanthan gum into Doogh samples improved the growth of *B. lactis*. Doogh is an Iranian type of ayran traditional fermented dairy drink, manufactured by addition of salt and plant extract like ginger, into yogurt and stirred it. Yilmaz-Ersan et al. (2017), found that gum arabic and Tara gum were a good type of gums to increase counts and availability of *B.lactis.* Flaxseed mucilage is wealthy in dietary fiber which form a new source of prebiotic product to use in food industry (Roberfroid and Slavin, 2000; Fodje et al., 2009).

Use of 0.03, 0.05 and 0.1% flaxseed mucilage and 0.2% of gum arabic caused to increase in the viability of *B. lactis* from 0 day up to 21st days during storage (Table 3.3). *B. lactis* counts were higher when gum arabic and flaxseed mucilage added both than adding flaxseed mucilage or gum alone (Figure 3.2). Also, gum arabic had significant ($P<0.05$) positive effect on *B. lactis* counts when we add it with flaxseed mucilage rather than when it alone compared to control sample. It may be due to that when we add flaxseed mucilage and gum arabic altogether to kefir samples its work synergistically as prebiotic with probiotic to increase the counts of kefir samples. Moreover, the highest bacterial counts were observed at 0.1% of flaxseed mucilage at 14th day of storage time, the magnitude was 7.24 log CFU/mL (Figure 3.2).

3.3 pH value

The variations in pH value in samples were measured at all days of storage and their results are shown in Tables 3.5 and 3.6. Statistical analysis indicated that addition of specified concentration of flaxseed mucilage (0.03, 0.05 and 0.1%) and gum arabic (0.2%) lead to drop ($P<0.05$) in pH level (Table 3.5).

The addition of 0.03, 0.05 and 0.1% of flaxseed mucilage effect pH level at all days of storage in comparison to control (Table 3.6). Enriched kefir samples with 0.03, 0.05 and 0.1% of flaxseed mucilage caused to significant decreased in pH level at all storage period compared to control (Table 3.5). Smolová et al. (2017) observed that the pH dropped in all kefir samples of flaxseed variety composition into milk correlates with the cell count very well, in case of lactobacilli, the pH drops below 4.0, which is evidence of over fermentation. This result was in good agreement with

Basiri et al. (2018). They reported that the pH value decrease in all samples of yoghurt enriched with flaxseed mucilage and the acidity increase which correlated to action of probiotic bacteria. However, the samples of 0.03 and 0.05% of flaxseed mucilage shows the same effect on pH value at 0 day and during 7^{th}, 14^{th} and 21^{st} days which had lower pH value than 0.1% samples of flaxseed mucilage (Figure 3.3). The samples prepared with 0.03, 0.05 and 0.1% of flaxseed mucilage show the same effect on pH value at 28^{th} day of storage (Figure 3.3).

The addition of 0.2% of gum arabic showed significant ($P<0.05$) effect on the pH value at all days during storage in comparison to control (Table 3.6). Use of 0.2% of gum arabic caused to significant decreased in the pH level at all days compared to control (Table 3.5 and Figure 3.3). Niamah et al. (2016) recorded the percentage of the pH value decreased and total acidity increased in yoghurt samples when increasing the concentration of added gum arabic. The gum arabic contains variety of carbohydrates which probiotic bacteria can fermented it (Osman, et al., 1993).

Use of 0.03% yoghurt culture and 0.06% *Bifidobacterium* caused to significant ($P<0.05$) decreased in pH value in comparison with control at all days during storage. Moreover, the lowest pH value was recorded for these samples (yoghurt culture and *B. lactis*) and it were the same effect as gum arabic at 0, 7^{th}, 14^{th} and 28 days of storage (Figure 3.3). This is due to rise in activity of bacteria, which lead to high acidic environment.

Mixing of flaxseed mucilage and gum arabic had significant ($P<0.05$) effect on the pH value during all storage days compared to control (Table 3.6). Use of 0.03, 0.05 and 0.1% flaxseed mucilage and 0.2% of gum arabic significantly decrease the pH level in comparison with control at all days during storage period (Table 3.5). Mixing samples of flaxseed mucilage with gum arabic showed the same effect on pH value at 7^{th}, 14^{th} and 28^{th} days during storage time (Figure 3.3).

Table 3.5 Effect of addition of flaxseed mucilage, gum arabic and yogurt culture and *B. lactis* at different concentration on pH value in kefir samples

	Time (day)					
Product	0 day	1st day	7th day	14th day	21st day	28th day
1	6.21±0.00 ^{g}	4.56±0.02 ^{f}	4.49±0.01 ^{d}	4.51±0.04 ^{e}	4.48±0.00 ^{h}	4.44±0.01 ^{e}
2	5.06±0.02 ^{a}	4.03±0.03 ^{a}	3.98±0.01 ^{a}	4.02±0.01 ^{a}	4.04±0.00 ^{a}	3.92±0.01 ^{a}
3	5.48±0.01 ^{b}	4.04±0.03 ^{a}	4.04±0.00 ^{a,b}	4.03±0.00 ^{a}	4.08±0.00 ^{b}	3.95±0.01 ^{a}
4	5.55±0.01 ^{c}	4.21±0.01 ^{c}	4.11±0.01 ^{a,b,c}	4.23±0.11 ^{b,c}	4.15±0.00 ^{c}	4.05±0.00 ^{b}
5	5.52±0.03 ^{c}	4.09±0.01 ^{b}	4.08±0.01 ^{a,b,c}	4.15±0.00 ^{b}	4.14±0.00 ^{c}	4.07±0.00 ^{b,c}
6	5.71±0.01 ^{d}	4.25±0.00 ^{d}	4.37±0.00 ^{c,d}	4.30±0.01 ^{c,d}	4.29±0.01 ^{f}	4.09±0.01 ^{b,c}
7	5.81±0.00 ^{e}	4.26±0.00 ^{d}	4.08±0.36 ^{a,b,c}	4.32±0.00 ^{d}	4.27±0.00 ^{e}	4.18±0.06 ^{d}
8	5.80±0.00 ^{e}	4.30±0.01 ^{e}	4.30±0.00 ^{b,c,d}	4.27±0.00 ^{c,d}	4.31±0.00 ^{g}	4.15±0.00 ^{d}
9	5.95±0.00 ^{f}	4.23±0.01 ^{c,d}	4.32±0.01 ^{b,c,d}	4.26±0.02 ^{c,d}	4.22±0.00 ^{d}	4.13±0.00 ^{c,d}

Different small letters show statistical difference among samples at each time at α=0.05 level

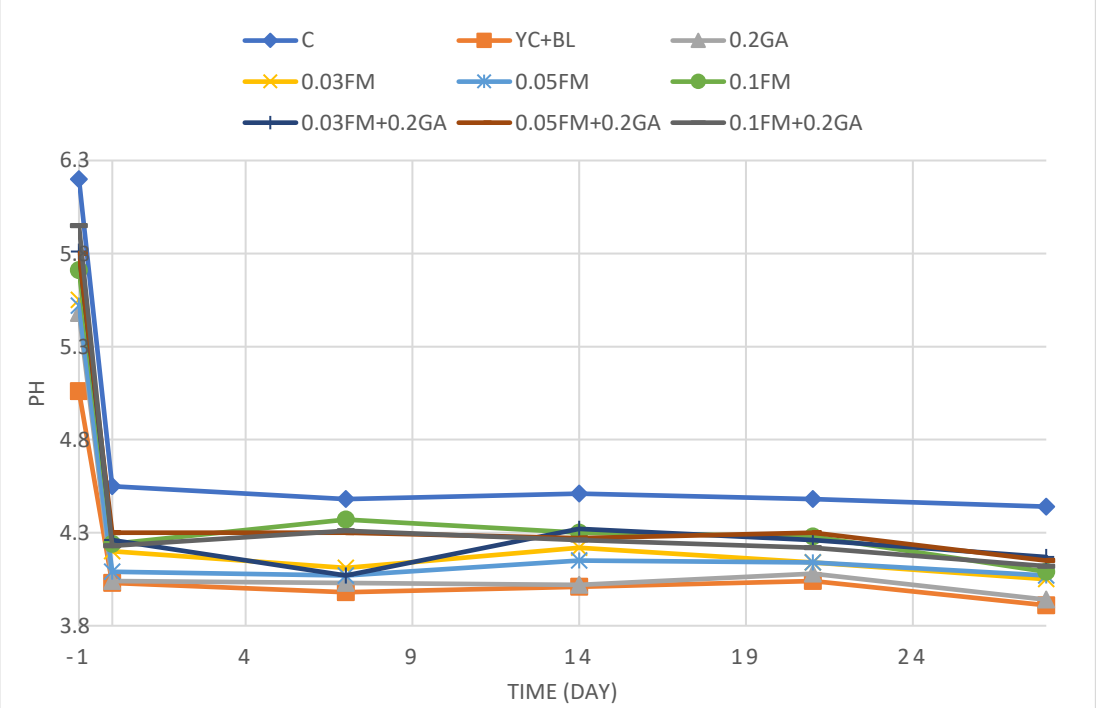

Figure 3.3 Effect of FM, GA and *B. lactis* + YC on the pH at different concentrations and through fermentation and storage time (FM; flaxseed mucilage, GA; gum arabic, YC; yogurt culture, BL; *B. Lactis*)

