PRODUCTION OF KEFIR FROM BOVINE AND OAT MILK MIXTURE

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ABSTRACT

PRODUCTION OF KEFIR FROM BOVINE AND OAT MILK MIXTURE

During recent years non-dairy milk types such as cereal and grain milks have been an increased demand from consumers due to their functional properties. The cereal and grain milks do not contain cholesterol or lactose; therefore, these milk types are preferred by consumers who are vegetarians, have special diets or are lactose intolerant.

In this study, different concentrations of oat milk (0, 15, 30, 45, and 60%), blueberry aroma(9, 12, 15, 18, and 21%), and kefir culture(1, 2, 3, 4, and 5%) were used for the optimization of the kefir production and samples were stored at 4ºC for 21 days. The response surface methodology was used for the optimization process. Sensory characteristics, the pH changes and microbial characteristics of the kefir samples were determined during storage and the concentrations of the oat milk, blueberry aroma and kefir culture for the best three kefir products were chosen based on the optimization results

According to optimization results, three kefir samples which contained the highest level of oat milk with optimum organoleptic characteristics, optimum pH and optimum microbial counts were selected and produced. Based on the organoleptic results, kefir samples composed of 20% oat milk, 4% kefir culture and 10% aroma concentration, were produced. According to the pH results kefir samples within 15% oat milk, 4% kefir culture and 9% aroma concentration were produced. Based on the microbiological results kefir samples within 30% oat milk, 3% kefir culture and 15% aroma concentration were produced. The pH, titratable acidity, dry matter, fat, protein, phenol content, beta-glucan content, whey off, viscosity, volatile and organic acid profile of samples, color change, microbiological characteristics and sensory characteristics of these samples were investigated during 21 days of storage.

ÖZET

İNEK VE YULAF SÜTÜ KARIŞIMINDAN KEFİR ÜRETİMİ

Son yıllarda hububat ve tahıl gibi hayvansal kaynaklı olmayan sütler, yüksek seviyedeki fonksiyonel özelliklerinden dolayı tüketiciler tarafından tercih edilmekte ve tüketimi artmaktadır. Ayrıca bu sütler kolesterol ve laktoz içermediklerinden dolayı diyet uygulayan kişiler, vejetaryenler, laktoz intoleransı olan kişiler tarafından süt tüketiminde tercih edilmektedir.

Bu çalışmada, farklı miktarda yabanmersini aroması (9-12-15-18 ve %21), kefir kültürü (1-2-3-4 ve %5) ve yulaf sütü (0-15-30-45 ve %50) içeren kefir ürünlerinin optimizasyonu yapılmış ve 4 C de 21 gün boyunca depolanmıstır. Optimizasyonda yüzey tepki yöntemi kullanılıp, örneklerin mikrobiyolojik, pH ve duyusal karakteristikleri incelenmiştir. Optimizasyon sonucunda ideal seviyede mikrobiyolojik, pH ve duyusal özellik gösteren kefir örneklerinden en fazla yulaf sütü içeriğine sahip olan üç örnek tekrar üretilip, bu örneklerde fiziksel, kimyasal mikrobiyolojik, duyusal ve aroma özellikleri depolama süresi boyunca analiz edilmiştir.

Optimizasyon sonuçlarına göre en ideal duyusal özelliklere sahip olan %20 yulaf sütü, %4 kefir kültürü ve %10 aroma konsantrasyonundaki ürün üretilmiştir. pH sonuçlarına göre %15 yulaf sütü, %4 kültür ve %9 aroma konsantrasyonuna sahip olan ürün üretilmiştir. Mikrobiyolojik analizlere göre %30 yulaf sütü, %3 kültür ve %15 aroma içeren ürün ideal ürün olarak üretilmiştir.

Optimizasyon sonrası üretilen örneklerin pH, titreedilebilir asitlik, kuru madde, yağ, protein, fenol, beta-glukan, su salma, viskozite, uçucu ve organik asit bileşenleri, renk değişimi, mikrobiyal özellikleri ve duyusal karakteristikleri depolanma süresi boyunca incelenmiştir.

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CHAPTER 1

INTRODUCTION

Traditional foods have been consumed locally or regionally for many generations around the world (European Commission, 2007; EuroFIR, 2009). They have been produced for ancient times and fermented foods had longer shelf-life and improved nutritional values compared to their unfermented equivalents.

The most important fermented food products are fermented milk products which are dairy foods that have been fermented with yeast and lactic acid bacteria such as *Lactobacillus* ssp., *Lactococcus* ssp. and *Leuconostoc* ssp. Fermenting milk provides many advantages to food. These advantages are the extension of shelf-life of products, improvement in taste and digestibility of products (Rasic and Kurmann 1978; Pederson, 1979).

Kefir is one of the important fermented milk product which was originated in central Asia between the Caucasus Mountains and Mongolia, and is very popular in many countries nowadays, such as Turkey, Russia, Poland, Czech Republic, Slovakia, Hungary, Bulgaria, Scandinavian countries, The United States, Brazil and Japan (Marshall and Cole 1985; Duncan, 1986; Koroleva, 1988b; Libudzisz and Piatkiewicz 1990; Saloff-Coste, 1996; Dzwolak and Ziajka, 2000; Grønnevik, 2011). It is a carbonated fermented milk product made by using a complex mixture of microorganisms known as kefir grains. It is a refreshing fermented milk beverage that has an alcoholic flavor (Güzel-Seydim, et al. 2000a).

Today alternative to animal source milk types, non-animal source milk types such as oat milk, rice milk, mill milk, coconut milk, peanut milk and soymilk, are used in fermented milk production. Oat is a good source of many compounds that show antioxidant activity with their vitamins, phenolic acids, avenanthramides, flavonoids, sterols and phytic acid (David, 2000). Oat milk also contains high percentage of fibre, vitamins A, D, E and B1, minerals such as calcium, potassium, sodium, magnesium and iron. This composition of oat and oat milk provides more functionality to food such as improving beneficial effects for digestive system and preventing against colonorectal cancer and helping to maintain an optimal weight due to high fibre content. Also oat

milk exhibits cholesterol and lipid-lowering effects (Wood, 1991; Behall, Scholfield, and Hallfrisch, 1997; Ognning, et al. 1998; Ognning, et al. 1999; Murphy, et al. 2004).

In the light of above-mentioned facts, the main objective of this study is to develop a functional fermented food product by optimizing composition and production methods of bovine-oat milk mixture kefir with investigating the chemical, microbiological and organoleptic characteristics. The spesific objective is to investigate the chemical, physical, microbiological and sensorial changes of the developed kefir products during storage.

CHAPTER 2

LITERATURE REVIEW

2.1. Milk

Milk is a fluid lacteal secretion obtained by the female of all mammals. Milk has an important function because it is a source of the essential nutrients for the proper development and maintenance of the human body. It must supply amino acids, vitamins, and minerals. It is very beneficial to balance human diet. Because milk has good quality protein such as caseins and serum proteins, it also has good amount of calcium and vitamins, specially vitamin A, B and C, riboflavin, niacin, and folic acid.

Hence, milk is an ideal nutrient for both infants and adults (Yetişemeyen, et al. 2007). Moreover, milk contains bio-protective molecules which are afford health security to humans including antimicrobial substances such as immunoglobulin, lactoperoxidase and lactotransferrin and it also contains enzymes and enzyme inhibitors, vitamin-binding carrier proteins (Fox, et al. 2000). Further it contains trace elements such as nickel, selenium, zinc and iron (Tekinşen, 2000).

The composition of milk differs from each other according to milk producing species. In addition to the species, geographical location and requirement for the neonates affect the composition of milk. This difference is clarified especially in milk proteins and fats (Tamime, 2006; Yetişemeyen, et al. 2007). Also, genetic constitution and age of the individual species, stage of lactation, number and time of milkings and disease conditions, seasons, and motion affect the milk composition (Üçüncü, 2005).

The usage of milk from different species depends on geographical conditions. There are more than 4,000 species (Fox, et al. 2000). The cow is the main source of milk and it has been the major dairying species in many regions of the world. Buffaloes are used significantly to milk production in the Indian subcontinent and Egypt. Ewe and goat are important in the Mediterranean regions, parts of the Middle East and some regions of Africa. The camel is substantial source of milk in desert regions of North and East Africa, and the Middle East (Tamime, 2006). Horses are also used in milk production in East Europe and Middle Asia (Yetişemeyen, et al. 2007).

2.2. Oat Milk

During recent years non-dairy milk types, such as soymilk, coconut milk, almonds milk, mill milk, rice milk and oat milk, have been an increased demand from consumers due to their high functional properties. The cereal and grain milks also do not contain cholesterol or lactose; hence, these milk types are preferred by someone who are vegetarians, have special diet or who are lactose intolerant (Durand, et al. 2002).

Oat is a good source of many compounds that present antioxidant activity with its vitamins, phenolic acids, avenanthramides, flavonoids, sterols and phytic acid (David, 2000). Oat beverage is also a good source for fiber compounds such as beta-glucan, which are beneficial for digestive system and preventive against colonorectal cancer help to maintain an optimal weight due to high fibre content and exhibits cholesterol and lipid-lowering effects (Wood, 1991; Behall, Scholfield, and Hallfrisch, 1997; Ognning, et al. 1998; Ognning, et al. 1999; Murphy, et al. 2004). It has been reported that the use of oat supported the growth of lactic acid bacteria such as *Lactobacillus plantarum* to probiotic levels (Kedia, et al. 2008).

Oat milk contains high percentage of fibre, vitamins A, D, E and B1, and minerals (calcium, potassium, sodium, magnesium and iron). Oat milk is consumed as drink, shakes, may be used for cooking and baking, for sauces and soups, pancakes and cakes. Chemical composition of oat milk is given in Table 2.1. Oat also used in mill milk has a smilar composition as oat milk. Composition of mill milk is given in Table 2.2.

Table 2.1. Chemical composition of oat milk

Component	(g/250 ml)
Protein (g)	4.4
Carbohydrates (g)	13.5
of which sugar (g)	9.8
Fat (g)	4.7
of which saturates (g)	0.8
Total fibre (g)	2
β-glucan (g)	0.8
Sodium (mg)	0.25

Table 2.2. Chemical composition of mill milk (medium)
(Source: Olof Masrtensson, et. al. 2000)

Component	Mill milk medium (g/100 g)
Protein (g)	1.1
Fat (g)	1.5
Maltose (g)	4.2
Maltodextrin (g)	2.7
Dry matter (%)	11
Total fibre (g)	0.8
β-glucan (g)	0.4
α-tocopherol (mg)	0.1
Thiamin (mg)	0.04
Riboflavin (μg)	9.6
Niacin (mg)	0.1
Folic acid (μg)	3.3
Pyridoxine (mg)	0.01
Iron (mg)	0.1
Magnesium (mg)	4.7
Manganese (mg)	0.1
Phosphorus (mg)	27
Sodium (mg)	11
Zinc (mg)	0.1

Figure 2.1. Oat milk powder

Oat milk powder is given in Figure 2.1. which is used in oat milk production. Oat milk can also be produced from extraction of oat.

2.3. Fermented Dairy Products

Fermentation has being used in foods for thousands of years according to archaeological evidence. From time to time fermentation started to use for longer shelf life, and higher nutritional values. Today this technique is commonly using in vegetables, fruits, cereals, meat, milk and fish and the fermented prodcuts are consumed around the world (Farnworth, 2005).

Fermented dairy products are important part of fermented foods and their beneficial effects on health were investigated a hundred years ago. It was reported that consuming fermented dairy products caused to longevity (Amer and Lammerling 1983).

Bacteria, especially probiotics, grown during fermentation have positive effects on health such as improvement on digestive system, lowering effects on cholesterols, improvement in immune system, beneficial for lactose intolerance, and having antimutagenic effects (Fanworth 2005; Seydim, et al. 2011).

It is exposed that fermented milk products has antimutagenic activity for mammalian cell system and mutagens can binding by lactic acid bacteria (Nadathur, et al. 1996; Pool Zobel, et al. 1993; Guzel-Seydim, et. al.2006).

These beneficial effects of fermented dairy products and researches cause awareness rising on consumer choice. Today lots of people chose fermented dairy products around the world. Wide range of fermented dairy products are manufactured and consumed in the world. Lots of traditional fermented milk products are also produced and some of these are given in Table 2.3.

Table 2.3.Fermented dairy products
(Source: Ertekin, 2008)

Traditional Name	**Country/ Region**
Yogurt, buttermilk	Turkey
Kefir, kefer, knapon kephir,kiaphur,kepi,kippi	Caucasus
Koumiss, kumiss, kymys, kymys	Central Asia
Skyr, Súrmjólk	Iceland
Busa	Turkestan
Kissel mleka/ naja/ yaourt	Balkan Peninsula
Urgotnic	the Balkan Mountains
Leban/labanya or rayeb	Lebanon, Arab countries
Zabady/zabade	Egypt and Sudan
Lassi and Dahi/dadhi/dahee	India
Doogh/dough/mast	Iran, Afghanistan
Roba/rob	Iraq
Mazun/matzoon,matsun,matsoni,madzoon	Armenia
Katyk	Transcaucasia
Yiaourti	Greece
Tarho/taho	Hungary
Iogurte	Brazil and Portugal
Leben	Israel
Donskaya/varenetes/kurugna/ryzhenka/gulsyanka	Russia
Matsoni, matson, matsoon	Georgia
Dadiah, Dadih	Indonesia
Viili, Piimä	Finland
Shosim/sho/sho/thara	Nepal
Blaand	Scotland
Lapte batut	Romania
Gruzovina	Yugoslavia
Filmmjolk/fillbunke/filbunk/surmelk/taettemjolk /tettemelk	Scandinavia
Kiselo mleko	Macedonia

(cont. on next page)

Table 2.3. (cont.)

Traditional Name	Country/ Region
Clabber	USA
Amasi	South Africa
Cieddu	Italy
Mezzoradu	Sicilia
Kwaśne mleko/ Zsiadłe mleko	Poland
Tarag	Mongolia

Kefir includes all nutrients of milk and has lots of beneficial effects on health. Consumption rate around the world and research areas are increasing nowadays because of these beneficial effects of kefir.

2.4. Kefir

Kefir is a carbonated fermented milk product made of using a complex mixture of microorganisms known as kefir grains. It is a refreshing fermented milk beverage that has an alcoholic flavor (Güzel-Seydim, et al. 2000a).

2.4.1. Historical Background

It is believed that kefir was originated in Central Asia between Mongolia and Caucasus mountains. (Kurman, et al.1992). Kefir is termed from Turkish word “keyf” or “kef” which means feeling good and pleasant taste. (Kurman, et al. 1992; Chaitow and Trenev 2002). Kefer, knapon kephir, kiaphur, kepi and kippi are also used as a kefir term (Koroleva 1988a). Kefir is defined that it is the yogurt of the 21st century (Gorski, 1994, Frengova, et al. 2002). It is also described as dairy champagne and the champagne of cultured dairy products. (Kemp, 1984; Mann, 1989).

Nowadays kefir is a popular drink especially in Europe. It is produced in Turkey, Poland, Hungary, Russia, Finland, Sweden, Norway, and Germany (Marshall and Cole 1985; Koroleva, 1988b; Libudsisz and piatkiewicz 1990, Heidi Grønnevik, 2011) Kefir is also produced in America, Japan, and Brazil. (Saloff-Coste, 1996)

2.4.2. Kefir Grains

2.4.2.1. Chemical Composition and Appearance

Kefir grains range in size from 0.3 to 2.0 cm or more in diameter, and are characterized by forming an irregular, folded or uneven surface; the grains resemble cauliflower florets in shape and color. They are elastic and white or slightly yellow in color, and have a characteristic smell. Kefir grains have a specific structure and biological function. When the grains are seeded in milk, they grow and pass their properties to the following generations of newly formed grains (Guzel-Seydim, 2000b; Saloff-Coste, 2002; Simova et al. 2002).