Table 3. 6 Effect of fermentation and storage time on pH value in kefir samples

	Product								
Time	1	2	3	4	5	6	7	8	9
0 day	6.21±0.00 ^{D}	5.06±0.02 ^{D}	5.48±0.01 ^{D}	5.55±0.01 ^{D}	5.52±0.03 ^{C}	5.71±0.01 ^{E}	5.81±0.00 ^{B}	5.80±0.00 ^{D}	5.95±0.00 ^{E}
1st day	4.56±0.02 ^{C}	4.03±0.03 ^{C}	4.04±0.03 ^{B}	4.21±0.01 ^{B,C}	4.09±0.01 ^{A}	4.25±0.00 ^{B}	4.26±0.00 ^{A}	4.30±0.01 ^{C}	4.23±0.01 ^{B}
7th day	4.49±0.01 ^{A,B}	3.98±0.01 ^{B}	4.04±0.00 ^{B}	4.11±0.01 ^{A,B}	4.08±0.01 ^{A}	4.37±0.00 ^{D}	4.08±0.36 ^{A}	4.30±0.00 ^{C}	4.32±0.01 ^{D}
14th day	4.51±0.04 ^{B,C}	4.02±0.01 ^{B,C}	4.03±0.00 ^{B}	4.23±0.11 ^{C}	4.15±0.00 ^{B}	4.30±0.01 ^{C}	4.32±0.00 ^{A}	4.27±0.00 ^{B}	4.26±0.01 ^{C}
21st day	4.48±0.00 ^{A,B}	4.04±0.00 ^{C}	4.08±0.00 ^{C}	4.15±0.00 ^{B,C}	4.14±0.00 ^{B}	4.29±0.01 ^{C}	4.27±0.00 ^{A}	4.30±0.00 ^{C}	4.22±0.00 ^{B}
28th day	4.44±0.01 ^{A}	3.92±0.01 ^{A}	3.95±0.01 ^{A}	4.05±0.00 ^{A}	4.07±0.00 ^{A}	4.09±0.01 ^{A}	4.18±0.06 ^{A}	4.15±0.00 ^{A}	4.13±0.00 ^{A}

Different capital letters show difference among time at each product at α=0.05 level. (1: C, 2: YC+B.L, 3: 0.2GA, 4: 0.03FM, 5: 0.05FM, 6: 0.1FM, 7: 0.03FM+0.2GA, 8: 0.05FM+0.2GA, 9:0.1FM+0.2G)

However, the lowest pH value was obtained for samples of kefir enriched with 0.2% gum arabic and samples of kefir enriched with 0.03% yoghurt culture + 0.06% *Bifidobacterium* which was 3.95 and 3.92, respectively (Figure 3.3). However, number of *L. acidophilus* and *Bifidobacterium* in these samples, were lesser than control, so, this may be due to post-acidification due to persistent metabolic activity of product's microflora that decreased lactic acid bacteria count.

3.4 Titratable Acidity

Changes in titratable acidity in all kefir samples were measured within storage time and their results are shown in Tables 3.7 and 3.8. Statistical analysis indicated that addition of specified concentration of flaxseed mucilage (0.03, 0.05 and 0.1%) and gum arabic (0.2%) lead to increase ($P<0.05$) in titratable acidity (Table 3.7).

Fortified kefir with 0.03, 0.05 and 0.1% of flaxseed mucilage showed significant effect on the titratable acidity at all days in comparison to control (Table 3.8). Use of 0.03, 0.05 and 0.1% of flaxseed mucilage led to increase the titratable acidity at 0, 1,st 7,th 14,th 21st and 28th days in comparison to control (Figure 3.4). Samples of flaxseed mucilage at 0.03, 0.05 and 0.1% concentration showed the same effect on titratable acidity at 0 day, 14th, 21st and 28th days during storage which was higher than that of control (Figure 3.4).

The addition of 0.2% gum arabic showed significant ($P<0.05$) effect on the titratable acidity at all days in comparison to control (Table 3.8). Use of 0.2% of gum arabic lead to significant increase in titratable acidity at all days (Figure 3.4).

Samples of 0.03% yoghurt culture and 0.06% *Bifidobacterium* had significant ($P<0.05$) increase in titratable acidity compared to control at all days of storage (Table 3.8). Moreover, samples of 0.2% of gum arabic and samples of 0.03% yoghurt culture and 0.06% *Bifidobacterium* showed the similar behavior of increasing acidity level at all days of storage except at 7th days of storage the samples of yoghurt culture and *Bifidobacterium* had higher acidity than those of 0.2% of gum arabic (Figure 3.4).

The mixing of flaxseed mucilage and gum arabic significantly ($P<0.05$) affected the titratable acidity at all days of storage in comparison to control (Table 3.8). Use of

0.03, 0.05 and 0.1% flaxseed mucilage and 0.2% of gum arabic significantly increase the titratable acidity compared to control at all storage period (Table 3.7).

The similar behavior of increasing acidity level was observed in 0.03, 0.05 and 0.1% of flaxseed mucilage with 0.2% of gum arabic at 14th and 21st days of storage (Figure 3.4). However, the higher level of titratable acidity was observed at 14th days in all samples of kefir except control sample which the higher acidity was observed at 28th day of storage (Figure 3.4). HadiNezhad et al. (2013) observed at termination of refrigerated storage, samples of kefir that fortifying with pure FM can caused to increase acidic media compared to kefir samples without mucilage establishing that pure mucilage prefer the viability of lactic acid bacteria. Furthermore, the action of probiotic of pure mucilage may produce more short-chain fatty acids which much rather in dissociated form, causing higher titratable acidity, but this action is not necessarily caused to lower pH value.

Although, Irigoyen et al. (2005) investigated that the counts of yeast and acetic acid bacteria keep completely proportional in kefir during cold storage which caused to more acidic conditions.

Table 3.7 Effect of addition of flaxseed mucilage, gum arabic, yogurt culture and *B. lactis* at different concentration on titratable acidity value in kefir samples

	Time (day)					
Product	0 day	1st day	7th day	14th day	21st day	28th day
1	0.23±0.01 ^{a}	0.71±0.01 ^{a}	0.76±0.01 ^{a}	0.74±0.03 ^{a}	0.73±0.01 ^{a}	0.78±0.04 ^{a}
2	0.58±0.01 ^{d}	0.92±0.02 ^{c,d}	0.95±0.01 ^{d,e}	0.97±0.01 ^{b,c}	0.96±0.00 ^{c,d}	0.94±0.05 ^{b}
3	0.59±0.03 ^{d}	0.95±0.00 ^{d}	0.90±0.03 ^{b,c}	0.97±0.03 ^{b,c}	0.95±0.01 ^{c,d}	0.88±0.03 ^{a,b}
4	0.39±0.03 ^{c}	0.90±0.01 ^{c}	0.91±0.01 ^{c,d}	0.96±0.04 ^{b,c}	0.93±0.00 ^{b,c,d}	0.87±0.05 ^{a,b}
5	0.39±0.00 ^{c}	0.93±0.01 ^{c,d}	0.99±0.01 ^{e}	1.01±0.03 ^{c}	0.98±0.02 ^{d}	0.87±0.06 ^{a,b}
6	0.39±0.00 ^{c}	0.94±0.00 ^{d}	0.90±0.03 ^{b,c,d}	0.98±0.02 ^{b,c}	0.95±0.01 ^{c,d}	0.93±0.06 ^{b}
7	0.34±0.05 ^{b,c}	0.87±0.02 ^{b}	0.86±0.03 ^{b}	0.91±0.04 ^{b}	0.89±0.02 ^{b}	0.78±0.06 ^{a}
8	0.33±0.04 ^{b}	0.90±0.01 ^{c}	0.86±0.03 ^{b}	0.96±0.03 ^{b,c}	0.90±0.04 ^{b}	0.89±0.04 ^{a,b}
9	0.35±0.01 ^{b,c}	0.92±0.00 ^{c,d}	0.89±0.01 ^{b,c}	1.00±0.03 ^{c}	0.97±0.04 ^{d}	0.94±0.05 ^{b}

Different small letters show statistical difference among samples at each time at α=0.05 level

Table 3.8 Effect of fermentation and storage time on titratable acidity value in kefir samples

	Product								
Time	1	2	3	4	5	6	7	8	9
0 day	0.23±0.01 ^{A}	0.58±0.01 ^{A}	0.59±0.03 ^{A}	0.39±0.03 ^{A}	0.39±0.00 ^{A}	0.39±0.00 ^{A}	0.34±0.05 ^{A}	0.33±0.04 ^{A}	0.35±0.01 ^{A}
1st day	0.71±0.01^{B}	0.92±0.02 ^{B}	0.95±0.00 ^{B,C}	0.90±0.01 ^{B,C}	0.93±0.02 ^{B,C}	0.94±0.00 ^{B,C}	0.87±0.02 ^{B,C}	0.90±0.01 ^{B,C}	0.92±0.00 ^{B,C}
7th day	0.76±0.01^{B,C}	0.95±0.01 ^{B}	0.90±0.03 ^{B}	0.91±0.01 ^{B,C}	0.99±0.01 ^{C}	0.90±0.03 ^{B}	0.86±0.03 ^{B,C}	0.86±0.03 ^{B}	0.89±0.01 ^{B}
14th day	0.74±0.03^{B,C}	0.97±0.01 ^{B}	0.97±0.03 ^{C}	0.96±0.04 ^{C}	1.01±0.03 ^{C}	0.98±0.02 ^{C}	0.91±0.04 ^{B}	0.96±0.03 ^{C}	1.00±0.03 ^{D}
21st day	0.73±0.01^{B,C}	0.96±0.00 ^{B}	0.95±0.01 ^{B,C}	0.93±0.00 ^{B,C}	0.98±0.02 ^{C}	0.95±0.01 ^{B,C}	0.89±0.02 ^{B}	0.90±0.04 ^{B,C}	0.97±0.04 ^{C,D}
28th day	0.78±0.04 ^{C}	0.94±0.05 ^{B}	0.88±0.03 ^{B}	0.87±0.04 ^{B}	0.87±0.06 ^{B}	0.93±0.06 ^{B,C}	0.78±0.06 ^{B}	0.89±0.04 ^{B,C}	0.94±0.05 $^{B,C.D}$

Different capital letters show difference among time at each product at α=0.05 level. (1: C, 2: YC+B.L, 3: 0.2GA, 4: 0.03FM, 5: 0.05FM, 6: 0.1FM, 7: 0.03FM+0.2GA, 8: 0.05FM+0.2GA, 9:0.1FM+0.2GA).