Kefir grain's appearance and electron micrograph of a kefir grain are given in Figure 2.2.a and 2.2.b.

(a) **(b)**

Figure 2.2. Kefir grains (a) SEM view of kefir grain (b)
(Source: a) Farnworth, 2005, b) Güzel-Seydim, et al. 2005a)

The FAO/WHO (2001) has recommended a definition of kefir based on the microbial composition of both kefir grains (the starter culture used to produce kefir) and the final kefir product is given in Table 2.4.

Table 2.4. Codex Alimentarius description of kefir (Source: *Codex Standard for Fermented Milks CODEX STAN 243-2003 FAO/WHO 2001)*

Composition	Amount
Milk protein (100 g^{-1})	> 2.8
Milk fat (%100 g^{-1})	*<10*
Titratable acidity, expressed as % of lactic acid (100 ml^{-1})	> 0.6
Ethanol (% vol./w)	not stated
Sum of specific microorganisms constituting the starter culture (cfu/g, in total)	$>10^7$
Yeasts (cfu /g)	$> 10^4$

According to kefir standards, kefir which is prepared with milk should contain higher than 2.8% milk protein, lower than 10% milk fat, higher than 0.6% titratable acidity which is expressed as % of lactic acid, higher than 10^7 cfu/g total sum of specific microorganism which are lactic acid and acetic acid bacteria, higher than 10^4 cfu/g yeast. However, ethanol content not stated, can be change according to yeast population.

2.4.2.2. Microflora

Source of kefir grains are not know but Motaghi and his friends stated that kefir grains could be produced by using traditional method of handling milk (Motaghi, et al. 1997)

Kefir and kefir grains contain several bacteria. Microbial flora of kefir mainly includes lactobacilli and lactococci species. Kefir grains also contain streptococci, enterococci, leuconostocs, acetic acid bacteria and other bacteria types. Isolated bacteria from kefir grains are given in Table 2.5.

Table 2.5. Bacteria found in kefir grains and kefir

***Lactobacilli* ssp.**	**Reference**
Lactobacillus kefir	Koreleva, 1991; Pintado, et al. 1996; Kandler and Kunath 1983; Takizawa, et al. 1994; Garrote, et al. 2001; Santos, et al. 2003; Angulo, et al. 1993; Mobili, et al. 2008.
Lactobacillus delbrueckii	Koreleva, 1991; Simova, et al. 2002; Santos, et al. 2003;
Lactobacillus kefiranofaciens	Fujisawa, et al. 1988; Takizawa, et al. 1994; Santos, et al. 2003; Wang, et al. 2008; Vinderola, et al., 2007
Lactobacillus rhamnosus	Koreleva, 1991; Angulo, et al. 1993.
Lactobacillus kefirgranum	Takizawa, et al. 1994;
Lactobacillus casei	Simova, et al. 2002; Ergullu and Ucuncu 1983; Karagozlu, 1990
Lactobacillus parakefir	Takizawa, et al. 1994; Garrote, et al. 2001;
Lactobacilli paracasei	Santos, et al. 2003;
Lactobacillus brevis	Ottogalli, et al. 1973 Simova, et al. 2002; Santos, et al. 2003; Angulo, et al. 1993; Mobili, et al. 2008.
Lactobacillus fructivorans	Yoshida and Toyoshima 1994;
Lactobacillus plantarum	Garrote, et al. 2001; Santos, et al. 2003;
Lactobacillus hilgardii	Yoshida and Toyoshima 1994
Lactobacillus helveticus	Koreleva, 1991; Lin, et al. 1999; Simova, et al. 2002; Valasaki, et al., 2007**.**
Lactobacillus fermentum	Angulo, et al. 1993; Garbers, et al. 2004
Lactobacillus acidophilus	Ottogalli, et al. 1973; Santos, et al. 2003; Angulo, et al. 1993.
Lactobacillus viridescens	Angulo, et al. 1993.
Lactobacillus gasseri	Angulo, et al. 1993.
Lactobacillus mesenteroides	Garbers, et al. 2004
Lactobacillus crispatus	Garbers, et al. 2004
Lactococci	
Lactococcus lactis subsp. *lactis*	Koreleva, 1991; Pintado, et al. 1996; Yuksekdag , et al. 2004; Dousset and Caillet 1993; Ottogalli, et al. 1973; Simova, et al. 2002; Yoshida and Toyoshima 1994; Garrote, et al. 2001; Angulo et al. 1993, Ergullu and Ucuncu 1983; Kojic, et al., 2007; Mainville, et al. 2005.
Lactococcus lactis subsp. cremoris	Koreleva 1991; Yuksekdag, et al. 2004; Dousset and Caillet 1993, Mainville, et al., 2005
Lc. lactis subsp. *Lactis biovar.diacetylactis*	Garrote, et al. 2001
Streptococci	
Streptococcus thermophilus	Yuksekdag, et al. 2004; Simova, et al. 2002;
Streptococcus cremoris,	Ergullu and Ucuncu 1983; Karagozlu, 1990
Streptococcus faecalis	Ergullu and Ucuncu 1983; Karagozlu, 1990
Streptococcus durans	Yuksekdag, et al. 2004
Enterococci	
Enterococcus durans	Rosi, 1978; Yuksekdag, et al. 2004

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Table 2.5. (cont.)

Leuconostocs	
Leuconostoc sp.	Angulo, et al. 1993.
Leuconostoc mesenteroides	Koreleva, 1991; Lin, et al. 1999; Ottogalli, et al. 1973; Garrote, et al. 2001
Acetic acid bacteria	
Acetobacter sp.	Garrote, et al. 2001;
Acetobacter pasteurianus	Ottogalli, et al. 1973
Acetobacter aceti	Koreleva, 1991; Rosi, 1978;
Other bacteria	
Bacillus sp. *Micrococcus* sp.	Angulo, et al. 1993.
Bacillus subtilis Escherichia coli	Angulo, et al. 1993.

Simova et al. 2002 reported that kefir grains contained 83-90% lactic acid bacteria and *Lactobacillus bulgaricus* and *Lactobacillus helveticus* species are mainly found in *Lactobacillus* ssp.

Kefir and kefir grains also include yeasts such as *Saccharomyces* ssp., *Candida* ssp. and *Kluyveromyces marxianus.* These isolated yeasts are given in Table 2.6.

Kluyveromyces spp. are mainly responsible for the yeasty aroma in kefir (Engel, et al. 1986; Seiler and Kummerle, 1997). It is also reported that lactose negative yeasts are present in kefir (Angulo, et al.1993; Simova, et.al.2002). Yeasts are responsible for production of ethanol and CO_2 in kefir (Wouters, et. al. 2002).

Table 2.6. Yeasts found in kefir grains and kefir

Kluyveromyces marxianus	Koreleva, 1991; Lin, et al. 1999;Ottogalli, et al. 1973; Simova, et al. 2002; Wyder and Puhan 1997, 1999; Yoshida and Toyoshima 1994; Engel, et al. 1986; Garrote, et al. 2001; Angulo, et al. 1993; Rohm, et al. 1992
Candida friedrichii	Rohm, et al. 1992
Saccharomyces sp.	Garrote, et al. 2001;
Candida pseudotropicalis	Ottogalli, et al. 1973;
Saccharomyces cerevisiae	Koreleva, 1991; Rosi, 1978; Dousset and Caillet 1993; Ottogalli, et al. 1973; Simova, et al. 2002; Engel, et al. 1986; Angulo, et al. 1993; Rohm, et al. 1992
Saccharomyces unisporus	Pintado, et al. 1996; Wyder and Puhan 1997, 1999; Engel, et al. 1986; Angulo, et al. 1993;

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Table 2.6 (cont.)

Candida tenuis	Ottogalli, et al. 1973;
Candida inconspicua	Simova, et al. 2002;
Candida maris	Simova, et al. 2002;
Saccharomyces exiguus	Iwasawa, et al. 1982;
Saccharomyces turicensis	Wyder and Puhan 1997, 1999;
Candida lambica	Engel, et al. 1986;
Saccharomyces delbrueckii	Rosi, 1978; Engel, et al. 1986
Candida tannotelerans	Dousset and Caillet 1993;
Saccharomyces dairensis	Rohm, et al. 1992
Candida valida	Dousset and Caillet 1993;
Torulaspora delbrueckii	Koreleva, 1991; Wyder and Puhan 1997, 1999;Angulo, et al. 1993;
Candida kefyr	Koreleva, 1991; Engel, et al. 1986; Rohm ,et al. 1992
Brettanomyces anomalus	Wyder and Puhan 1997, 1999;
Candida holmii	Engel, et al. 1986; Angulo, et al. 1993;
Issatchenkia occidentalis	Engel, et al. 1986;
Pichia fermentans	Lin, et al. 1999; Angulo, et al. 1993; Rohm, et al. 1992

2.4.3. Kefir Production

In industrial production of kefir, bovine milk is mainly used but ewe milk, goat milk, camel milk and buffalo milk are also used. Besides coconut, soy and rice milks could be used in kefir production (Mann, 1985; Ötles and Çagındı, 2003; Powell, 2006).

Traditional kefir production flow chart is given in Figure 2.3. Kefir has been produced at home conditions in this method. Firstly milk is boiled then is cooled to 25^{o}C. Kefir grains (3-3,5%) are inoculated to milk and fermented at room temperature for 18-24 hours. Kefir grains are separated from curd and milk. Kefir grains are cleaned with water and stored at 4^{o}C until the next fermentation.

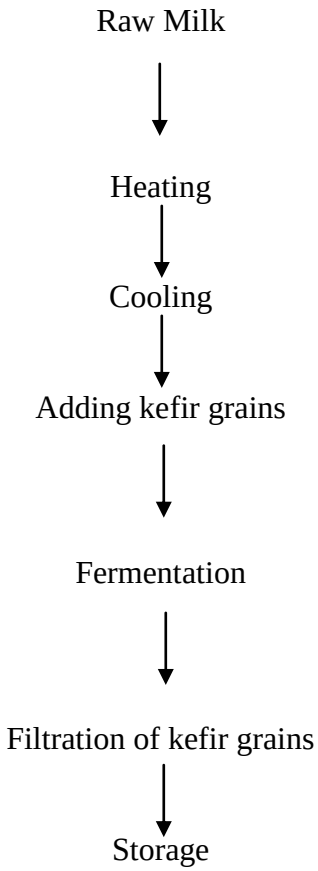


Figure 2.3. Traditional kefir production flowchart

Traditional production has few differences from industrial production. Kefir grains or kefir culture lyophilized could be used in industrial kefir production. Industrial kefir production flowchart is given in Figure 2.4.

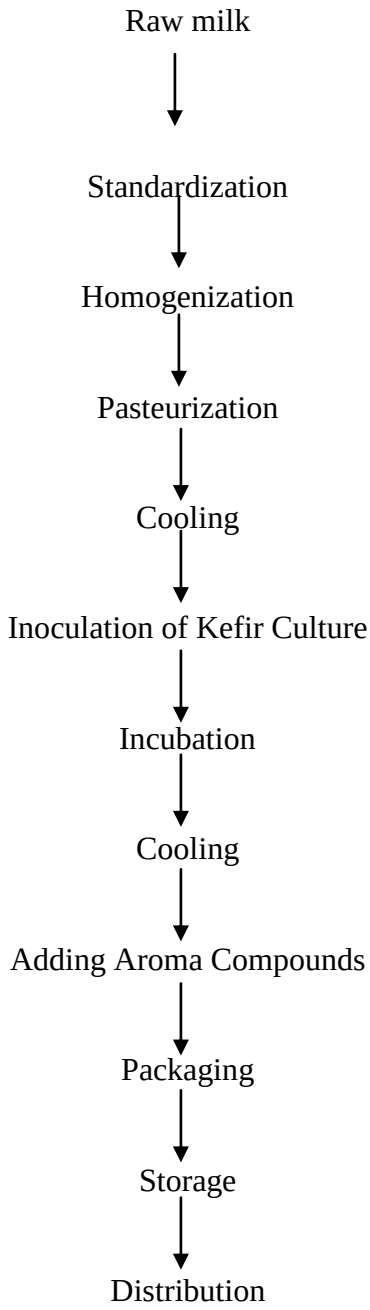


Figure 2.4. Industrial kefir production flowchart

In industrial kefir production firstly, milk is standardized and homogenized. Then milk is pasteurized and cooled to 25^{o}C. Kefir culture is inoculated to milk and incubated at 25^{o}C for 18-24 hours until the kefir's pH 4.60-4.65.If aroma is used in kefir

production, aroma is added after incubation step. Finally, kefir is packaged and storage at 4^{o}C. Glass or plastic materials are used in packaging.

2.4.4. Chemical and Physical Characteristics of Kefir

Characteristics of kefir change according to kefir grains, microbial content, composition of milk, and production method. Composition of kefir also changes in different countries.

It was reported that typical composition of kefir consists of 3-3.4% protein, 1.5 % fat, and 2-3.5% lactose. Lactic acid amount may change between 0.6 % and 1%, alcohol level 0 to 0.1% (Bottazzi, et al. 1994; Halle, et al. 1994). Standards of kefir are given in Table 4 (*Codex Standard for Fermented Milks CODEX STAN 243-2003 FAO/WHO 2001).*

Chemical and microbiological characteristics of kefir in Poland are described as protein content not less than 2.7%, fat level less than 10%, titratable acidity not less than 0.6%, yeast count not less than 10^2 cfu/g bacterial count not specified (Anonymous 2002).

The pH of kefir decreases with the increase of homofermentative lactic acid bacteria and the growth of *Lactobaciusl* ssp. also decreases pH and induce to decrease of streptococcus enumeration. During fermentation lactic acid bacteria are more effective on development of aroma in kefir than yeasts and acetic acid bacteria, (Koroleva, 1982).

Microbiological, physicochemical and sensory characteristics of kefir were analyzed during storage and it was reported that yeasts and acetic acid bacterial counts investigated certain. However, lactic acid bacteria counts decreased between 7 and 14 days of storage. Total fat, lactose, dry matter and pH were investigated constant until to 14th day of storage. Sensory characteristics of kefir were also investigated and best scores were obtained in the first day of storage (Irigoyen, et. al. 2005).

Gronnevik, et. al. (2011) investigated microbiological and chemical properties of Norwegian kefir during storage and it was reported that lactic acid bacteria decreased during 4 weeks of storage. However, yeast numbers increased in this period. The increase of yeast population also caused increase in CO_2 and ethanol during storage. Glutamic acid was also reduced.

Chemical composition, structure and microbial communities of Brazilian kefir analyzed and it was explained that during fermentation lactic acid bacteria were more predominant then yeasts and Gram-negative bacteria. Increase of lactic acid bacteria caused an increase on lactic acid amount, whereas increase in yeast population increased ethanol amount. Chemical characteristics investigated on the first day of fermentation such as pH 4.42, protein 3.91%, total titratable acidity (TTA) 93, fat 2.34%, calcium 0.22% and dry matter of kefir grain was 9.62% (Magalhaes, et. al. 2011).

Physicochemical attributes of kefir under different cultural conditions were analyzed by Ismaiel, et al.(2011). Increase in titratable acidity was dependent on lactic acid production. Final pH was investigated more acidic (2.91 – 4.04). Highest growth and kefiran production of kefir were investigated using skimmed cows' milk.

2.4.5. Sensory Profile of Kefir

Kefir has a different sensory characteristics based on the production method. The usage of kefir grains or starter culture affects the sensory properties. The starter culture preparation, raw materials properties and fermentation conditions might cause changes in sensory characteristics of kefir. The traditional sensory properties of kefir made with kefir grains have acidic, but pleasant and refreshing taste, balanced and yeasty aroma, white or yellowish color and rather thick, but not gluey, with an elastic consistency texture (Duitschaever, et. al. 1987; Assadi, et. al. 2000; Wszolek, et. al. 2001).

Lactic acid content, volatile acids, acetic acid, ethanol, aldehydes, formic, orotic and propionic acids affect the taste of kefir (Muir, et al. 1999; Robinson, et al. 2002; Beshkova, et al. 2003). The main aroma forming compounds are diacetly and acetaldehyde in kefir. Their level affects the aroma and depends on the production method. Yeast level, and type fermentation time kefir grain or starter culture type affect the alcohol content of kefir. Kefir may contain 0.1-1.0 g 100 mL alcohol (Molska, 1988; Robinson, et al. 2002)

2.4.6. Beneficial Effects of Kefir

Fermented dairy products have lots of health benefits and kefir has had a long history of being beneficial to health in Eastern European countries, where it is associated with general wellbeing. It is easily digested (Alm, 1982c)

The chemical composition of kefir provides the nutritional value of products. A typical compositional analysis of kefir consists of protein 3-3.4%, fat 1.5% and lactose 2.0-3.5%, lactic acid content 0.6-1.0% (Bottazzi, et. al. 1994; Halle, et. al. 1994). Kneifel and Mayer (1991) reported that the vitamins in kefir made using grains and milk from different species of mammals increased by 20% such as thiamine (B_1) in ewe's milk kefir, pyridoxine(B_6) in kefir made with ewe's, goat's and mare's milk. Folic acid and orotic acid content was reduced in kefir production during fermentation.

Kefir is beneficial for improving lactose tolerance, improving immune system, improving gastrointestinal system it has cholesterol lowering effects, anticarcinogenic properties, antimicrobial properties, probiotic and prebiotic properties. Kefir has positive effects on cholesterol metabolism. Kefir grains could assimilate and reduce cholesterol (Vujicic, et al.1992). L. acidophilus, L. plantarum and L. paracasei and some bifidobacterium strains performed cholesterol assimilation activity (Yoon, et al.1998).

Kefir has great antibacterial activity and antibacterial activity of kefir has been reported by many researchers (Garg, 1989; Serot, et al. 1990; Cevikbas, et al. 1994; Zacconi, et al. 1995, 2003; Atanassova, et al. 1999; Gulmez and Guven 2003a, b, c; Santos, et al. 2003; Yoon, et al. 2003).

Kefir has beneficial for improvement of the digestion of the milk proteins, hydrolysis of lactose, treatment of severe intestinal infections and the correction of dysbiosis in children (Sukhov, et al. 1986; Vrese, et al. 1992; Murashova, et al. 1994; Safronova, et al. 2001; Hertzler and Clancy 2003). It has also some anti-tumour activity (Shiomi, et al. 1982; Murofushi, et al. 1983; Furukawa, et al. 1990, 1991; Cevikbas, et al. 1994).

Therefore some kefir microorganisms can bind mutagenic substances, such as indole and imidazole (Hosono, et al. 1990; Miyamoto, et al. 1991; Tamai, et al. 1995, 1996). And it was researched that kefir and sphingomyelin obtained from kefir lipids may stimulate the immune system in young, but not old rats (Furukawa, et al. 1991;

Osada, et al. 1994; Thoreux and Schmucker 2001; see also Nagira, et al. 2003; Teruya, et al. 2003).

Kefir has also antimicrobial activity against Gram-positive and Gram negative bacteria and fungi (Garrote, et al. 2000).

2.5. Blueberry

Blueberries are members of the Ericaceae or Heath family, the genus is very various which is containing about 400 species. It has lots of wild species and they mostly found in tropical and high elevation regions. Also they can grow over temperate and boreal regions (Ratnaparkhe, 2007).

Blueberry is a small fruit crop. Appearance of blueberry is given in Figure 2.5. Crops have blue-black color.

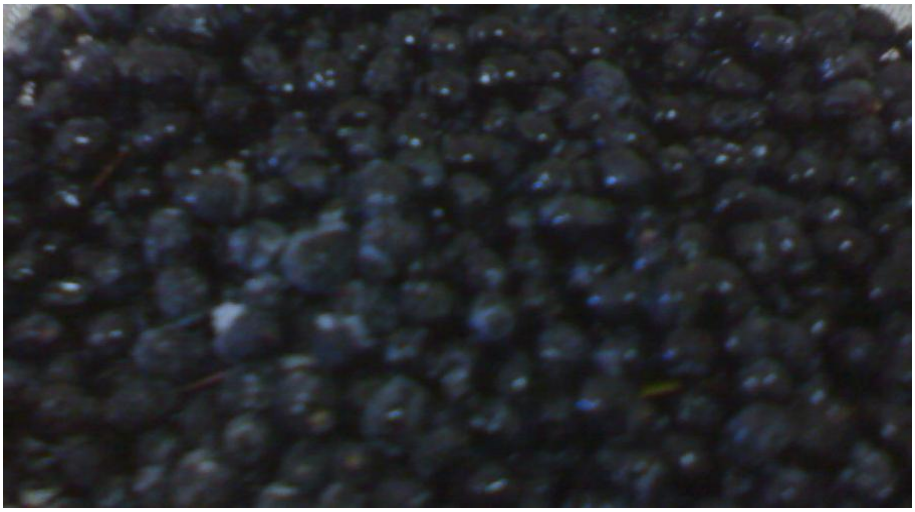


Figure 2.5. Blueberry

Blueberry was domesticated during 20th century. Major producer of blueberry are USA and Canada. Also Poland, Netherlands, France, Italy, Mexico, New Zealand and Lithuania are producer of blueberry. Blueberries were commercially divided to five types which are highbush blueberry, rabbiteye blueberry, the wild or lowbush blueberries, southern highbush blueberry and halfhigh blueberries. The highbush blueberry, rabbiteye blueberry and the lowbush blueberries are economically important blueberry types (Ratnaparkhe, 2007).

Consumption of blueberries is on the increase from day to day. Blueberries contain anthocyanins, flavonoids and polyphenols so blueberries have highest antioxidant capacity in fruit and vegetables. This antioxidant effect is correlated with anthocyanin and phenolic content (Prior, et al. 1998). Blueberry has also rich phenol content in fruits (Kahkönen, et al. 1999; Vinson, et al. 2001). Moreover, fresh blueberries are good source of vitamin C (Matzner, 1967).

Beneficial effects of blueberries were studied and it was reported that blueberries has protective effects against cancer and vascular diseases and blueberry has also antitumor effects (YI, et al. 2005; Schmidt, et al. 2006; Seraam, et al. 2006; Catherine and Neto 2007).

It was analyzed that blueberry had good effect on improving memory function in older adults (Krikorian, et al. 2010)

It was reported that blueberries inhibited lipid oxidation in leptosomes and LDL oxidation in vitro and in vivo (Heinonen, et. al. 1998; Marniemi, et al. 2000; Smith, et al. 2000; Viljanen, et al. 2004)

CHAPTER 3

MATERIALS AND METHODS

3.1. Materials

3.1.1. Chemicals and Media

The chemicals and media used in the study are listed in Appendix A.

3.1.2. Reagents and Solutions

Preparations of reagents and solutions used in this study are given in Appendix B.

3.1.3. Samples

In the first part of the study, thirty kefir samples, which were consisted of bovine and oat milk, were produced according to different storage time, aroma, culture and milk concentration (Table 3.1). In optimization step pH, microbiological and sensory properties were investigated. Analyses were done at 1^{st}, 6^{th}, 11^{th}, 16^{th} and 21^{th} days of storage. Samples were stored under refrigeration conditions during storage for pH, microbiological and sensory analysis.

In the second part of the study, three kefir samples were produced according to optimization results (Table 3.2). In this part samples were produced twice for replication. Chemical, physical microbiological, aroma and sensory properties were investigated during 1^{st}, 6^{th}, 11^{th}, 16^{th} and 21^{th} storage days.

Table 3.1.Produced kefir samples in optimization

Sample No	Storage days	Culture Con.	Aroma Con.	Oat Milk Con.
1	1	3%	15%	30%
2	6	2%	12%	15%
3	6	2%	12%	45%
4	6	2%	18%	15%
5	6	2%	18%	45%
6	6	4%	12%	15%
7	6	4%	12%	45%
8	6	4%	18%	15%
9	6	4%	18%	45%
10	11	1%	15%	30%
11	11	3%	9%	30%
12	11	3%	15%	0%
13	11	3%	15%	30%
14	11	3%	15%	30%
15	11	3%	15%	30%
16	11	3%	15%	30%
17	11	3%	15%	30%
18	11	3%	15%	30%
19	11	3%	15%	60%
20	11	3%	21%	30%
21	11	5%	15%	30%
22	16	2%	12%	15%
23	16	2%	12%	45%
24	16	2%	18%	15%
25	16	2%	18%	45%
26	16	4%	12%	15%
27	16	4%	12%	45%
28	16	4%	18%	15%
29	16	4%	18%	45%
30	21	3%	15%	30%

Table 3.2. Produced kefir samples according to optimization results

Sample no	Culture Con.	Aroma Con.	Oat Milk Con.
1	4%	10%	20%
2	4%	9%	15%
3	3%	15%	30%

3.2. Methods

3.2.1. Kefir Production

3.2.1.1. Preparation of Kefir Culture

Kefir culture was prepared with reconstituted milk which consists of 12% dry matter of skimmed milk powder (Pınar, İzmir). It was heated at 90^{o}C for 10 minutes and cooling to 25^{o}C. For 500 ml reconstituted milk Kefir DC1 (Danisco, Poland) starter culture was (1.65 g) inoculated at 25^{o}C. Culture sample was held until its pH 4.65 and its curd was broken. Holding time was recorded (18 hours). Culture was stored at 4^{o}C for 24 hours then it was used in kefir production.

3.2.1.2. Preparation of Oat Milk

Oat milk powder was used in oat milk preparation. The oat milk powder (13 g) was added to 100 ml water. The oat milk was pasteurized at 90^{o}C for 10 minutes then filtered and it was used in kefir production.

3.2.1.3. Preparation of Aroma

Blueberry was used in aroma preparation. Blueberries were washed and cleaned. Sucrose (75 g) and water (50 ml) were added to blueberry (100 g). Mixture was heated at 65^{o}C for 10 minutes. Soft pressing and stirring was implemented. Mixture was cooled

to room temperature and filtered. Liquid part of filtration was added kefir samples before storage.

3.2.1.4. Kefir Production and Sampling

Kefir production was given in Figure 3.1. Whole milk (3.4%) and oat milk mixture were used in kefir production. Thirty samples were produced in optimization step (Table 3.1) Kefir culture was inoculated at 25^{o}C. Samples were incubated until their pH 4.65. Holding time was recorded (17-20 hours). After fermentation was completed blueberry aroma was added to the samples. Kefir samples stored at 4^{o}C up to 21 days. The second part of this study 3 kefir samples were produced (Table3.2). Samples were stored at 4^{o}C up to 21 days. The experiment was repeated twice.

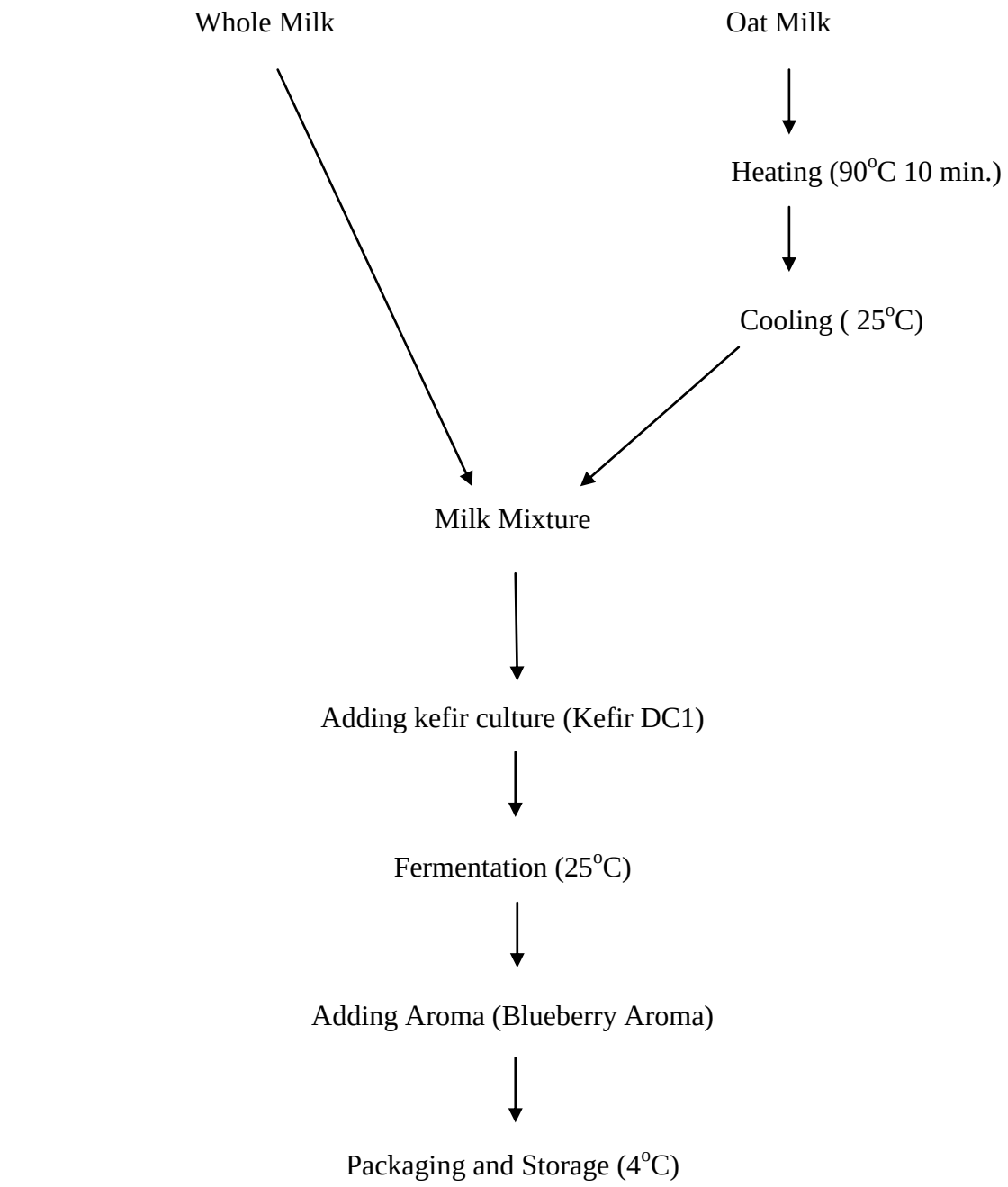


Figure 3.1. Kefir Production flowchart

3.2.2. Raw Materials Properties

Bovine milk's properties were measured using Lactostar (Funke Gerber, Berlin). Chemical and volatile properties were investigated in oat milk and aroma of blueberry.

3.2.3. Chemical and Physical Analysis

3.2.3.1. pH and Titratable Acidity

The pH of the kefir was determined using a pH meter (Hanna HI 221,Germany). Measurements were done twice and average values were reported.

For determination of titratable acidity, 2.5 g kefir was weighed and diluted with distilled water to 25g. Three drops of phenolphthalein were added to the solution and titrated with 0.1 N NaOH until the first permanent pink color appeared. All measurements were done in duplicate and average values were reported. Titratable acidity values were calculated according to equation 3.1.