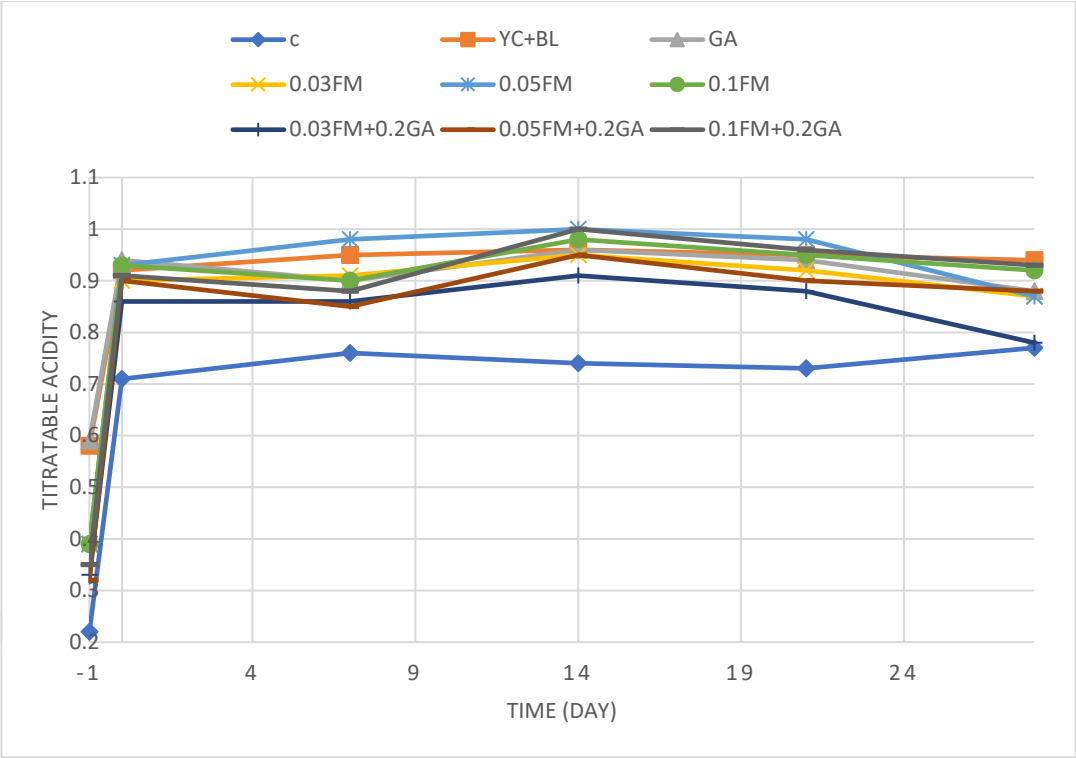

Figure 3.4 Effect of FM, GA and *B. lactis* + YC on the titratable acidity at different concentrations and through fermentation and storage time (FM; flaxseed mucilage, GA; gum arabic, YC; yogurt culture, BL; *B. Lactis*)

3.5 The viscosity

Statistical analysis indicated that concentrations of flaxseed mucilage, gum arabic and yogurt culture with *Bifidobacterium* had significant effect ($P<0.05$) on viscosity value are shown in Tables 3.9 and 3.10. Use of 0.03, 0.05 and 0.1% of flaxseed mucilage led to significant ($P<0.05$) increased in viscosity value in comparison with control samples at all days of storage (Figure 3.5). Sodini et al. (2004) investigated that increased viscosity can be due to the interactions between polysaccharides in flaxseed mucilage and dairy protein. However, as concentration of flaxseed mucilage increase in the viscosity value decreased at all days during storage (Figure 3.5). This may be due to increase in concentration of flaxseed mucilage that cause to decrease in viscosity and constituent of new kefir product due to protein rearrangement or protein–protein contact. As Anema et al. (2004) explained that variations in viscosity values in dairy products may be due to variations in pH value and acidity level through storage time. It also may be due to symbiotic effect of effectivity of probiotic bacteria and prebiotic components.

Table 3.9 Effect of addition of flaxseed mucilage, gum arabic and yogurt culture and *B. lactis* at different concentration on viscosity value (cp) in kefir samples

	Time (day)					
Product	0 day	1st day	7th day	14th day	21st day	28th day
1	13.86±1.29 [a]	1242±83.4[a]	1623±94.0[a]	1738±61.2 [a,b]	1554±41.7 [a]	1634±49.5 [a]
2	369±43.1 [b]	1947±84.9 [b]	2127±74.2[c]	2375±61.5 [c,d]	2166±0.35 [c]	2308±74.2 [c]
3	449±30.1 [b,c]	2108±129 [b]	1903±77.4[b]	2309±99.7 [c]	1911±86.6 [b]	1993±85.6 [b]
4	453±62.8 [b,c]	2563±114 [c]	2807±120 [f]	2549±120 [d,e]	2485±96.5[d]	2952±111 [d]
5	502±69.3 [c,d]	2100±94.0 [b]	2377±100 [d,e]	2305±87.7[c]	2021±95.5 [b,c]	2364±106 [c]
6	689±35.3 [e]	1403±19.4 [a]	1466±30.4 [a]	1637±86.6 [a]	1515±55.2 [a]	1853±69.3 [b]
7	585±57.6 [d,e]	2148±118 [b]	2539±61.2 [e]	2692±26.2 [e]	2410±79.9 [d]	1969±15.9 [b]
8	830±61.4 [f]	2067±83.4[b]	2215±110 [c,d]	2496±78.5 [c,d]	2093±51.3 [c]	2481±104 [c]
9	667±50.7 [e]	1986±79.5 [b]	2082±72.1 [b,c]	1832±78.5 [b]	1861±73.2 [b]	2351±142 [c]

Different small letters show statistical difference among samples at each time at α=0.05 level.

Fortified kefir samples with 0.2% of gum arabic had significantly (P<0.05) effect on viscosity value (Table 3.9). Addition of 0.2% of gum arabic increased the viscosity value from 0 day until the end days of storage compared to control samples (Figure 3.5). It also showed the similar effect on viscosity in samples of yogurt culture and *Bifidobacterium* at 0, 1st and 14th days of storage. However, at 7th, 21st and 28th days, the viscosity of samples of yogurt culture and *Bifidobacterium* had higher viscosity value than that of 0.2% of gum arabic samples (Figure 3.5). Azarikia and Abbasi (2010) investigated that the gum tragacanth could increase viscosity value of Doogh samples could be as a result of its functional properties of its soluble and insoluble fractions.

Kefir samples enriched with mixing of flaxseed mucilage and gum arabic caused to significant (P<0.05) increase in viscosity at all storage days in comparison with control (Table 3.10). Use of 0.03, 0.05 and 0.1% flaxseed mucilage and 0.2% of gum arabic significantly increase in viscosity value at 0 day and until end days of storage in comparison with control sample (Table 3.9). At 0 day all of mixing samples

Table 3.10 Effect of fermentation and storage time on viscosity value (cp) in kefir samples

	Product								
Time	1	2	3	4	5	6	7	8	9
0 day	13.86±1.29^{A}	369±43.1 ^{A}	449±30.1^{A}	453±62.8 ^{A}	502±69.3 ^{A}	689±35.3 ^{A}	585±57.6 ^{A}	830±61.4 ^{A}	667±50.7 ^{A}
1st day	1242±83.4 ^{B}	1947±84.9 ^{B}	2108±129 ^{B,C}	2563±114 ^{B,C}	2100±94.0 ^{B,C}	1403±19.4 ^{B}	2148±118 ^{C}	2067±83.4 ^{B}	1986±79.5 ^{B,C}
7th day	1623±94.0 ^{C,D}	2127±74.2 ^{C}	1903±77.4^{B}	2807±120 ^{C,D}	2376±100 ^{D}	1466±30.4 ^{B}	2539± 61.2 ^{D,E}	2215±110 ^{B}	2082±72.1^{C}
14th day	1738±61.1^{D}	2375±61.5 ^{E}	2309±99.7 ^{C}	2549±120 ^{B,C}	2305±87.7 ^{C,D}	1637±86.7 ^{C}	2692±26.2 ^{E}	2496±78.5 ^{C}	1832±78.5 ^{B}
21st day	1554±41.7^{C}	2166±0.35 ^{C,D}	1911±86.6 ^{B}	2485±96.5 ^{B}	2021±95.5 ^{B}	1515±55.2 ^{B,C}	2410±79.9 ^{D}	2093±51.3 ^{B}	1861±73.2 ^{B,C}
28th day	1634±49.5 ^{C,D}	2308±74.2 ^{D,E}	1993±85.6 ^{B}	2952±111 ^{D}	2364±106 ^{D}	1853±69.3 ^{D}	1968±15.9 ^{B}	2481±104 ^{C}	2351±142 ^{D}

Different capital letters show difference among time at each product at α=0.05 level. (1: C, 2: YC+B.L, 3: 0.2GA, 4: 0.03FM, 5: 0.05FM, 6: 0.1FM, 7: 0.03FM+0.2GA, 8: 0.05FM+0.2GA, 9:0.1FM+0.2GA)

showed similar effect on increasing viscosity, but at 7th day 0.03% of FM and 0.2% of GA had higher viscosity than those of 0.05 and 0.1% of FM mixed with 0.2 of GA. After 14th and 21st days, as concentration of flaxseed mucilage increased the viscosity value decreased in samples. This drop in viscosity value could be considered as a result of the action of a bacterial enzyme on the casein micelle matrix throughout the storage time (Kosikowski, 1982).