% lactic acid = 0.1 N NaOH amount (ml) x 0.009 x 100) / Sample amount (g) (3.1)

3.2.3.2. Total Dry Matter Content

Total dry matter content of the kefir samples were determined gravimetrically by drying a sample to constant weight in an oven at 105ºC (IDF, 1986). Empty dishes were heated at 105ºC for 3 hours and cooling to room temperature in desiccator. 3 g kefir sample was added to predried weighing dish. Samples were heated at 105ºC for 4 hours and cooling to room temperature in desiccator. The difference in weight before and after drying gives the results of total dry matter content. Results were calculated by percentage according to equation 3.2

$$\% \, \mathbf{DM} = \left(\frac{\mathbf{W_1 - W}}{\mathbf{W_2 - W}}\right) \mathbf{X\ 100} \qquad (3.2)$$

W = Weight of predried dish (g)

W_1 = Weight of predried dish and dried sample (g)

W_2 = Weight of predried dish and sample (g)

3.2.3.3. Total Protein Content

For determination of total protein Kjeldahl method was used (IDF, 1993). Kefir sample (2.5 g), catalyst, antifoaming agent and H_2SO_4 (20 ml) were added to Kjeldahl tubes and they were placed into digestion unit (Gerhardt Kjeldaterm, Germany). The digestion was done at 420ºC until the solution in tubes became transparent. After the solution in tubes was cool, the tubes were placed into the distillation unit (Gerhardt Vapodest 50S, Germany). 80 ml distilled water; 50 ml H_3BO_3 and indicator were added into distillation unit. % protein content was observed with distillation, the flask was titrated with 0.1 N HCl. All measurements were done twice and average values were reported.

3.2.3.4. Total Fat Content

Fat content of samples were determined by Gerber method. The vessel was filled with 10 ml H_2SO_4 (d: 1.82 g /ml). Kefir sample (10 ml) was added into a butyrometer vessel and then 1 ml amyl alcohol was added. Butyrometer vessel was completed to level with distilled water. After that, the butyrometers were centrifuged in Gerber centrifuge for 10 min. The oil level was read as percentage oil in kefir from butyrometer vessel (IDF, 1997).

3.2.3.5. Determination of Whey off

100 ml kefir samples were added to graduated cylinders (100ml) after fermentation. Graduated cylinders were stored at 4^oC. Graduated cylinders checked during storage and phase separation was determined by percentage.

3.2.3.6. Rheological Analysis

The viscosity measurements were carried out using a viscometer Haake Viscotester (VT) 550 (Thermo Inc. Germany). Concentric cylinder MV-DIN sensor was used for analyses. A small amount of sample (about 75 ml) was placed into the center of cylindrical container. Rheological properties were measured at 30 °C. The following

procedure was performed: an increasing sequence from 0 to 1032 s^{-1} in a period of 10 min, followed by 1 min at the maximum value and the corresponding decreasing sequence in 3 min. Apparent viscosity (μ) was calculated at mPa.s. (Garrote, et al. 2001). All measurements done in duplicate and average values were reported.

3.2.3.7. Color Analysis

For color analysis, Minolta CR400 (Tokyo, Japan) colorimeter was used with a reading area of 8 mm. Kefir samples were transferred into quartz glass case and five readings were performed. The colorimeter directly calculated three color features of *L** (lightness), *a** (red–green component), and *b** (yellow–blue component). All analyses were performed in duplicate with five readings for each replicated sample and average values were estimated.

3.2.3.8. Volatile Compound Analysis

For the determination of volatile compounds GC-MS (Agilent 6890) was used. For this purpose, a fibre, provided by Supelco (57348-U, PA, USA) coated with the following sorbent material: Divinylbenzene/Carboxen/Polydimethylsiloxane was used. Samples were defrosted at 4ºC before the day of analysis. Five milliliters of samples were added into a 20 ml headspace vial, and a PTFE/butyl septum was immediately sealed with an aluminum crimp seal. Sample was equilibrated at 65ºC at 400 rpm for 30 min. Then, fiber was inserted into headspace of the vial using SPME fiber holder. The sample was agitated at 400 rpm at 65ºC while the fiber was inside the vial. After 30 minutes, the fiber was inserted into the Gas chromatography injector and held for 5 min. The temperature of the injector port was 220ºC. Agilent 6890 N / 5973 N Network GC / MSD System equipped with Agilent 5973 Mass Selective Detector (S/SL inlet) was used. The oven was temperature programmed as follows: hold at 40ºC for 6 minutes, then the temperature was raised to 110ºC (5 ºC/min, held 2 min) to a final temperature 220ºC (10ºC/min, held for 2 min). Carrier gas was He with 1 ml/min flow rate. 30m×0.25 mm ID-BP20×0.25 capillary column was used. The analysis was done in duplicate. Identification was done with comparing GC/MS mass spectral data, retention time and aroma with standards and Massa Spectral Library.

3.2.3.9. Organic Acid Compound Analysis

Determination of organic acid compounds of kefir samples were done with HPLC (Lombardi, et al. 1994). Perkin Elmer (PE) series 200 autosamplers, PE series 200 pump (Norwalk CT 06859), PE series 200 column heater, PE series 200 EP diode array detector (DAD) HPLC system were used. Acid separation was performed with an AMINEX HPX-87H ion exchange column (Biorad Labs). Calibration curve was prepared with organic acid standards. 0,018 M H_2SO_4 was used as a mobile phase. 10 ml samples were diluted to 50 ml with mobile phase and centrifuged at 6000 rpm for 5 minutes. The resulting supernatants were filtered first through whatman no 1 filter paper and then through a 0.45 μm membrane filter. Of the resulting filtrate were filled up to 2ml vial. All vials put in autosampler. 20μl sample was injected in the chromatograph (WatersTM 717, Millipore). Analyses were performed at a flow rate of 0.6 ml/min at 60°C using as 0.018 M H_2SO_4 the mobile phase. HPLC grade reagents were used as standard acids (Sigma Chemical Co., St. Louis, MO, USA). Solvents were degassed under vacuum. Organic acid identification was based on matching the retention times with standard acids. Analyses were done duplicate and average values were represented.

3.2.3.10. Total Phenol Content of Kefir Samples

Total phenol of the kefir samples were determined with the use of Folin-Ciocalteau micro method, a method derived from total phenol analysis (Slinkard and Singleton 1977).

3 ml kefir samples were diluted 45 ml to with distilled water. Samples were centrifuged at 6000 rpm for 5 minutes. 40 μL supernatants were added into different tubes and 3.16 mL of distilled water was added. 200 μL of Folin- Ciocalteau reagent was added and immediately mixed. After waiting for 3 minutes, 600 μL of sodium carbonate solution was added and mixed. The solutions were kept for 2 hours in a dark place at room temperature then the absorbance of each solution was read against the blank at 765 nm with a spectrophotometer (Schimadzu UV-2450, Japan). Results were expressed as μg gallic acid equivalent per ml of sample according to equation 3.3. Standart curve of gallic acid was given in Appendix D.

$$y = 0.012x + 0.0124 \quad (3.3)$$

3.2.3.11. Total Beta-Glucan Content

Total beta-glucan content of kefir samples were determined with modification of AOAC method 995.16 and AACC Method 32-23.

Megazyme enzyme kit (mixed-linkage beta-glucan, Ireland) was used in analyses. 80-120 mg of kefir samples were added to glass centrifuge tubes and 0.2 ml ethanol (50% v/v) was added to all tubes. Then 4 ml sodium phosphate buffer (20 mM, pH6.5) was added to tubes and tubes were stirred with a vortex. Samples were immediately placed into boiling water bath and incubated for 60 seconds. Next tubes stirred on vortex and incubated at 100^{o}C again for 2 minutes. Then tubes holding at 50^{o}C water bath to get equilibrium. After that step 0.2 ml lichenase (10 U) was added to tubes and samples were stirred. Tubes sealed with parafilm and incubated at 50^{o}C for 1 hour. Samples were stirred for 4-5 times during incubation on this step. 5 ml sodium acetate buffer (200mM, pH 4.0) was added all tubes and tubes were mixed. Next tubes were holded at room temperature for 5 minutes to get equilibrium then centrifuged at 1000 g for 10 minutes. 0.1 ml supernatants of centrifuged samples were added to three test tubes. 0.1 ml β-glucosidase (0.2 U prepared with 50mM sodium acetate buffer pH 4.0) was added to two test tubes to measure the reaction and 0.1 ml acetate buffer (50mM, pH 4.0) was added the other tube for measure the blank and all three test tubes for all samples were incubated at 50^{o}C for 10 minutes. 3 ml GOPOD reagent was added to each tube and incubated at 50^{o}C for 20 minutes on last step. All tubes removed from water bath and their absorbance was measured at 510 nm within 1 hour. All analyses were done in duplicate and average values were represented. Total beta-glucan of samples were calculated according to equation 3.4.

$$\beta\text{-glucan (\%w/w)} = \Delta A \times F \times 94 \times \left(\frac{1}{1000}\right) \times \left(\frac{100}{W}\right) \times 162 \div 180 \qquad (3.4)$$

ΔA = Absorbance after β-glucosidase treatment minus reaction blank absorbance

F = A factor for the conversion of absorbance values to μg of glucose

$$F = \frac{100\ (\mu g\ \text{of D-glucose})}{(\text{absorbance of } 100\ \mu g\ \text{D-glucose})}$$

94 = Volume correction factor

$\frac{1}{1000}$ = Conversion from μg to mg

$\frac{100}{W}$ = Factor to express β-glucan content as a percentage of dry weight

W = Calculated dry weight of the sample analyzed in mg

$\frac{162}{180}$ = A factor to convert from free D-glucose, as determined to anhydro-D-glucose, as occurs in β-glucan.

3.2.4. Microbiological Analysis

Serial dilutions in sterile 0.1% peptone water were prepared for bacterial analysis (Psoni, et al. 2003). For *Lactobacillus* ssp. and *Lactococcus* ssp. pour plate method was used whereas spread plate technique was used for yeast. After the incubation, the plates with colony forming units (CFU) ranging from 30 to 300 were selected for enumeration. After the colony counting, the numbers were expressed in logarithmic scales (log $CFUg^{-1}$). Two measurements were carried out and average values were reported.

3.2.4.1. *Lactobacillus* spp. Enumeration

MRS agar was used for the enumeration of *Lactobacillus* spp. Plates were incubated at 37°C for 48 hours in sealed jar containing anaerogen sachet.

3.2.4.2. *Lactococcus* spp. Enumeration

M17 agar was used for the enumeration of *Lactococcus* spp.. Plates were incubated at 37°C for 48 hours.

3.2.4.3. Yeast Enumeration

Yeast glucose chloromophenical agar was used for yeast enumeration. Plates were incubated at 25°C for 48 hours.

3.2.5. Sensory Analyses

In sensory analyses 10 trained panelists (ages ranged from 24 to 48, 4 males and 6 females) were tasted kefir samples. The panelists were staff and graduate students in the Department of Food Engineering at Izmir Institute of Technology. The panelists identified and defined the flavor terms from representative kefir.

Firstly panelists were trained by tasting kefir, kefir with blueberry aroma and then they started to taste experiment samples and scored sample 1 to 10 according to personal liking. They scored sample's flavor, odor, consistency, appearance and overall acceptability in optimization step.

After optimization step panelist were tasted 3 kefir samples during storage (1^{st}, 6^{th}, 11^{th}, 16^{th}, 21^{th} days). In this part sensory analyses were done in duplicate.

Panelists were provided with water and unsalted bread to cleanse the palate between samples. The kefir samples were presented in plastic cups and coded with three-digit numbers. Sensory evaluation ballot was given in Appendix F.

3.2.6. Statistical Analysis

Design Expert® 7.0 software was used in optimization step. Response surface methodology (RSM) with central composite design (CCD) was used. 4 numeric factors (Storage time, culture concentration, aroma concentration and oat milk concentration) with 5 levels (plus and minus axial, factorial points and center point) were used (Table 3.3)

Table 3.3. Chosen factors and levels for optimization

Points (axial, factorial, center)	-2	-1	0	1	2
Storage (days)	1	6	11	16	21
Culture concentration (%)	1	2	3	4	5
Aroma conenctration (%)	9	12	15	18	21
Oat milk concentration (%)	0	15	30	45	60

After optimization, three samples were chosen having optimum pH, optimum microbiological characteristic and optimum sensory characteristics with a highest oat milk concentration. Mean values, standard deviations, maximum and minimum values were calculated for all the determined parameters. Analysis of variance was performed to investigate the differences ($p<0.05$) in characteristics during storage.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1. Raw Materials Properties

Bovine milk used in this study consisted of 3.22% fat, 3.09% protein, 5.01% lactose. Its freezing point was 0.100^{o}C based on Lactostar results. The dry matter, protein content, and pH of the milk were 11%, 2.91%, and 6.6, respectively.

The chemical composition of oat milk was 1.6% fat, 0.95% protein, 8.4% dry matter and the pH was 7.03. Volatile compounds and organic acid profile of oat milk were given in Appendix E.

The chemical composition of blueberry aroma was 0.1% fat, 0.05% protein, 15% dry matter and pH was 3.67. Volatile compounds and organic acid profile of blueberry aroma were given in Appendix E.

4.2. Optimization of Kefir Samples

Thirty kefir samples were produced according to storage time, culture concentration, aroma concentration and oat milk concentration. Sensory characteristics, pH and microbiological characteristics of kefir samples were investigated in optimization step. According to results three kefir samples were chosen having optimum sensory characteristic, pH, and microbiological characteristic. In this step, our main aim was choosing the best sample, which contained the highest level of oat milk concentration.

4.2.1. Sensory Analyses

Thirty kefir samples were scored by panelists according to their appearance, odor, taste, consistency and overall acceptability attributes. According to the results, oat milk concentration was the most effective factor, culture concentration and storage were

also effective factors. Aroma concentration was less effective than other factors. Only in odor characteristics aroma concentration is more effective then culture concentration and storage. All sensory results analyzed separately and together. All factors and their interactions were investigated to produce optimum kefir sample.

4.2.1.1. Appearance

The results for the appearance attributes of samples were given in Table 4.1. According to the results model was significant, so there was a difference between samples. Oat milk concentration was the most effective factor in differences during storage. Other factors were inefficient in appearance. Interactions of factors also analyzed and they were found insignificant. Lack of fit was determined insignificant and it was good for model.

Table 4.1. Anova table for apperance

Response	**Appearance**					
ANOVA for Response Surface Quadratic Model						
Analysis of variance table [Partial sum of squares - Type III]						
Source	Sum of Squares	df	Mean Square	F Value	P-Value Prob > F	
Model	54.33	14	3.88	4.23	0.044	**significant**
A-Storage	1.71	1	1.71	1.86	0.1929	
B- Culture Conc.	1.60	1	1.60	1.75	0.2063	
C- Aroma Conc.	0.060	1	0.060	0.065	0.8017	
D- Oat Conc.	37.50	1	37.50	40.86	0.0001	
Residual	13.77	15	0.92			
Lack of Fit	6.54	10	0.65	0.45	0.8663	Not significant
Pure Error	7.23	5	1.45			
Cor Total	68.09					
Std Dev.	0.96		R-Squared	0.7978		
Mean	5.54		Adj R-Squared	0.6091		
C.V. %	17.29		Pred R-Squared	0.2941		
PRESS	48.06		Adeq Precision	8.913		

* Prob>F less than 0.100 indicate model terms significant

Effects of oat milk concentration due to storage were given with contour plots and response surface in Figures 4.1 and 4.2. It was easily seen that increase of oat milk concentration lead to disliking in appearance.