For all samples viscosity value increased at all storage days and all of them was higher viscosity value than that of control except sample of 0.1% of flaxseed mucilage its approximately had similar effect as control (Figure 3.5). The highest viscosity value was obtained for samples of 0.03% of flaxseed mucilage at all days of storage. However, kefir samples with 0.03% of flaxseed mucilage and 0.03 of flaxseed mucilage with 0.2% of gum arabic showed the similar increasing in viscosity value at 14th and 21st days (Figure 3.5).

Actually, the change in viscosity value may be due to alterations in the volume of casein micelles (supramolecule of colloidal size) during storage, which can be a result of many factors such as existence and number of fats, proteins or micelle-binding factors (Bienvenue et al., 2003).

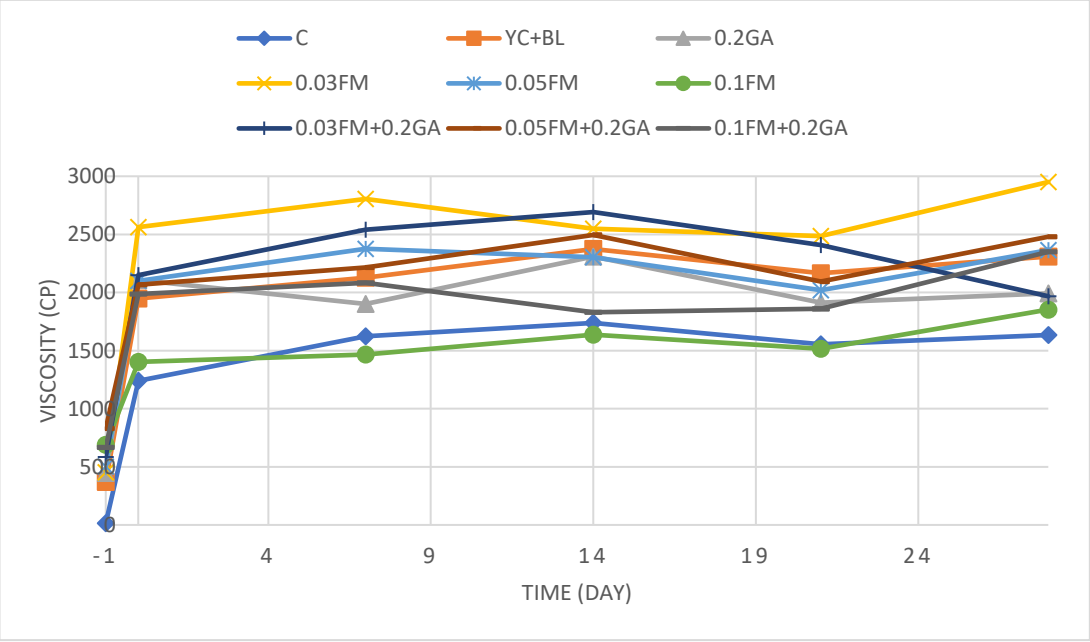

Figure 3.5 Effect of FM, GA and *B. lactis* + YC on the viscosity at different concentrations and through fermentation and storage time (FM; flaxseed mucilage, GA; gum arabic, YC; yogurt culture, BL; *B. Lactis*)

3.6 Color parameters (L*, a*and b*)

Hunter color parameters; a* red- green, b* yellow- blue, L* light- dark have already recorded to show the gradation of visual colors (Garza et al., 1999). Whiteness in dairy products is related to colloidal particles such as casein micelles and milk fat globules when light hits these casein micelles it causes the light to refract and scatter resulting in milk appearing white (Garcıa-Perez et al., 2005).

Use of 0.03, 0.05 and 0.1% of flaxseed mucilage effect in L* value (lightness) compared to control at all days (Tables 3.11 and 3.12). Fortified kefir samples with 0.03, 0.05 and 0.1% of flaxseed mucilage could increase ($P<0.05$) L* value in comparison with control at 0 day and 14^{th} days of storage (Figure 3.6). However, as concentration of flaxseed mucilage increased the L* value decreased. Similar study conducted the same result; the L* value of the semi-fat yoghurt decreased as concentrations of flaxseed mucilage ($p<0.01$) increased interpreting that flaxseed mucilage donated a darkening effect, may be due to that the FM could absorb water (Arabshahi- Deloueea, et al., 2020). Use of 0.2% of gum arabic had significant increase in L* value in comparison with control at 0, 14^{th}, 21^{st} and 28^{th} days during storage period (Table 3.11), but at 0 day it had decrease in L* value compared to control.

Mixing of 0.03, 0.05 and 0.1% of flaxseed mucilage with 0.2% of gum arabic had significant ($P<0.05$) increase in L* value in comparison with control at 0, 14^{th} and 21^{st} days of storage (Table 3.11). However, at 0 day the mixing samples had decreased in L* value, at 7^{th} day comparing to control. The 0.1% of FM and 0.2% of GA also showed significant decreased in L* value compared to control (Figure 3.6).

Use of 0.03, 0.05 and 0.1% of flaxseed mucilage showed significant ($P<0.05$) effect in a* value (redness) compared to control at all storage days. Addition of 0.03, 0.05 and 0.1% of flaxseed mucilage increased ($P<0.05$) in a* value compared to control at all days (Table 3.13). The addition of 0.1% of flaxseed mucilage had higher a* value than those of 0.03 and 0.05% of FM at 0, $1,^{st}$ $7,^{th}$ 21^{st} and 28^{th} days during storage (Figure 3.7). This result is similar to study conducted by Arabshahi- Deloueea et al. (2020). They found that a* value of the yoghurt increased ($p<0.01$) as the amount of flaxseed mucilage increased.

Addition of 0.2% of gum arabic into kefir samples had significant increased in a* value compared to control at 0, 1st and 7th days of storage. At 14th day showed the similar effect on a* value compared to control, but at 21st and 28th days showed decreased (P<0.05) effect in a* value compared to control (Figure 3.7).

Mixing of 0.03, 0.05 and 0.1% of flaxseed mucilage with 0.2% of gum arabic had significant (P<0.05) increased in a* value compared to control at all days (Tables 3.13 and 3.14). However, at 0 day the mixing had significant decreased in a* value comparing to control. At 7th days kefir samples of 0.1% of FM and 0.2% of GA also showed significant decreased in a* value compared to control (Figure 3.7). Mixing of 0.03, 0.05 and 0.1% of flaxseed mucilage with 0.2% of gum arabic had similar effect on a* value at 0 day of storage. However, the mixing of 0.03% of FM with 0.2% of GA had higher effect on a* value compared to those of 0.05 and 0.1% of FM mixed with 0.2% of GA at 7th, 14th and 21st days (Figure 3.7).

Addition of flaxseed mucilage and gum arabic had significant (P<0.05) effect on b* value in kefir samples. Use of 0.03, 0.05 and 0.1% of flaxseed mucilage had significant increase in b* value (yellowness) comparing to control at all days of storage (Table 3.15). Samples of 0.05% of flaxseed mucilage had higher b* value compared to those of 0.03 and 0.1% of FM at 0, 1,st 7th and 14th days of storage (Figure 3.8). However, at 21st day of storage all samples of flaxseed mucilage (0.03, 0.05 and 0.1%) had similar effect on b* value (Figure 3.8). Use of 0.2% of gum arabic had significant (P<0.05) effect on b* value compared to control. Samples of 0.2% of gum arabic showed significant increase in b* value compared to control at 0, 14th and 28th days of storage. However, at 0, 7th and 21st days, it showed the similar effect on b* value compared to control (Figure 3.8).

Mixing of 0.03, 0.05 and 0.1% of flaxseed mucilage with 0.2% of gum arabic had significant (P<0.05) increased in b* value compared to control at all storage days (Tables 3.15 and 3.16). As mixing of 0.03, 0.05 and 0.1% of flaxseed mucilage with 0.2% of gum arabic increased, the b* value also increased at all days of storage (Figure 3.8). The similar effect was showed by Arabshahi- Deloueea et al. (2020) on flaxseed mucilage with yogurt samples were a* and b*values of the yoghurt increased (p<0.01) with increasing the amount of flaxseed mucilage and samples containing 0.2% of flaxseed mucilage had the maximum a* and b*value among the

samples. This is may be due to release of some pigments from flaxseed mucilage that made the product more yellow. Moreover, pasteurization have role in inducing destabilization of the casein micelles which increases a* and b* values. Other study conducted by Garcıa-Perez et al. (2005) found that the supplement of fiber to the yoghurt samples had effect on L* value (decreased) and on b* value (increased) of the yoghurt during fermentation and storage.

Table 3.11 Effect of addition of flaxseed mucilage, gum arabic and yogurt culture and *B. lactis* at different concentration on color L* value in kefir samples

	Time (day)					
Product	0 day	1st day	7th day	14th day	21st day	28th day
1	85.64±0.03 ^{a}	88.45±0.01 ^{f}	87.83±0.04 ^{b}	87.00±0.09 ^{a}	87.42±0.11^{a,b}	86.93±0.04 ^{a}
2	86.29±0.13 ^{b}	87.97±0.09 ^{c}	88.45±0.02 ^{f}	88.29±0.03 ^{f}	87.99±0.11^{e}	88.00±0.02 ^{f}
3	86.24±0.12 ^{b}	88.10±0.05 ^{d}	87.74±0.04 ^{b}	87.61±0.06 ^{d}	88.10±0.02^{f}	88.56±0.02 ^{g}
4	88.10±0.08 ^{f}	88.83±0.03 ^{g}	88.34±0.12 ^{e}	87.74±0.02 ^{e}	88.33±0.02^{g}	88.72±0.05 ^{h}
5	87.05±0.05 ^{d,e}	88.52±0.11 ^{f}	88.21±0.06 ^{d}	87.67±0.01 ^{d,e}	87.85±0.05 ^{d}	87.88±0.06 ^{e}
6	86.54±0.06 ^{c}	88.35±0.03 ^{e}	87.12±0.02 ^{a}	87.19±0.07 ^{b}	87.34±0.05^{a}	87.46±0.04 ^{d}
7	87.16±0.09 ^{e}	87.40±0.03 ^{a}	88.02±0.05 ^{c}	87.72±0.03 ^{e}	87.54±0.02^{c}	87.36±0.13 ^{c}
8	86.91±0.08 ^{d}	87.69±0.00 ^{b}	87.97±0.09 ^{c}	87.48±0.03 ^{c}	87.52±0.02^{b,c}	87.08±0.01 ^{b}
9	86.29±0.05 ^{b}	87.75±0.03 ^{b}	87.08±0.02 ^{a}	87.24±0.05 ^{b}	87.49±0.02^{b,c}	86.97±0.02 ^{a}

Different small letters show statistical difference among samples at each time at α=0.05 level.

Table 3.12 Effect of fermentation and storage time on color L* value in kefir samples

	Product								
Time	1	2	3	4	5	6	7	8	9
0 day	85.64±0.03 ^{A}	86.29±0.13 ^{A}	86.24±0.12 ^{A}	88.10±0.08 ^{B}	87.05±0.05 ^{A}	86.54±0.06 ^{A}	87.16±0.09 ^{A}	86.91±0.08 ^{A}	86.29±0.05 ^{A}
1st day	88.45±0.01 ^{E}	87.97±0.09 ^{B}	88.10±0.05 ^{D}	88.83±0.03 ^{D}	88.52±0.11 ^{E}	88.35±0.03 ^{E}	87.40±0.03 ^{B}	87.69±0.00 ^{D}	87.75±0.03 ^{F}
7th day	87.83±0.04 ^{D}	88.45±0.02 ^{D}	87.74±0.04 ^{C}	88.34±0.12 ^{C}	88.21±0.06 ^{D}	87.12±0.02 ^{B}	88.02±0.05 ^{E}	87.97±0.09 ^{E}	87.08±0.02 ^{C}
14th day	87.00±0.09 ^{B}	88.29±0.03 ^{C}	87.61±0.06 ^{B}	87.74±0.02 ^{A}	87.67±0.01 ^{B}	87.19±0.07 ^{B}	87.72±0.03 ^{D}	87.48±0.02 ^{C}	87.24±0.05 ^{D}
21st day	87.42±0.11 ^{C}	87.99±0.11 ^{B}	88.10±0.02 ^{D}	88.33±0.02 ^{C}	87.85±0.05 ^{C}	87.34±0.05 ^{C}	87.54±0.02 ^{C}	87.52±0.02 ^{C}	87.49±0.02 ^{E}
28th day	86.93±0.04 ^{B}	88.00±0.02 ^{B}	88.56±0.02 ^{E}	88.72±0.05 ^{D}	87.88±0.06 ^{C}	87.46 ±0.04^{D}	87.36±0.12 ^{B}	87.08±0.01 ^{B}	86.97±0.02 ^{B}

Different capital letters show difference among time at each product at α=0.05 level. (1: C, 2: YC+B.L, 3: 0.2GA, 4: 0.03FM, 5: 0.05FM, 6: 0.1FM, 7: 0.03FM+0.2GA, 8: 0.05FM+0.2GA, 9:0.1FM+0.2G)

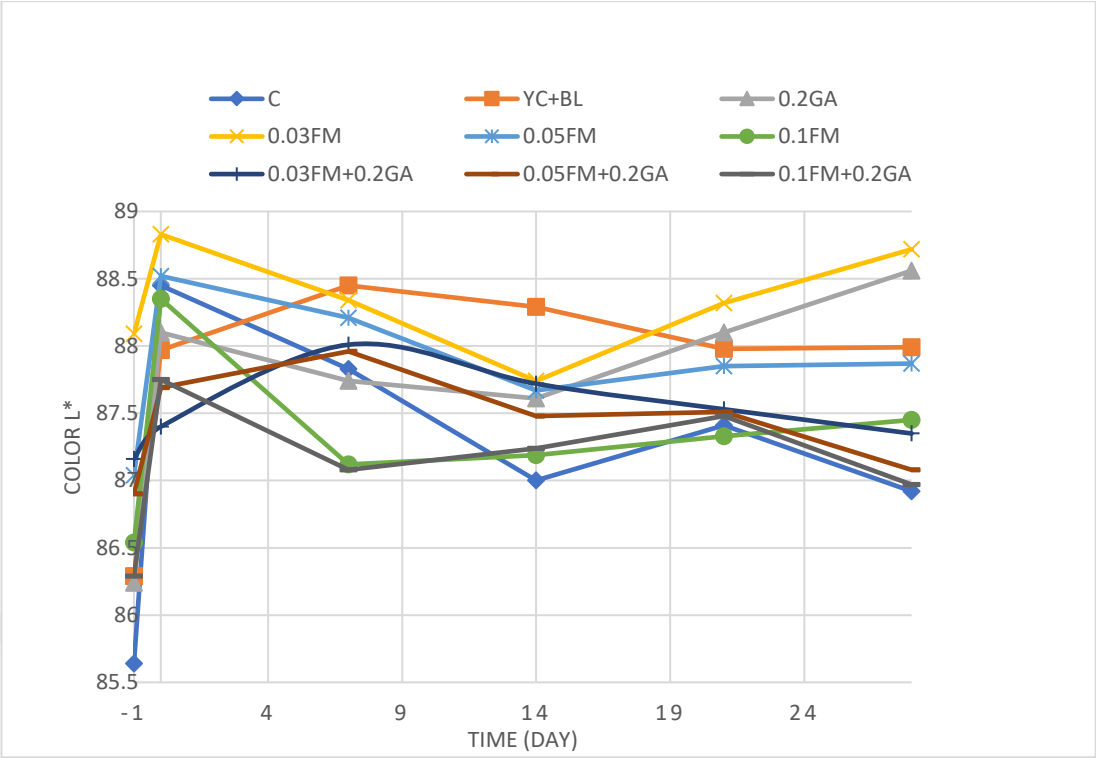

Figure 3.6 Effect of FM, GA and *B. lactis* + YC on the color L* at different concentrations and through fermentation and storage time (FM; flaxseed mucilage, GA; gum arabic, YC; yogurt culture, BL; *B. Lactis*)

Table 3.13 Effect of addition of flaxseed mucilage, gum arabic and yogurt culture and *B. lactis* at different concentration on color a* value in kefir samples

	Time (day)					
Product	0 day	1st day	7th day	14th day	21st day	28th day
1	-4.39±0.00 ^{a}	-3.85±0.05^{a}	-3.88±0.01 ^{a}	-3.77±0.06 ^{a}	-3.67±0.02 ^{b}	-3.63±0.03 ^{b}
2	-4.23±0.01 ^{b}	-3.74±0.02 ^{b}	-3.78±0.02 ^{b}	-3.78±0.02 ^{a}	-3.71±0.01 ^{b}	-3.77±0.01 ^{a}
3	-4.13±0.01 ^{c}	-3.75±0.01 ^{b}	-3.75±0.00 ^{b}	-3.75±0.02 ^{a}	-3.78±0.01 ^{a}	-3.82±0.01 ^{a}
4	-3.97±0.01 ^{e}	-3.24±0.03 ^{d}	-3.21±0.06 ^{d}	-3.39±0.03 ^{c}	-3.24±0.02 ^{c,d}	-3.18±0.03 ^{e}
5	-3.97±0.01 ^{e}	-3.32±0.03 ^{c}	-3.18±0.01 ^{d}	-3.27±0.00 ^{d}	-3.18±0.01 ^{d}	-3.01±0.03 ^{f}
6	-3.88±0.01 ^{f}	-2.87±0.04 ^{f}	-2.99±0.05 ^{e,f}	-3.45±0.05 ^{b}	-3.06±0.07 ^{e}	-2.99±0.01 ^{f}
7	-3.99±0.02 ^{d}	-3.15±0.05 ^{e}	-2.98±0.06 ^{f}	-3.24±0.06 ^{d}	-3.06±0.03 ^{e}	-3.28±0.07 ^{d}
8	-3.89±0.01 ^{f}	-3.13±0.09 ^{e}	-3.76±0.06 ^{e}	-3.41±0.04 ^{b,c}	-3.03±0.06 ^{e}	-3.14±0.02 ^{e}
9	-3.81±0.01 ^{g}	-3.15±0.02 ^{e}	-3.37±0.05 ^{c}	-3.29±0.02 ^{d}	-3.29±0.02 ^{c}	-3.39±0.14 ^{c}

Different small letters show statistical difference among samples at each time at α=0.05 level.