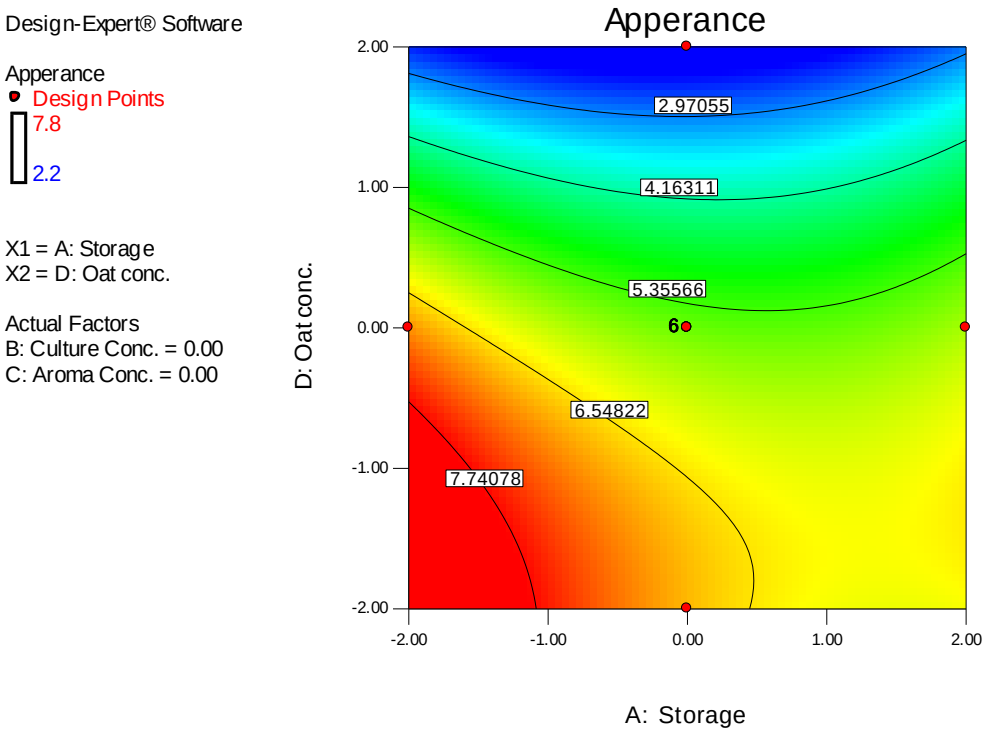


Figure 4.1. Contour plot for the effects of oat milk concentration and storage for appearance

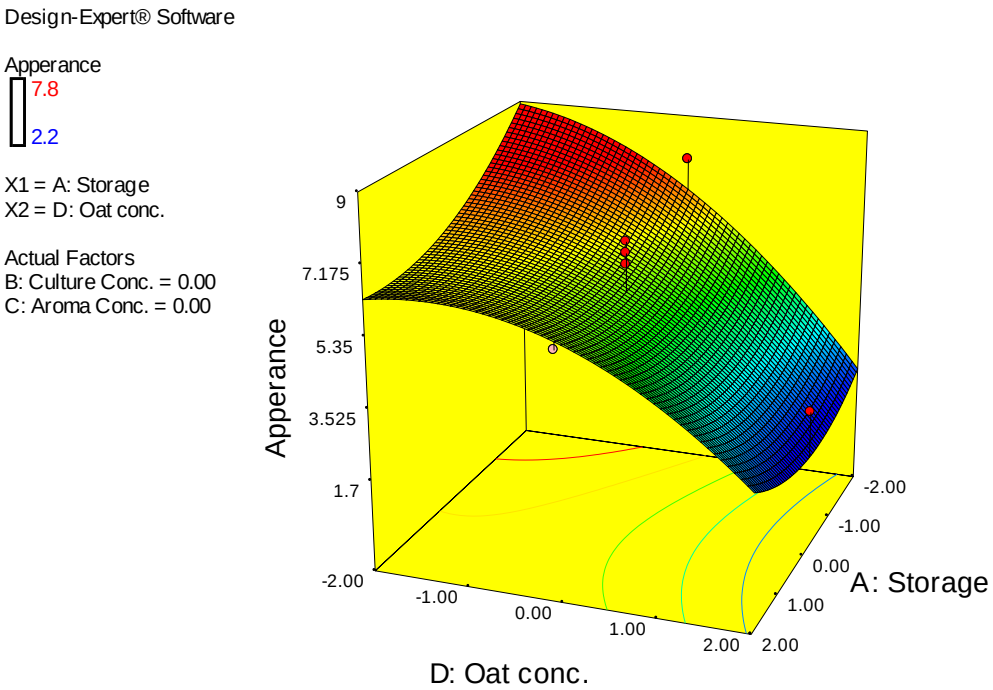


Figure 4.2. Response surface for the effects of oat milk concentration and storage for appearance

It was observed that there was a phase separation related to increase of oat milk concentration and this affected the appearance of samples negatively.

Increase of culture concentration affected appearance of samples positively. Storage affected aroma impact. Aroma concentration affected appearance after day 11. The samples having the highest aroma concentration preserved their color than the samples having the lowest concentration. In some samples which were consist of less blueberry aroma, color changed from red-pink to white-yellow and this affected the scores negatively.

4.2.1.2. Odor

The results of odor attributes of kefir samples were given in Table 4.2. There was a significant difference between samples ($p < 0.100$). Oat milk concentration was the most effective factor. Also aroma concentration affected the odor slightly. Culture concentration and straoge were insignificant in odor attributes of kefir samples. Interactions of factors also analyzed and they were found insignificant. Lack of fit was determined insignificant and it was good for model.

Table 4.2. Anova table for odor

Response		**Odor**				
ANOVA for Response Surface Quadratic Model						
Analysis of variance table [Partial sum of squares - Type III]						
Source	Sum of Squares	df	Mean Square	F Value	P-Value Prob > F	
Model	29.18	14	2.08	5.41	0.0012	**signicant**
A-Storage	0.54	1	0.54	1.40	0.2549	
B- Culture Conc.	0.17	1	0.17	0.43	0.5207	
C- Aroma Conc.	1.21	1	1.21	3.15	0.09161	
D- Oat Conc.	20.17	1	20.17	52.33	<0.0001	
Residual	5.78	1	0.39			
Lack of Fit	3.31	10	0.33	0.67	0.7239	Not.
Pure Error	2.47	5	0.49			
Cor Total	34.96	29				

Std. Dev.	0.62	R-Squared	0.8346
Mean	5.83	Adj R-Squared	0.6803
C.V %	10.65	Pred R-Squared	0.3525
PRESS	22.63	Adeq Precision	10.128

* Prob>F less than 0.100 indicate model terms significant

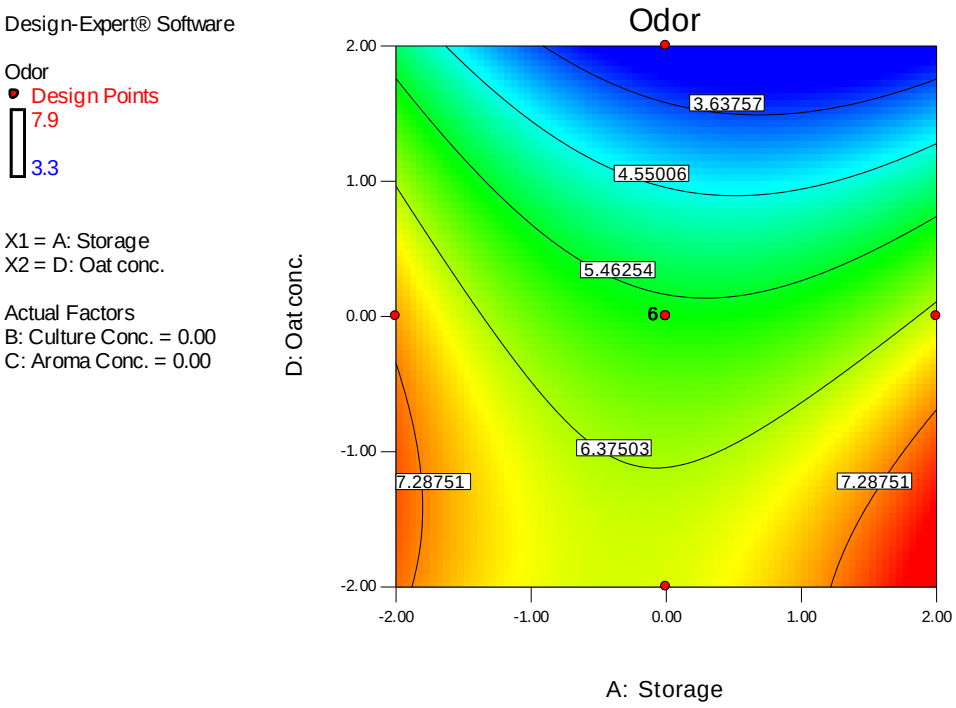


Figure 4.3. Contour plot for the effects of oat milk concentration and storage for odor

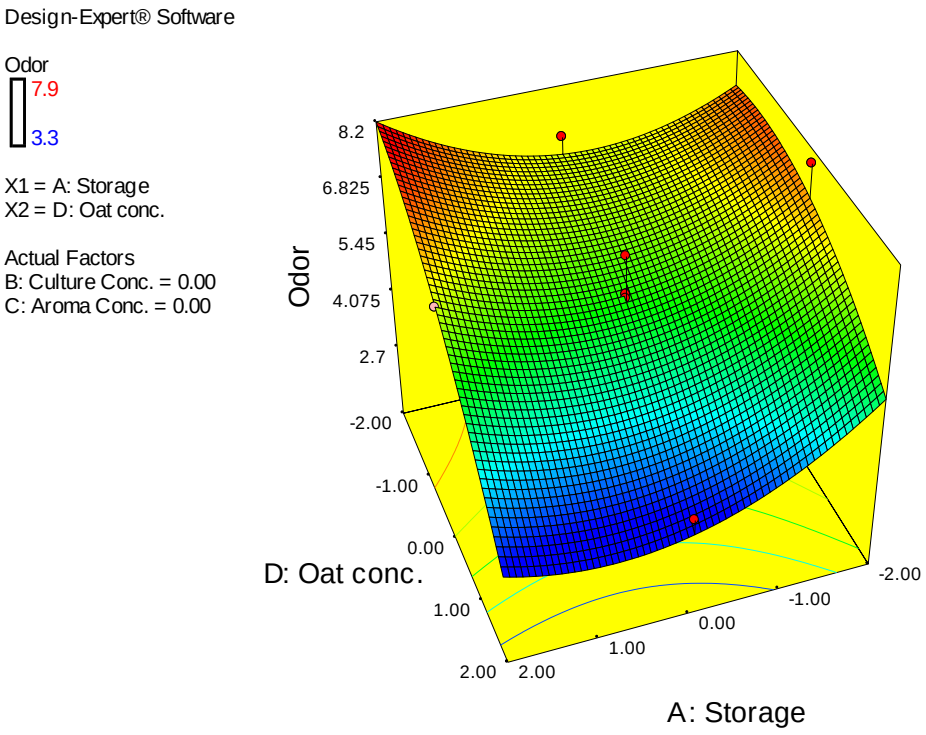


Figure 4.4. 3D surface for the effects of oat milk concentration and storage for odor

Contour plot and 3D surface plot were given for effect of oat milk concentration during storage in odor characteristic in Figures 4.3 and 4.4. Increase of oat milk concentration caused to more oat smell which was conduced to disliking in kefir samples. Increase of aroma concentration increased liking due to the blueberry's fruity smell. Storage also affected the odor. The samples started to lose their fruity smell after day 11. The sour smell occurred in some samples after day 16.

4.2.1.3. Flavor

Flavor was the most effective factor in sensory analyses. Flavor results of kefir samples were given in Table 4.3. As regards to results, model had 0.0003 p value so there was a great difference between samples. Oat milk concentration was the most effective factor. Besides culture concentration was also effective. Storage and aroma concentration were insignificant in flavor. Normally it was expected that aroma concentration affected flavor excessively; however, panelist scores showed that aroma concentration did not affect the flavor. We can say that 9% to 15% aroma concentrations were enough to mask undesirable flavor which came from oat milk.

Table 4.3. Anova table for flavor.

Response			**Flavor**			
ANOVA for Response Surface Quadratic Model						
Analysis of variance table [Partial sum of squares - Type III]						
Source	Sum of Squares	df	Mean Square	F Value	P-Value Prob > F	
Model	51.74	14	3.70	6.92	0.0003	**significant**
A-Storage	1.667	1	1.667	3.12	0.9562	
B- Culture Conc.	4.86	1	4.86	9.10	0.0087	
C- Aroma Conc.	0.015	1	0.015	0.028	0.8692	
D- Oat Conc.	36.02	1	36.02	67.42	<0.0001	
BC	2.10	1	2.10	3.94	0.0659	
Residual	8.01	15	0.53			
Lack of Fit	5.42	10	0.54	1.04	0.5132	Not significant
Pure Error	2.59	5	0.52			
Cor Total	59.75	29				
Std. Dev.	0.73	R-Squared		0.8659		
Mean	5.66	Adj R-Squared		0.7408		
C.V. %	12.92	Pred R-Squared		0.4151		
PRESS	34.95	Adeq Precision		10.586		

* Prob>F less than 0.100 indicate model terms significant

Interactions of factors also analyzed and they were found insignificant. Lack of fit was determined insignificant and it was good for model.

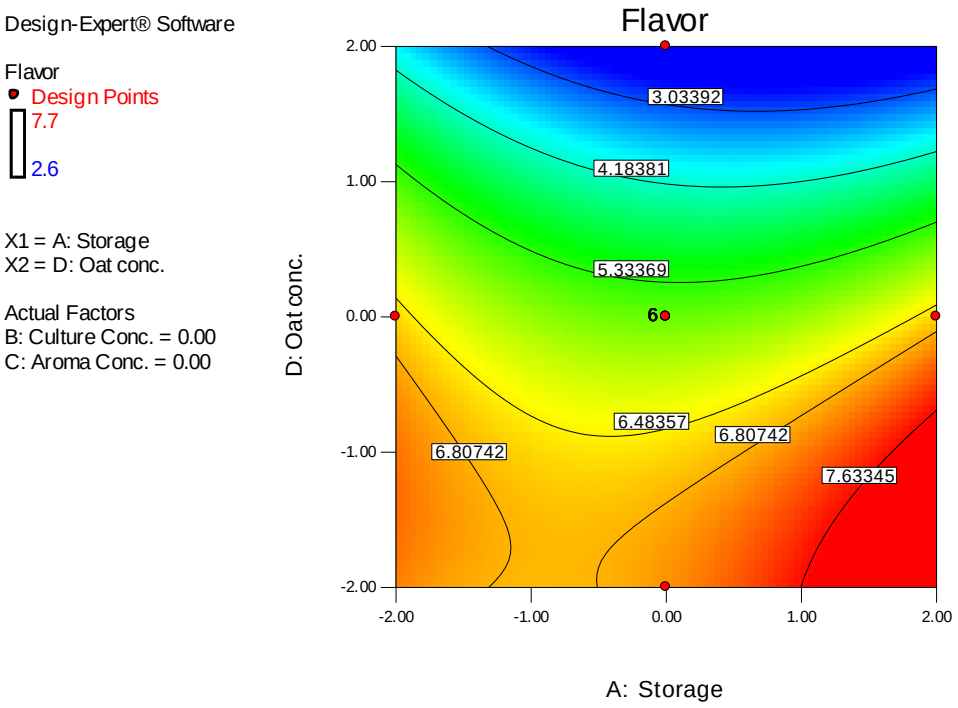


Figure 4.5. Contour plot for the effects of oat milk concentration and storage for flavor

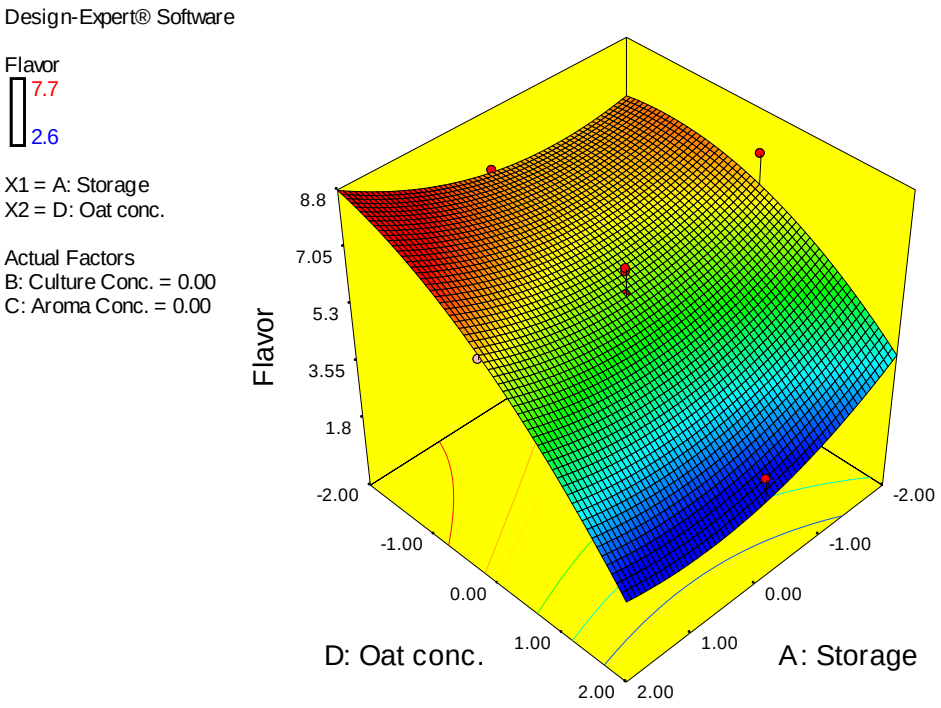


Figure 4.6. 3D surface for the effects of oat milk concentration and storage for flavor.