Table 3.14 Effect of fermentation and storage time on color a* value in kefir samples

	Product								
Time	1	2	3	4	5	6	7	8	9
0 day	-4.39±0.00 ^{A}	-4.23±0.01 ^{A}	-4.13±0.01 ^{A}	-3.97±0.01 ^{A}	-3.97±0.01 ^{A}	-3.88±0.01 ^{A}	-3.99±0.02 ^{A}	-3.89±0.01 ^{A}	-3.81±0.01 ^{A}
1st day	-3.85±0.05 ^{B}	-3.74±0.02 ^{C}	-3.75±0.01 ^{D}	-3.24±0.03 ^{C}	-3.32±0.03 ^{B}	-2.87±0.04 ^{D}	-3.15±0.05 ^{C}	-3.13±0.09 ^{C,D}	-3.15±0.02 ^{C}
7th day	-3.88±0.01 ^{B}	-3.78±0.02 ^{B}	-3.75±0.00 ^{D}	-3.21±0.06 ^{C}	-3.18±0.01 ^{D}	-2.99±0.05 ^{C}	-2.98±0.06 ^{D}	-3.07±0.06 ^{C,D}	-3.37±0.05 ^{B}
14th day	-3.77±0.06 ^{C}	-3.78±0.02 ^{B}	-3.75±0.02 ^{D}	-3.39±0.03 ^{B}	-3.27±0.00 ^{C}	-3.45±0.05 ^{B}	-3.24±0.06 ^{B}	-3.41±0.04 ^{B}	-3.29±0.02 ^{B}
21st day	-3.67±0.02 ^{D}	-3.71±0.01 ^{D}	-3.78±0.01 ^{C}	-3.24±0.02 ^{C}	-3.18±0.01 ^{D}	-3.06±0.07 ^{C}	-3.06±0.03 ^{C,D}	-3.03±0.06 ^{D}	-3.29±0.02 ^{B}
28th day	-3.63±0.03 ^{D}	-3.77±0.01 ^{B,C}	-3.82±0.01 ^{B}	-3.18±0.03 ^{C}	-3.01±0.03 ^{E}	-2.99±0.01 ^{C}	-3.28±0.07 ^{B}	-3.14±0.02 ^{C}	-3.39±0.14 ^{B}

Different capital letters show difference among time at each product at α=0.05 level. (1: C, 2: YC+B.L, 3: 0.2GA, 4: 0.03FM, 5: 0.05FM, 6: 0.1FM, 7: 0.03FM+0.2GA, 8: 0.05FM+0.2GA, 9:0.1FM+0.2GA)

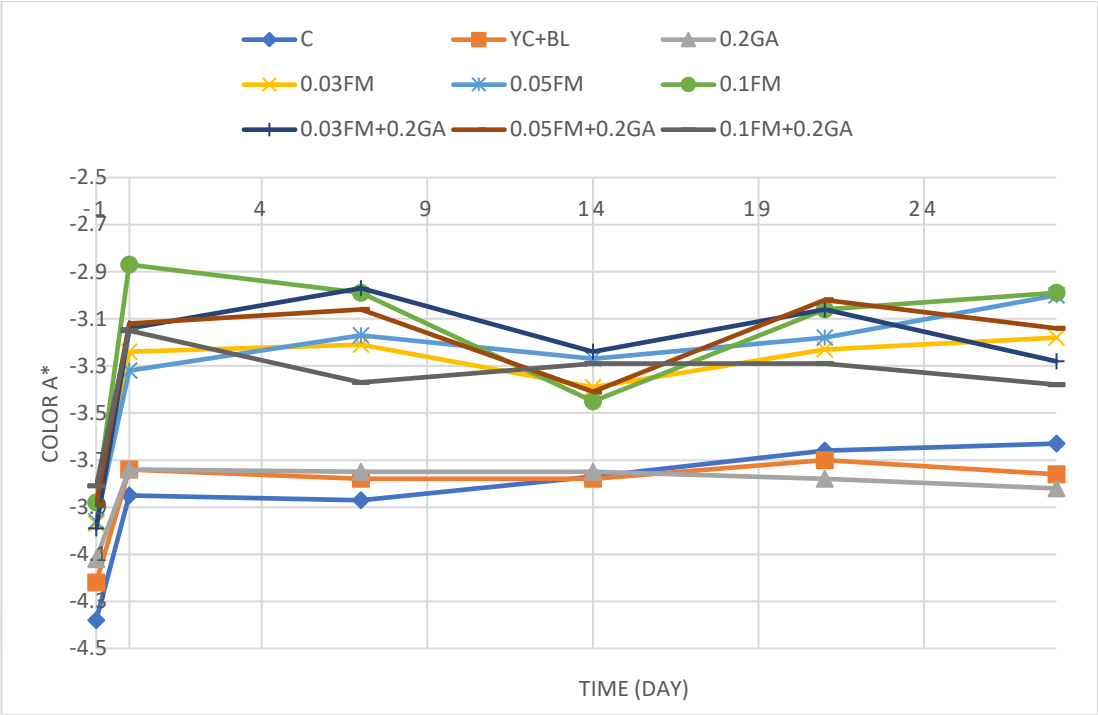

Figure 3.7 Effect of FM, GA and *B. lactis* + YC on the color a* at different concentrations and through fermentation and storage time (FM; flaxseed mucilage, GA; gum arabic, YC; yogurt culture, BL; *B. Lactis*)

Table 3.15 Effect of addition of flaxseed mucilage, gum arabic and yogurt culture and *B. lactis* at different concentration on color b* value in kefir samples

	Time (day)					
Product	0 day	1st day	7th day	14th day	21st day	28th day
1	1.59±0.01 ^{a}	4.79±0.09 ^{b,c}	3.78±0.02 ^{a}	3.02±0.26 ^{a}	3.74±0.14 ^{a}	3.41±0.11 ^{a}
2	2.04±0.16 ^{b}	4.20±0.11 ^{a}	4.67±0.04 ^{d}	4.61±0.11 ^{c}	5.26±1.53 ^{b}	4.86±0.02 ^{d}
3	2.28±0.20 ^{c}	4.58±0.03 ^{b}	3.94± 0.14 ^{a,b}	3.87±0.06 ^{b}	4.73±0.03^{a,b}	5.80±0.03 ^{g}
4	4.77±0.03 ^{f}	5.85±0.04 ^{e}	4.88±0.13 ^{d}	5.02±0.08 ^{d}	5.19±0.03 ^{b}	5.29±0.07 ^{e}
5	4.49±0.05 ^{e}	6.02±0.11 ^{e}	5.24±0.08 ^{e}	5.30±0.02 ^{e}	5.28±0.03 ^{b}	4.89±0.08 ^{d}
6	4.88±0.01 ^{f,g}	4.95±0.09 ^{c,d}	4.24±0.13 ^{c}	5.44±0.25 ^{e}	4.29±0.22 ^{a,b}	5.34±0.03 ^{e}
7	4.19±0.06 ^{d}	3.98±0.18 ^{a}	4.18±0.14 ^{b,c}	4.90±0.07 ^{d}	4.40±0.05 ^{a,b}	4.51±0.17 ^{b}
8	4.74±0.07 ^{f}	4.10±0.27 ^{a}	4.89±0.26 ^{d}	5.33±0.04 ^{e}	4.71±0.19 ^{a,b}	4.68±0.08 ^{c}
9	4.99±0.03 ^{g}	5.10±0.03 ^{d}	5.34±0.21 ^{e}	6.65±0.04 ^{f}	6.42±0.09 ^{c}	5.50±0.05 ^{f}

Different small letters show statistical difference among samples at each time at α=0.05 level.

Table 3.16 Effect of fermentation and storage time on color b* value in kefir samples

	Product								
Time	1	2	3	4	5	6	7	8	9
0 day	1.59±0.01 ^{A}	2.04±0.16 ^{A}	2.28±0.20 ^{A}	4.77±0.03 ^{A}	4.49±0.05 ^{A}	4.88±0.02 ^{B}	4.19±0.06 ^{A,B}	4.74±0.07 ^{B}	4.99±0.03 ^{A}
1st day	4.79±0.09 ^{E}	4.20±0.11 ^{B}	4.58±0.03 ^{C}	5.85±0.04 ^{D}	6.02±0.11 ^{D}	4.95±0.09 ^{B}	3.98±0.18 ^{A}	4.10±0.27 ^{A}	5.10±0.03 ^{A}
7th day	3.78±0.02 ^{D}	4.67±0.04 ^{B}	3.94±0.14 ^{B}	4.88±0.13 ^{A}	5.24±0.08 ^{C}	4.24±0.13 ^{A}	4.18±0.14 ^{A,B}	4.89±0.26 ^{B}	5.34±0.21 ^{B}
14th day	3.02±0.26 ^{B}	4.61±0.11 ^{B}	3.87±0.06 ^{B}	5.02±0.08 ^{B}	5.30±0.02 ^{C}	5.44±0.25 ^{C}	4.90±0.07 ^{D}	5.33±0.04 ^{C}	6.65±0.04 ^{D}
21st day	3.74±0.15 ^{D}	5.26±1.53 ^{B}	4.73±0.03 ^{C}	5.19±0.03 ^{C}	5.28±0.03 ^{C}	4.29±0.23 ^{A}	4.40±0.05 ^{B,C}	4.71±0.19 ^{B}	6.42±0.09 ^{C}
28th day	3.41±0.11 ^{C}	4.86±0.02 ^{B}	5.80±0.03 ^{D}	5.29±0.07 ^{C}	4.89±0.08 ^{B}	5.34±0.03 ^{C}	4.51±0.17 ^{C}	4.68±0.08 ^{B}	5.50±0.05 ^{B}

Different capital letters show difference among time at each product at α=0.05 level. (1: C, 2: YC+B.L, 3: 0.2GA, 4: 0.03FM, 5: 0.05FM, 6: 0.1FM, 7: 0.03FM+0.2GA, 8: 0.05FM+0.2GA, 9:0.1FM+0.2GA)

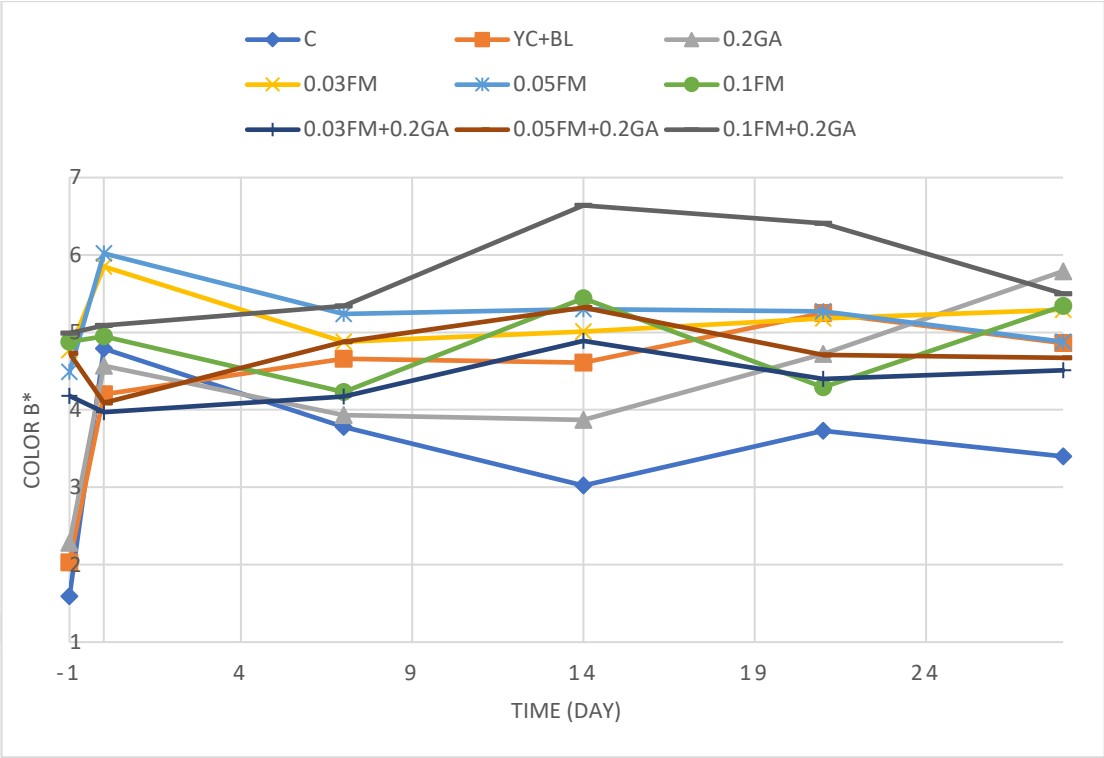

Figure 3.8 Effect of FM, GA and *B. lactis* + YC on the color b* at different concentrations and through fermentation and storage time (FM; flaxseed mucilage, GA; gum arabic, YC; yogurt culture, BL; *B. Lactis*)

3.7 Total solid measurements

Total solid of control, 0.2% of gum arabic, yoghurt culture and *Bifidobacterium* lactis, flaxseed mucilage in different concentrations (0.03, 0.05 and 0.1%), flaxseed mucilage in different concentrations (0.03, 0.05 and 0.1%) + (0.2%) was measured at the termination of storage period. Total solid results are shown gum arabic in Table 3.17.

Incorporation of flaxseed mucilage and gum arabic into kefir showed approximately the same total solid comparing to control and the peak of total solid was at 0.2% of gum arabic (Figure 3.9). Montenegro et al. (2012) found the gum arabic contents 78 - 88% of solid materials and essential amin acid. Also, another researcher Niamah et al. (2016) reported that increase concentration of gum arabic caused increased solid contents in yoghurt production. Also, Gul et al. (2018) demonstrated that the total solid contents of both the buffalo and cows' milk were 11.5 g/100 g.

Table 3.17 Effect of addition of flaxseed mucilage and gum arabic on total solid value after the storage time

	Total solid %
1	11.6294± 0.12 [a,b]
2	11.8766± 0.17 [b]
3	12.0532± 0.14 [b]
4	11.9101± 0.24 [b]
5	11.8617± 0.04 [b]
6	11.7527± 0.09 [a,b]
7	11.9754± 0.36 [b]
8	11.7457± 0.06 [a,b]
9	11.4195± 0.16 [a]

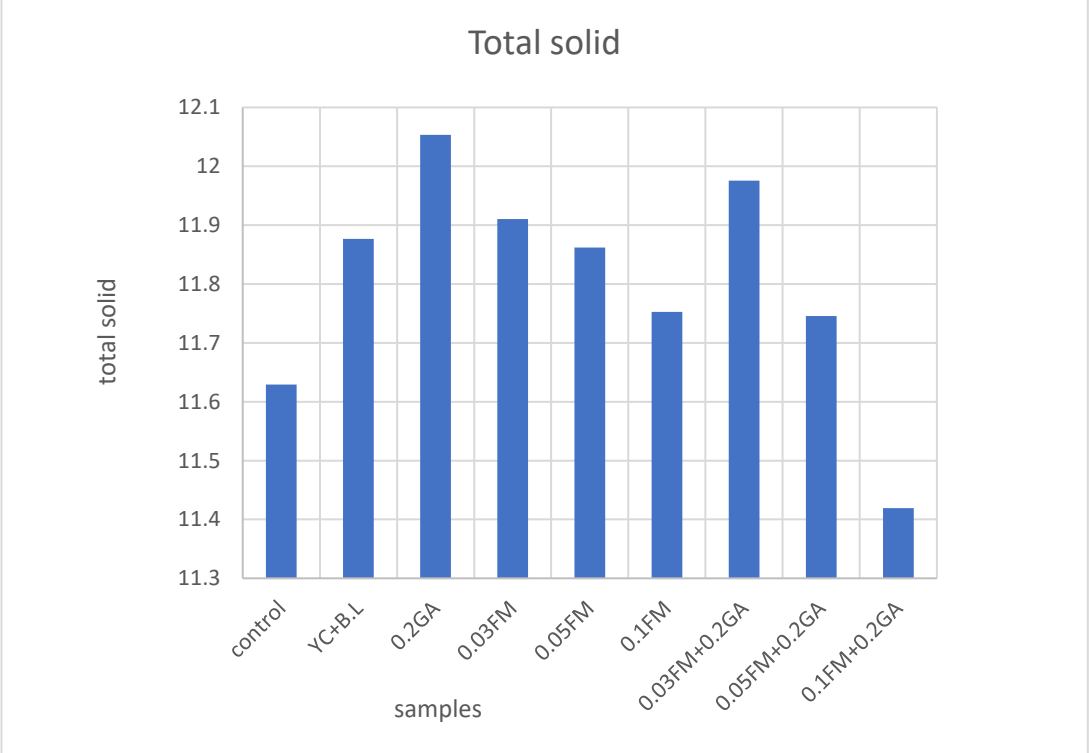

Figure 3.9 Effect of FM, GA and *B. lactis* + YC on the total solid at the end of storage (FM; flaxseed mucilage, GA; gum arabic, YC; yogurt culture)

3.8 Protein measurements

The results of protein content for control, 0.2% of gum arabic, yoghurt culture and *Bifidobacterium* lactis, flaxseed mucilage in different concentrations (0.03, 0.05 and 0.1%), flaxseed mucilage in different concentrations (0.03, 0.05 and 0.1%) + gum arabic (0.2%) was measured at the end of storage time. Protein contents are shown in Table 3.18.

All samples of kefir with flaxseed mucilage and gum arabic were shown the higher protein contents compared to control. Sample containing yogurt culture + *Bifidobacterium* and sample with gum arabic showed the same value of protein contents. Protein contents of kefir samples were dependent on the protein content of milk, protein values of kefir samples were 3.3g/100 mL (Renner and Renz, 1986; Halle et al., 1994). Also, Niamah et al. (2016) reported that percentage of proteins increased as gum arabic concentration increased in yogurt samples. For samples of flaxseed mucilage as concentration of FM increased the protein contents were also increased and the highest value observed at 0.1% of flaxseed mucilage. Daun et al. (2003) reported that flaxseed contained high protein content.