Effects of oat milk concentration during storage on flavor for kefir samples were given in Figures 4.5. and 4.6. Oat milk left a cereal taste in mouth. Increase of oat milk concentration over 35-40% reduced liking point. According to Figures 4.5 and 4.6, 20% oat milk concentration was acceptable. Results showed that increase in culture concentration increased liking.

4.2.1.4. Consistency

Consistency results were given in Table 4.4. According to results oat milk concentration was the most effective factor. Other factors were ineffective. Samples were significantly different from each other. Interactions of factors also analyzed and they were found as insignificant. Lack of fit was determined as insignificant and it was good for model.

Table 4.4. Anova table for consistency

Response	**Consistency**					
ANOVA for Response Surface Quadratic Model						
Analysis of variance table [Partial sum of squares - Type III]						
Source	Sum of Squares	df	Mean Square	F Value	P-Value Prob > F	
Model	59.08	14	4.22	4.28	0.0042	**significant**
A-Storage	0.43	1	0.43	0.43	0.5207	
B- Culture Conc.	2.41	1	2.41	2.44	0.1391	
C- Aroma Conc.	0.042	1	0.042	0.042	0.8399	
D- Oat Conc.	47.60	1	47.60	48.26	<0.0001	
Residual	14.80	15	0.99			
Lack of Fit	7.30	10	0.73	0.49	0.8446	Not significant
Pure Error	7.50	5	1.50			
Cor Total	73.88	29				
Std. Dev.	0.99		R-Squared	0.7997		
Mean	5.53		Adj R-Squared	0.6128		
C.V. %	17.97		Pred R-Squared	0.2849		
PRESS	52.83		Adeq Precision	8.923		

* Prob>F less than 0.100 indicate model terms significant

Contour plot and 3D surface plot were given the effect of oat milk concentration during storage on consistency of kefir samples in Figures 4.7 and 4.8.

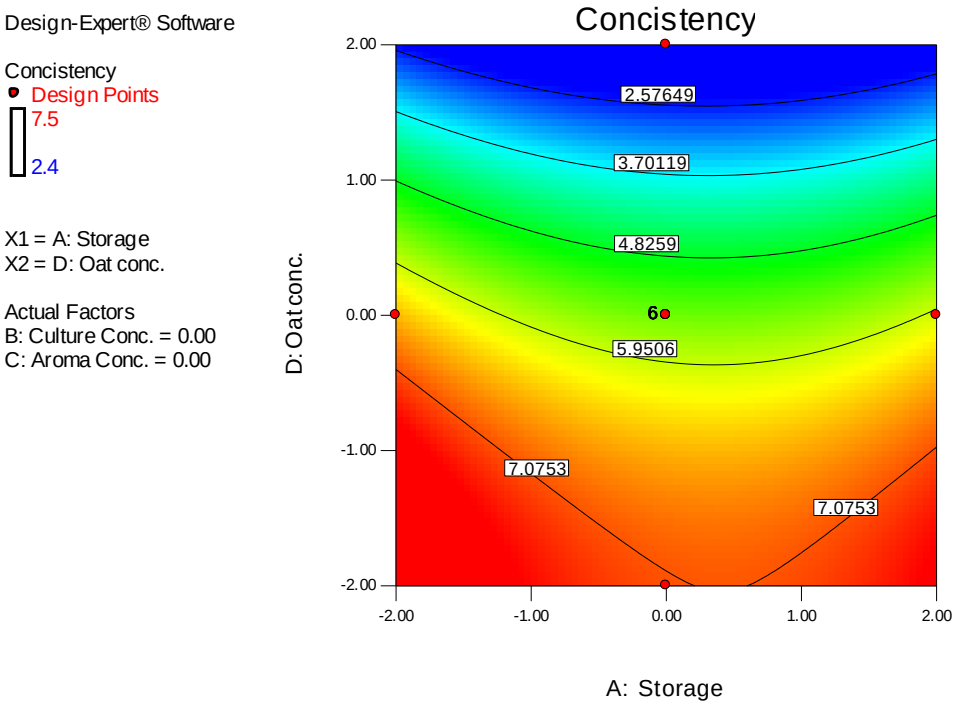


Figure 4.7. Contour plot for the effects of oat milk concentration and storage for consistency

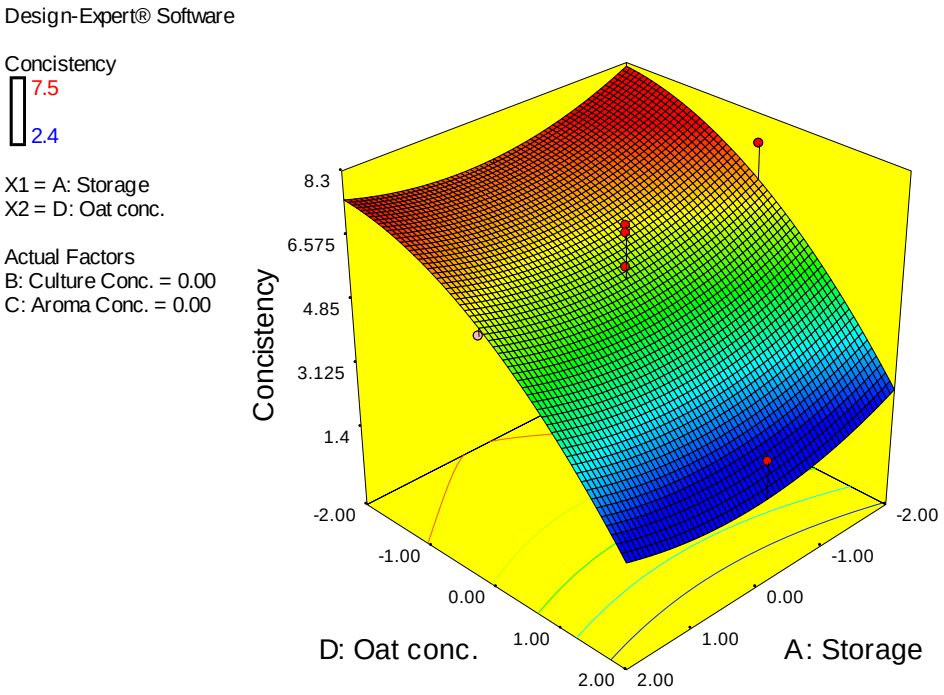


Figure 4.8. 3D surface for the effects of oat milk concentration and storage for consistency

Accoring to plots and 3D surface consistency scores decreased greatly when oat milk concentration was over 30%.

4.2.1.5. Overall Acceptability

For overall acceptability results were given in Table 4.5. According to results oat milk concentration was the most effective factor. The culture concentration also had a sligft effect on the overall acceptability. Storage and aroma concentration were insignificant. Interactions of factors also analyzed and they were found as insignificant. Lack of fit was determined as insignificant and it was good for model.

Table 4.5. Anova table for overall accepatbility

Response	**Overall Acceptability**					
ANOVA for Response Surface Quadratic Model						
Analysis of variance table [Partial sum of squares - Type III]						
Source	Sum of Squares	df	Mean Square	F Value	P-Value Prob > F	
Model	59.80	14	4.27	4.80	0.0023	**Significant**
A-Storage	0.073	1	0.073	0.082	0.7782	
B- Culture Conc.	2.45	1	2.45	2.76	0.1174	
C- Aroma Conc.	0.036	1	0.036	0.040	0.8440	
D- Oat Conc.	47.39	1	47.39	53.28	<0.0001	
Residual	13.34	15	0.89			
Lack of Fit	8.13	10	0.81	0.78	0.6564	Not significant
Pure Error	5.21	5	1.04			
Cor Total	73.14	29				
Std. Dev.	0.94		R-Squared	0.8176		
Mean	5.64		Adj R-Squared	0.6474		
C.V. %	16.72		Pred R-Squared	0.2574		
PRESS	54.31		Adeq Precision	9.322		

* Prob>F less than 0.100 indicate model terms significant

In Figures 4.9 and 4.10, contour plot and response surface were given for the effects of oat milk concentration and storage for overall acceptability. Increase of oat milk concentration caused a decrease of liking. According to figures, after 20% of oat milk, scores decreased sharply. Storage had an insignificant effect on overall acceptability. Overall acceptability and flavor scores were very close to each other. We can say that flavor was an important parameter for overall acceptability in sensory analyses.

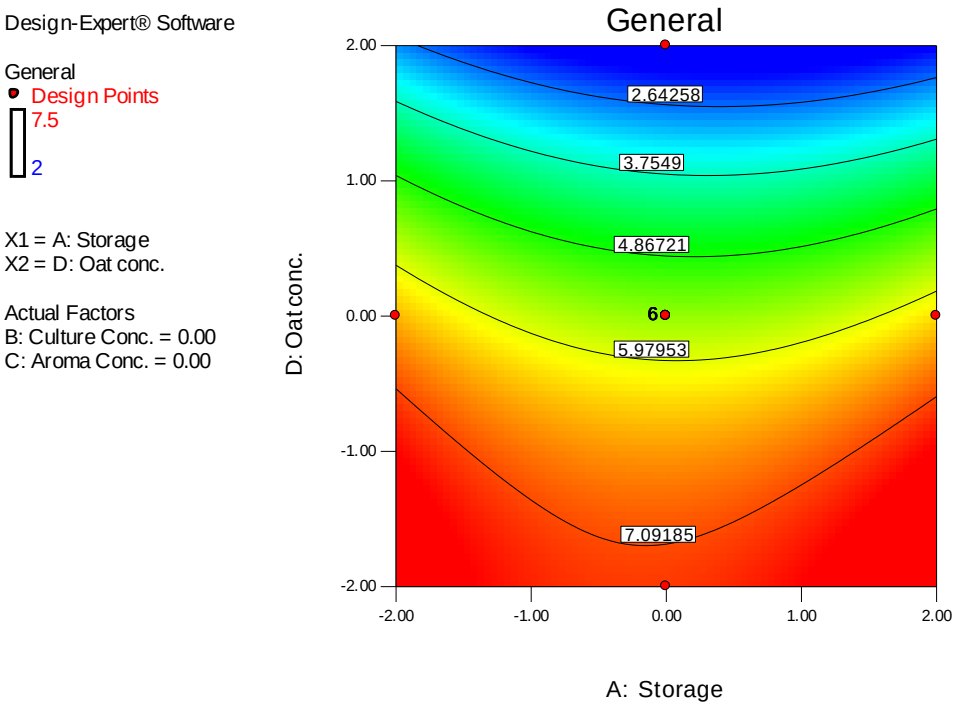


Figure 4.9. Contour plot for the effects of oat milk concentration and storage for overall acceptability

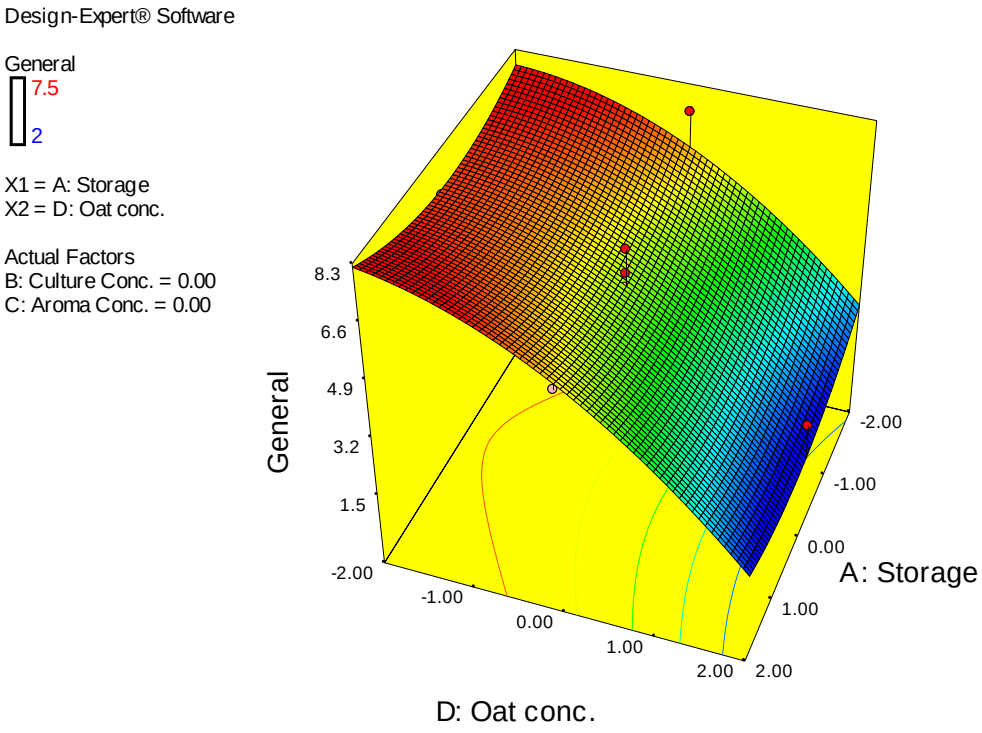
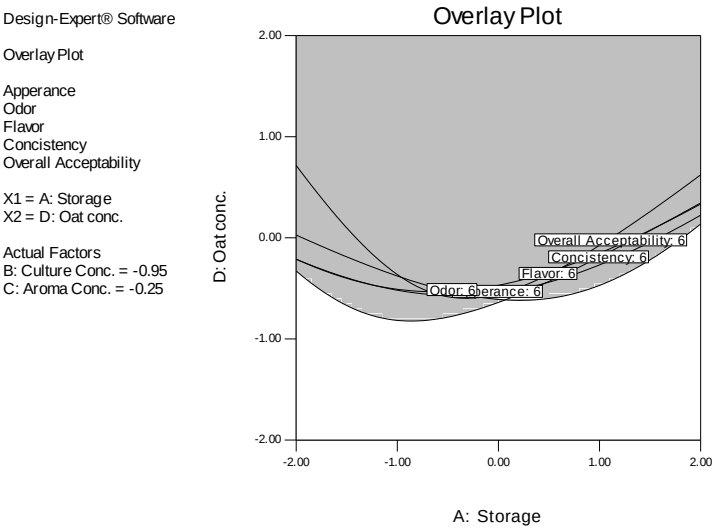
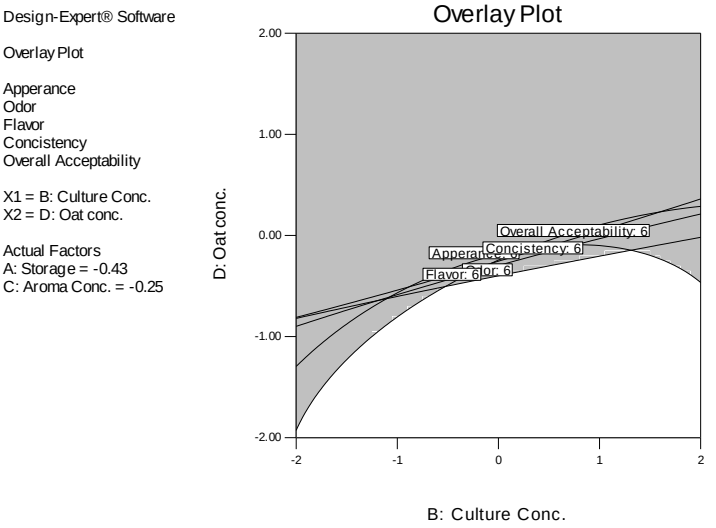