Table 3.18 Effect of addition of flaxseed mucilage and gum arabic on protein content after the storage time

	Protein %
1	12.33± 0.60 ^{a}
2	16.15± 0.00 ^{c,d}
3	16.15± 0.60 ^{c,d}
4	14.88± 0.60 ^{c}
5	15.51± 0.90 ^{c,d}
6	16.36± 0.30 ^{d}
7	13.60± 0.00 ^{b}
8	15.08± 0.30 ^{c,d}
9	15.30± 0.60 ^{c,d}

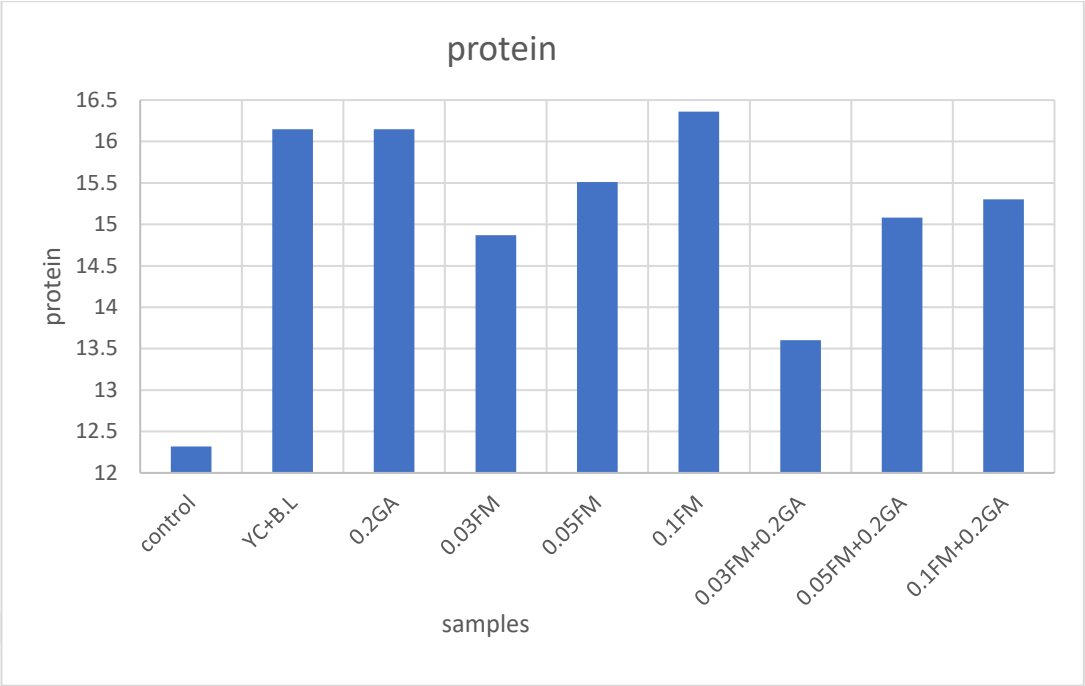

Figure 3.10 Effect of FM, GA and *B. lactis* + YC on the protein content at the end of storage time (FM; flaxseed mucilage, GA; gum arabic, YC; yogurt culture, BL; *B. Lactis*)

3.9 Sensory analysis

The mean scores of sensory evaluations of kefir are showed in Figure 3.11. Samples fortified with 0.2% of gum arabic had higher sensory scores in appearance and thickness compared to control. For color scores, samples of 0.2% of gum arabic was approximately similar to control sample but smell was less than that of control (Figure 3.11).

Incorporating of yogurt culture and *Bifidobacterium* into kefir samples had similar color and appearance scores compared to control but it slightly less than control in smell and thickness characteristics (Figure 3.11).

However, kefir samples enriched with flaxseed mucilage had effect on sensory scores; smell was highly influenced by addition of flaxseed mucilage in the kefir as the sample containing 0.1% of flaxseed mucilage alone and mixing with gum arabic received the least score by the panelists throughout storage period. Also, thickness scores dropped by increasing flaxseed mucilage concentration (Figure 3.11). For appearance, as concentration of flaxseed mucilage increased the appearance score

increased but still less than that of control. Color scores approximately similar to each other for 0.03, 0.05 and 0.1% samples of flaxseed mucilage.

In terms of mixing of 0.03, 0.05 and 0.1% of flaxseed mucilage with 0.2% of gum arabic, it had achieved the least sensory scores among other samples. All the mixed sample of flaxseed mucilage and gum arabic had received approximately the similar scores in appearance and color characteristics (Figure 3.11). Samples of 0.1% of flaxseed mucilage with 0.2% of gum arabic had the least smell score and samples of 0.05% of flaxseed mucilage with 0.2% of gum arabic had received the least thickness score (Figure 3.11).

Table 3.19 Effect of flaxseed mucilage and gum arabic addition on sensory attributes during storage time.

Characteristics	Samples								
	1	2	3	4	5	6	7	8	9
Appearance	2.25	2.23	2.66	1.95	2.21	2.28	1.96	1.87	1.89
Color	2.83	2.83	2.8	2.63	2.71	2.64	2.44	2.44	2.41
Smell	2.45	2.41	2.14	1.87	1.81	1.42	1.56	1.62	1.25
Thickness	2.56	2.46	2.63	2.31	2.26	2.15	2.08	1.84	1.92

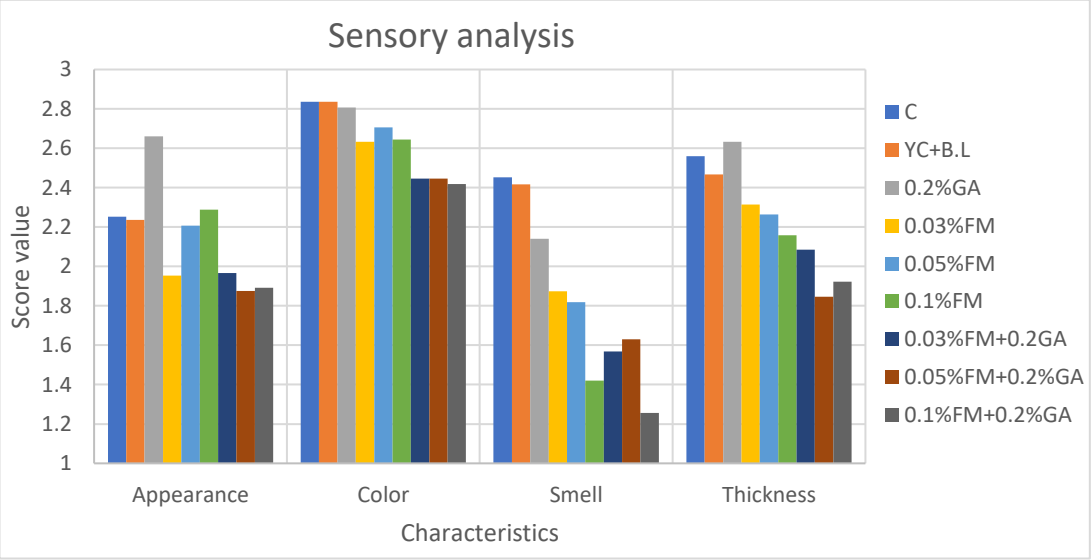

Figure 3.11 Effect of flaxseed mucilage and gum arabic on sensory evaluations during storage time. All scores are the mean of seven data n=31.

CHAPTER IV

CONCLUSION

In present study, effects of flaxseed mucilage in different concentrations (0.03, 0.05 and 0.1%) alone and mixed with gum arabic and yogurt culture with *Bifidobacterium lactis* on the growth changes in pH, titratable acidity, viscosity, Hunter L*, a* and b*, total solid and protein values and sensory analysis were investigated at 0, 1,st 7,th 14,th 21,st 28th days of storage.

- Addition of flaxseed mucilage at 0.03, 0.05 and 0.1% alone and mixed with 0.2% of gum arabic caused to increase the growth of *L. acidophilus* in comparison with control sample from 0 up to 21st days during storage.
- Fortified kefir with 0.03% yoghurt culture and 0.06% *Bifidobacterium* help in supporting the growth of *L. acidophilus* until 21st days of storage.
- The addition of flaxseed mucilage at 0.03, 0.05 and 0.1% alone and mixed with 0.2% of gum arabic caused to increase the growth of *Bifidobacterium lactis*, titratable acidity and viscosity in comparison with control sample start from 0 day until 28th days during storage.
- In contrast, enriched kefir with 0.2% of gum arabic, 0.03% yoghurt culture and 0.06% *Bifidobacterium* alone decreased the growth of *Bifidobacterium lactis* in comparison with control sample at all days of storage.
- Fortified kefir samples with 0.03, 0.05 and 0.1% of flaxseed mucilage alone and mixed with 0.2% of gum arabic, 0.03% yoghurt culture and 0.06% *Bifidobacterium* caused to drop in pH value at all days of storage compared to control
- Samples of 0.2% of gum arabic showed increase in L* value compared to control at 0, 14th, 21st and 28th days during storage.

- Addition of 0.03, 0.05 and 0.1% of flaxseed mucilage increased L* value compared to control at 0 and 14th days of storage. However, as concentration of flaxseed mucilage increased the L* value decreased.
- Addition of 0.03, 0.05 and 0.1% of flaxseed mucilage alone and mixed with 0.2% of gum arabic caused to increase in a* and b* value compared to control at all days of storage.
- Addition of 0.2% of gum arabic into kefir samples increased in a* value compared to control at 0, 1st and 7th days of storage.
- Samples of 0.2% of gum arabic showed significant increase in b* value compared to control at 0, 14th and 28th days of storage.
- Incorporation of flaxseed mucilage at 0.03, 0.05 and 0.1% concentrations and 0.2% of gum arabic into kefir samples was showed the same total solid contents in comparison with control and the highest total solid value was observed at 0.2% of gum arabic.
- All samples of kefir with flaxseed mucilage at 0.03, 0.05 and 0.1% alone and mixed 0.2% of gum arabic were shown the higher protein contents compared to control. Also, as concentration of FM increased the protein contents were increased and the highest value observed at 0.1% of flaxseed mucilage.
- Kefir samples enriched with flaxseed mucilage had effect on sensory scores; smell and thickness were highly influenced by addition of flaxseed mucilage and it received the least score by the panelists throughout storage period. For appearance, as concentration of flaxseed mucilage increased the appearance score increased but still less than that of control. Color scores approximately the same for all samples of flaxseed mucilage. In terms of mixing samples, it had achieved the least sensory scores among other samples.
- The taste and smell of mucilage is undesirable in kefir, so in future, we have to work on addition other substances that improve taste and have role in promotion of probiotics.

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