Figure 4.10. Response surface for the effects of oat milk concentration and storage for overall acceptability





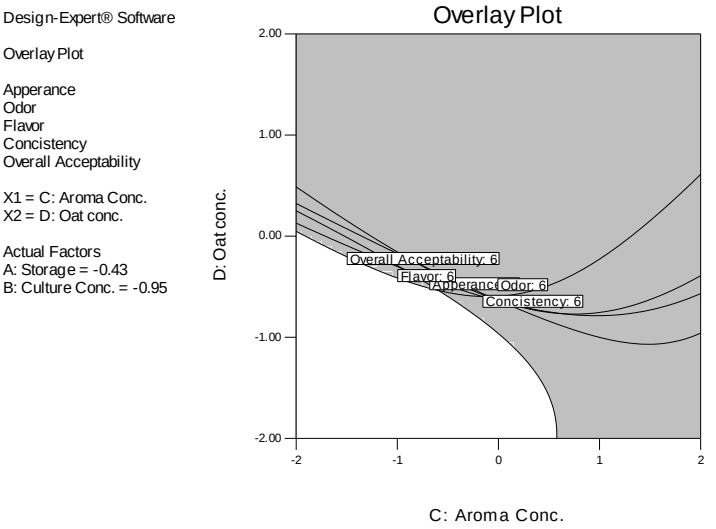


Figure 4.11. Overlay plots for sensory characteristics

In Figure 4.11, overlay plots were given for sensory responses. After analyzed all responses and their interactions in sensory analyses, optimum kefir produced sample was chosen as the sample consisting of 4% culture concentration, 10% aroma concentration and 20% oat milk concentration.

4.2.2. The pH Results

The pH results were given in Table 4.6. According to results storage and oat milk concentrations were effective factors. And also aroma concentration affected the pH due to blueberry's acidic characteristics. Interactions of factors were also analyzed and they were found as insignificant. Lack of fit was determined as insignificant and it was good for model.

Table 4.6. Anova table for pH

Response	**pH**					
ANOVA for Response Surface Quadratic Model						
Analysis of variance table [Partial sum of squares - Type III]						
Source	Sum of Squares	df	Mean Square	F Value	P-Value Prob > F	
Model	0.60	14	0.043	9.77	< 0.0001	**Significant**
A-Storage	0.43	1	0.43	98.31	< 0.0001	
B- Culture Conc.	1.276	1	1.276	0.29	0.5984	
C- Aroma Conc.	0.016	1	0.016	3.58	0.0781	
D- Oat Conc.	0.096	1	0.096	21.70	0.003	
Residual	0.066	15	0.004			
Lack of Fit	0.053	10	0.053	2.06	0.2206	Not significant
Pure Error	0.013	5	0.003			
Cor Total	0.67	29				
Std. Dev.	0.066		R-Squared	0.9012		
Mean	4.25		Adj R-Squared	0.8089		
C.V.%	1.56		Pred R-Squared	0.5143		
PRESS	0.32		Adeq Precision	11.538		

* Prob>F less than 0.100 indicate model terms significant

Contour plot and 3D surface plot were given for oat milk concentration and storage in Figures 4.12 and 4.13. Increase of oat milk and aroma concentration caused to decrease in pH .

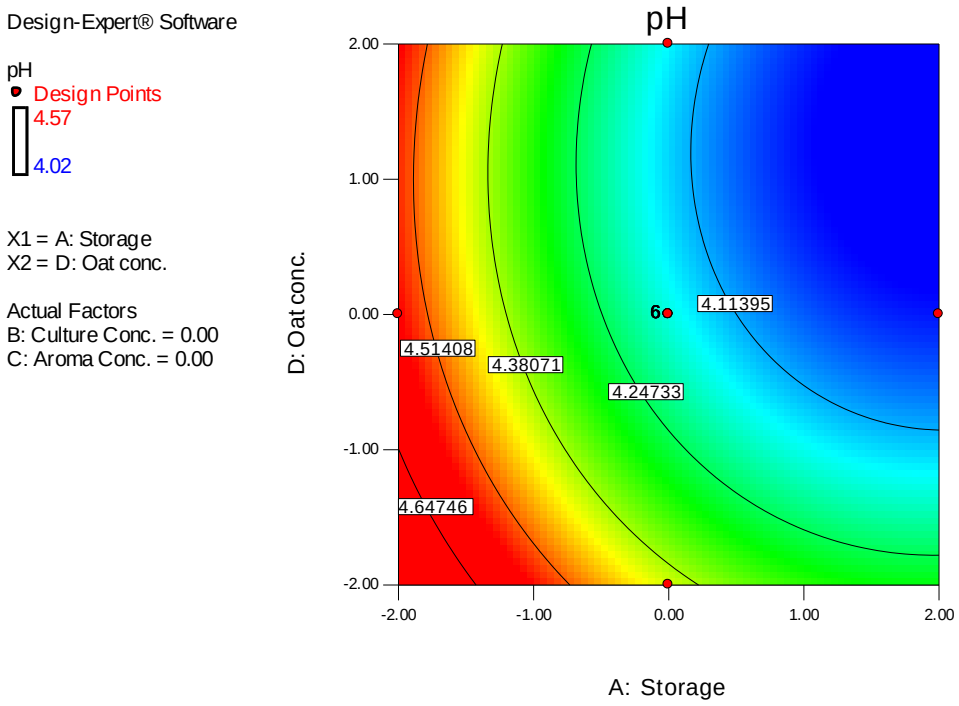


Figure 4.12. Contour plot for the effects of oat milk concentration and storage for pH

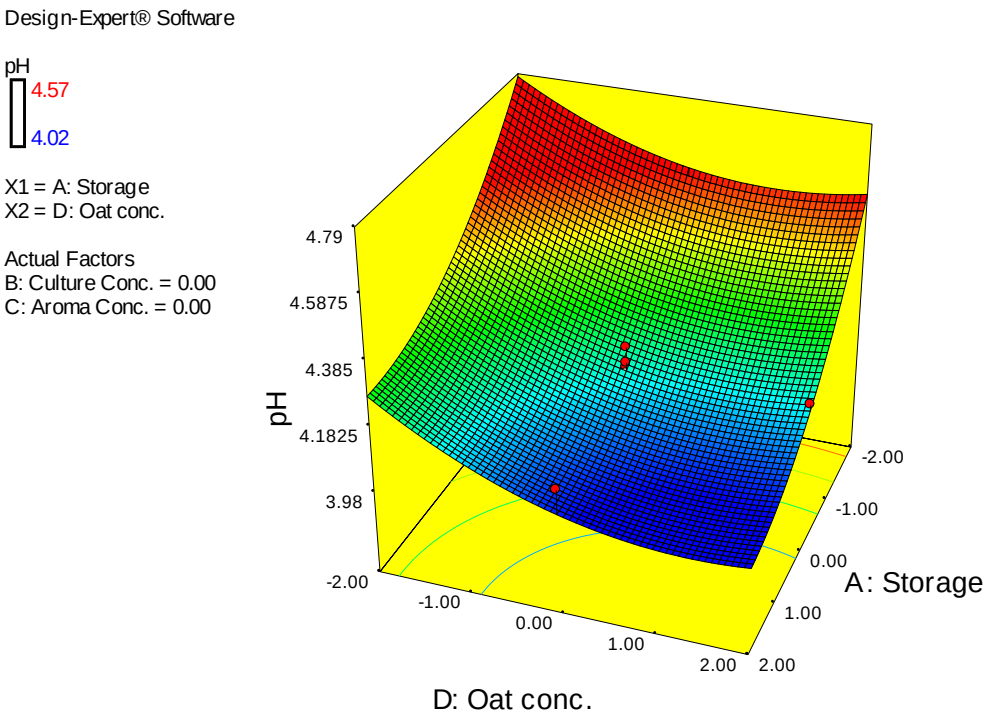


Figure 4.13. Response surface for the effects of oat milk concentration and storage for pH

According to figures oat milk concentration was not important on day 1; however, after day 6 the pH of the sample had the lowest oat milk concentration was more balanced than of the highest ones. Higher than 45-50% oat milk concentration, samples' pH decreased less than 4 units. Oat milk affected the nutritional content of samples so microflora of kefir changed and this caused a decrease in pH.

After analyzing all responses and their interaction in pH analyses, optimum kefir produced sample was chosen as the one consisting of 4% culture concentration, 9% aroma concentration and 15% oat milk concentration.

4.2.3. Microbiological Results

Microbiological results were given in Table 4.7. According to these results, storage and oat milk concentrations were effective factors. However, aroma and culture concentrations were affectless in microbiological results. Samples were significantly different each other (P<0.100). Interactions of factors also analyzed and they were found insignificant. Lack of fit was determined significant and it was not good for model.

Table 4.7. Anova table for *Lactobaillus* ssp. count

Response			***Lactobacillus* ssp.**			
ANOVA for Response Surface Quadratic Model (transform: base 10 log)						
Analysis of variance table [Partial sum of squares - Type III]						
Source	Sum of Squares	df	Mean Square	F Value	P-Value Prob > F	
Model	30.24	14	2.16	5.66	0.0010	**significant**
A-Storage	12.23	1	12.23	32.06	< 0.0001	
B- Culture Conc.	8.094	1	8.094	0.021	0.8861	
C- Aroma Conc.	8.795	1	8.795	0.023	0.8813	
D- Oat Conc.	2.97	1	2.97	7.790	0.0137	
Residual	5.72	15	0.38			
Lack of Fit	5.67	10	0.57	59.45	0.0001	Significant
Pure Error	0.048	5	0.009			
Cor Total	35.96					
Std. Dev.	0.62		R-Squared	0.8409		
Mean	6.43		Adj R-Squared	0.6924		
C.V.%	9.60		Pred R-Squared	0.0893		
PRESS	32.75		Adeq Precision	7.686		

* Prob>F less than 0.100 indicate model terms significant

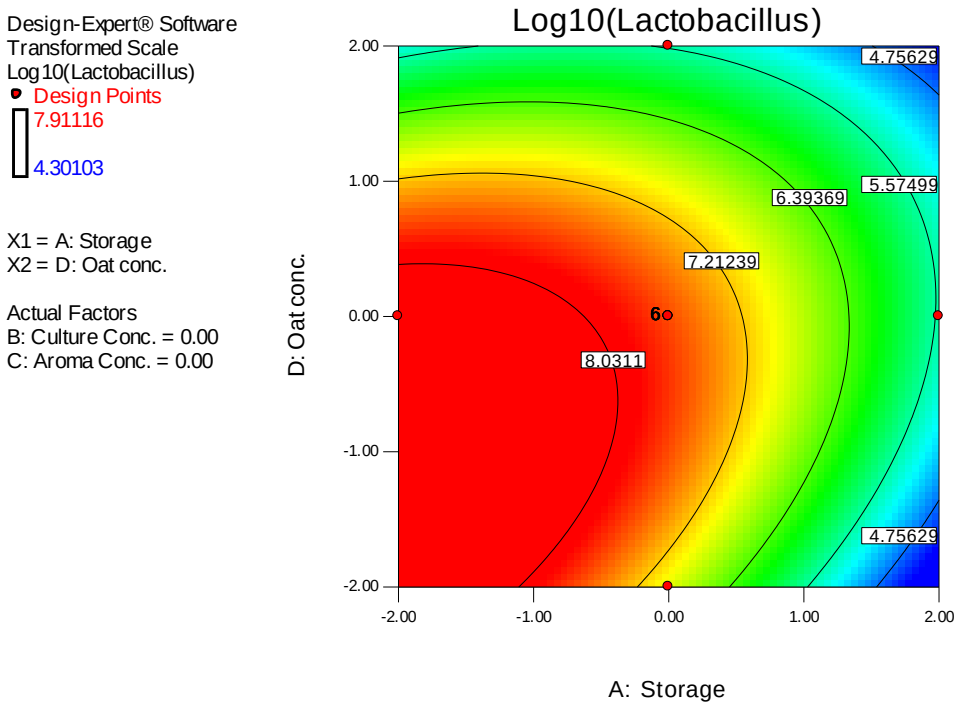


Figure 4.14. Contour plot for the effects of oat milk concentration and storage for *Lactobacillus* ssp. count

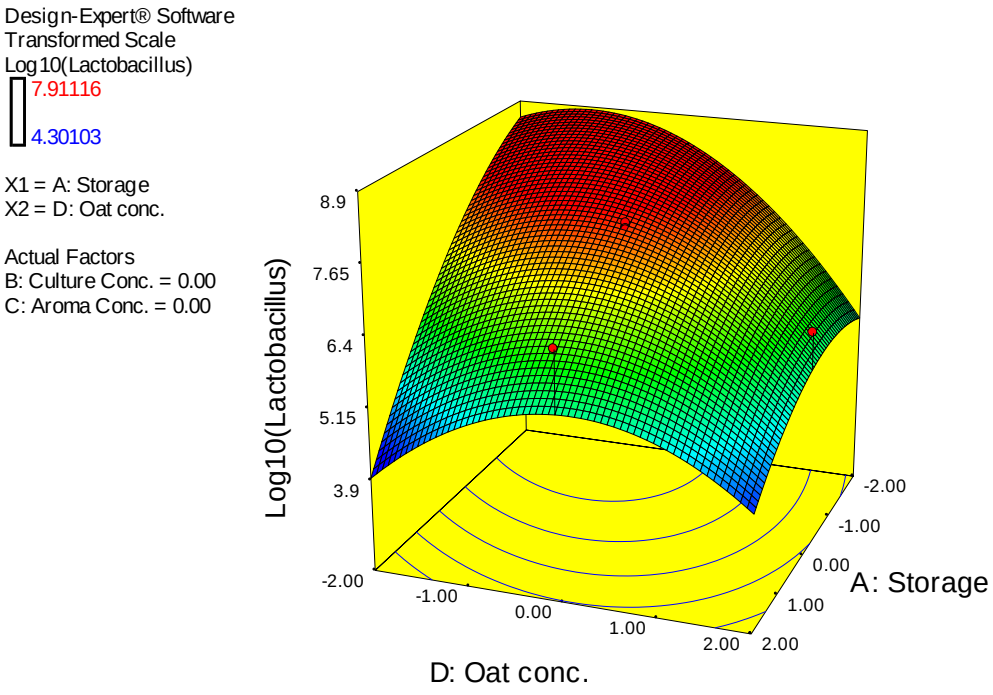


Figure 4.15. Response surface for the effects of oat milk concentration and storage for *Lactobacillus* ssp. count

In Figures 4.14 and 4.15, contour plot and 3D surface were given for the effects of oat milk concentration and storage for *Lactobacillus* ssp. count. According to these figures, *Lactobacillus* ssp. count decreased during storage. Decrease of oat milk concentration caused an increase in *Lactobacillus* ssp. count, but when we checked *Lactobacillus* ssp. count during storage from contour plot and 3D surface optimum concentration was detected at 30% oat milk concentrations. For this concentration, *Lactobacillus* ssp. count held greater than 10^6 though 16^{th} day of storage. Culture and aroma concentration were chosen according to that of oat milk concentration.

After optimization analysis including all responses and their interactions within microbiological experiment, it was decided to produce optimum kefir sample consisting of 3% culture concentration, 15% aroma concentration and 30% oat milk concentration.

4.3. Chemical and Physical Analyses Results

pH, titratable acidity, total dry matter contents, total protein contents , total fat contents , whey off, viscosity, color, volatile and organic acid profile, total phenol content, microbial characteristics and sensory profile analyses were done for 3 samples during storage. Samples were coded according to Table 3.2. Also in figures blue color referenced to sample 1, red color represented to sample 2 and green color indicated to sample 3.

4.3.1. The pH and Titratable Acidity Results

The pH values of kefir samples were given in Table C.1 (Appendix C). The pH values were investigated between 4.28 and 4.03. The average pH value for sample 1, sample 2, and sample 3 were 4.22, 4.26, and 4.08, respectively. The pH change was significantly different in sample 1, sample 2 and among the all samples during storage ($P<0.05$). However, in sample 3 no significant differences observed during storage ($P>0.05$).

The pH changes was given for all samples during storage in Figure 4.16. First day of storage pH values changed between 4.28 and 4.09.

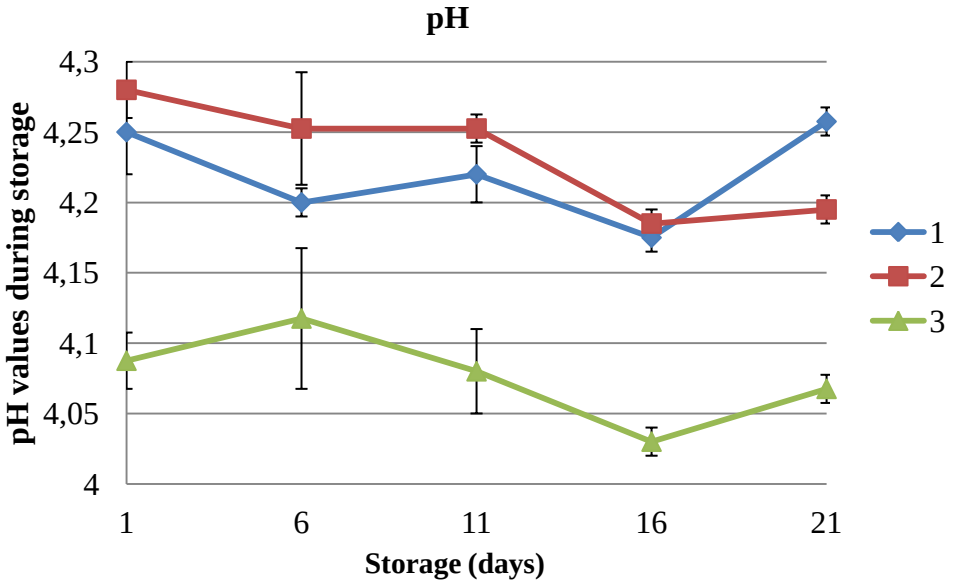


Figure 4.16. pH change in kefir samples during storage

For sample 1 and sample 2, the pH changes showed similarity. Both sample's pH balanced and decreased until the 16th day of storage. Increases in pH were observed on day 21.

The pH increased after first day then decreased until day 16 in sample 3. Low pH for the sample having 15% aroma concentration affected the pH in sample 3 on first day. The pH was balanced on the 6th day of storage and started to decrease down to day 16 and then increased on the 21th day of storage

For all samples it can be said that oat milk concentration and aroma concentrations affect the samples pH efficiently. Increase of oat milk concentration conduced to a decrease in pH. This might be related to microbial flora of samples. Over 10% aroma concentrations, an excessive pH decrease was observed in day 1.

After 16th day of storage all samples pH increased, Seydim (2001) informed that microbial proteolysis could increase pH after 14th day of storage in kefir.

In some studies it was investigated that no significant difference in pH changes was observed during storage (Irigoyen, et al. 2005; Tratnik, et al. 2006). However, it was explained that pH decreased significantly during the first week of storage in Norwegian kefirs (H. Grønnevik, et al. 2011).

Titratable acidity results of kefir were given in Table C.2 (Appendix C). Titratable acidity results were given by % lactic acid for all samples during storage in Figure 4.17.

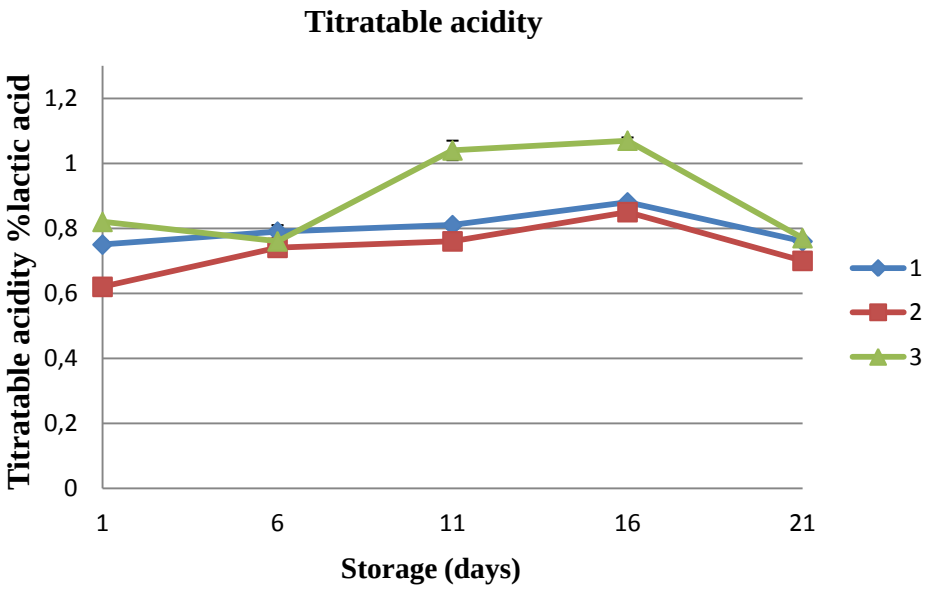


Figure 4.17. Titratable acidity change in kefir samples during storage

Kefir samples titratable acidity was observed between 0.62% and 0.82% in first day analyses. For sample 1 and 2, titratable acidity increased to 0.88% and 0.85% until the 16th day of storage, and then decreased to 0.76% to 0.70% on the 21th day of storage. Titratable acidity decreased on day 6, then increased to 1.07% till day 16 in sample 3, then decreased again to 0.77% on the 21th day of storage.

No differences were observed in all samples during storage ($p>0.05$). Significant differences was observed among the all samples during storage ($p<0.05$). Results were investigated in proportional with pH results.

According to standards of kefir, titratable acidity results found in the acceptable limits (Codex Standard for Fermented Milks CODEX STAN 243-2003 FAO/WHO 2001, Turkish Food Codex; Fermente Sütler Tebliği 2001).

4.3.2. Total Dry Matter Contents

Total dry matter (DM) results of kefir samples were given in Table C.3 (Appendix C). Samples dry matter contents were observed between 12.56% and 15.59%. Average DM content for kefir for sample 1, sample 2, and sample 3 were 13.64%, 14.58%, and 14.77%.

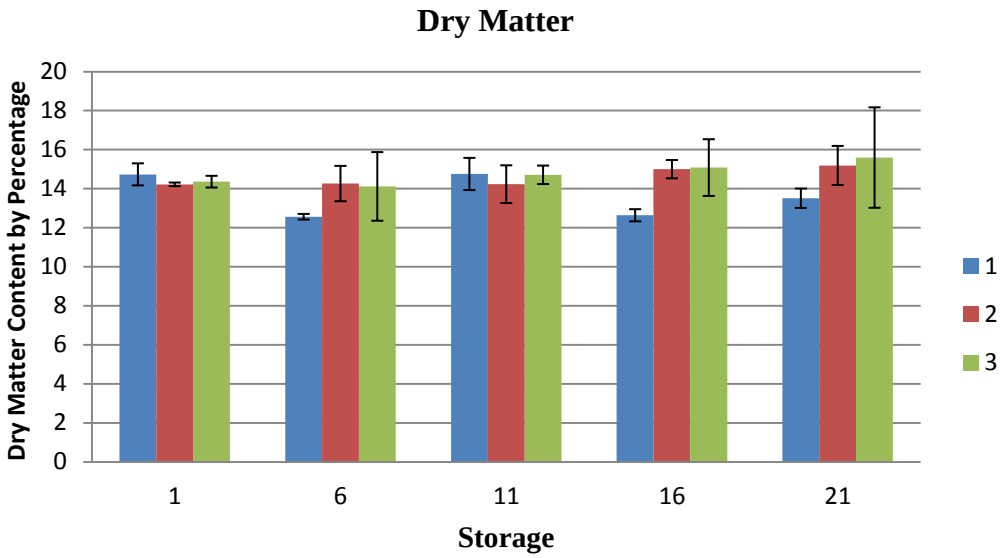


Figure 4.18. Dry matter content change in kefir samples during storage

The dry matter contents of kefir samples were observed close to each other during storage. No significant difference was observed in sample 2 and sample 3 ($P>0.05$); however, there was a difference determined in sample 1 ($P<0.05$). Among the all samples, analysis of variance results showed that significant differences were observed in each sample ($p<0.05$) As a reason of particle structure of oat milk and aroma differences might be seen in sample 1.

Irigoyen indicated that dry matter of kefir was found as 11.7% and no significant differences were observed in results (Irigoyen, et al. 2005).

In literature it was reported that dry matter content of kefir differed according to geographic origin and dry matter can be changed between 9.4% and 11.1% (Ottogalli, et al. 1973). Composition of oat milk and blueberry aroma affected the dry matter content.

4.3.2. Total Protein Contents

Total protein results of kefir samples were given in Table C.4 (Appendix C). Results were determined between 1.91% and 2.52% during storage. The average total protein values of sample 1, sample 2, and sample 3 were 2.24%, 2.37%, and 1.97%, respectively. Total protein changes were significantly different in sample 1 during

storage, and among the all samples (P<0.05). However, no significant differences observed in sample 2 and in sample 3 during storage (P>0.05).

Total protein changes were given for kefir samples during storage in Figure 4.19. According to figure oat milk concentration was the effective factor for total protein level. Increase of oat milk concentration induced to a decrease of protein percentage.

Aroma concentration also affected the total protein percentage of samples. Increase of aroma concentration caused to decrease in protein level.

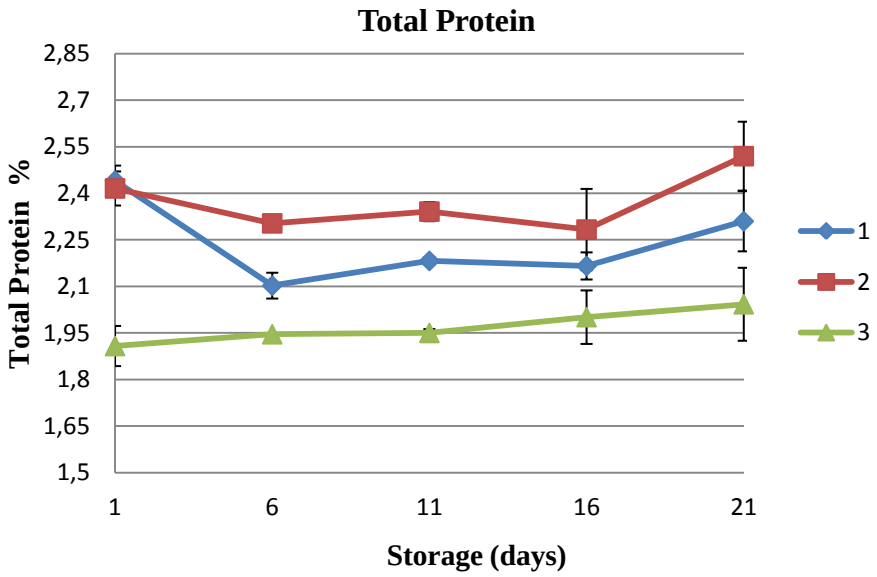


Figure 4.19. Total protein changes in kefir samples during storage

According to kefir standards, kefir should include higher than 2.8% (Codex Standard for Fermented Milks CODEX STAN 243-2003 FAO/WHO 2001, Turkish Food Codex; Fermente Sütler Tebliği 2001). Aroma and oat milk concentration decreased protein content greatly.

4.3.4. Total Fat Content

Average total fat content for kefir for sample 1, samples 2, and sample 3 were 1.85%, 1.95%, 1.35%, respectively. Analyses were done on the first day of storage. These results were in acceptable limit in kefir standards.

4.3.5. Whey Off

Whey off results of kefir samples were given in Table C.5 (Appendix C). Results were observed between 2% and 27% during storage. The average whey off values of sample 1, sample 2, and sample 3 were 11.2%, 5.7%, and 19.8%, respectively. Whey off change was significantly different in all samples and among the all samples during storage ($P<0.05$).

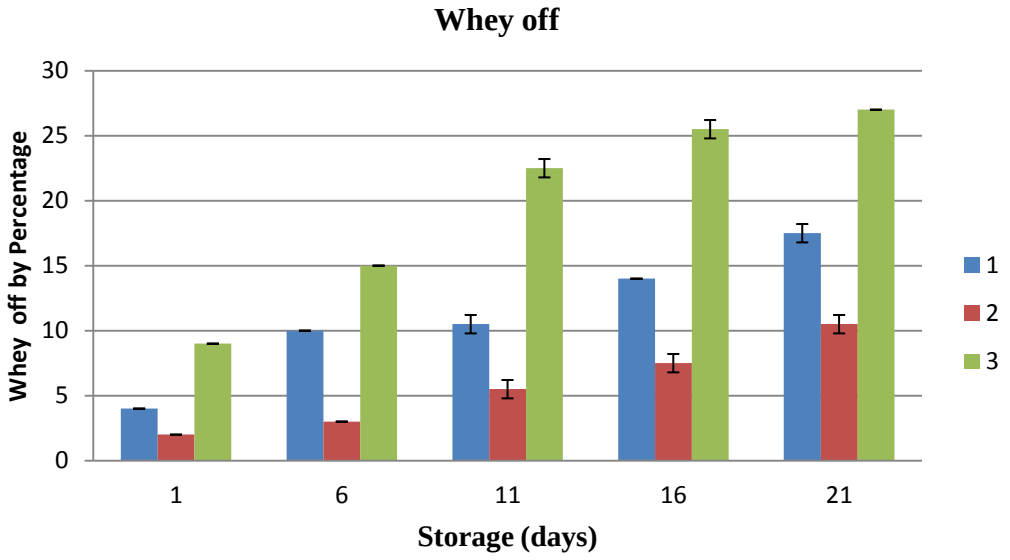


Figure 4.20. Whey off change in kefir samples during storage

Whey off changes were given in kefir samples during storage in Figure 4.20. According to figure, oat milk concentration was the effective factor for phase separation. Increase of oat milk concentration induced to more phase separation in samples. Also oat milk concentrations affected the differences between the 1st and 21th days of storage's results. During storage, the biggest differences were observed in sample 3, which was 18%. Besides, lowest differences determined in sample 2, which was 8.5%. Aroma concentration might influence whey off, too. Usage of water in preparation of aroma led to more phase separation in samples.

4.3.6. Rheological Results

Kefir samples viscosity results were given in, Table C.6 (Appendix C). For all samples equations were calculated related to apparent viscosity (Pa.s) and shear rate (1/sec) changes and R^2 of sample were determined between 0.9972 and 0.8747. The R^2 values changed between 0.9957 and 0.9692 in sample 1, 0.9972 and 0.9861 in sample 2, and 0.9892 and 0.8747 in sample 3. Sample 1 and sample 2 showed more coherence to model than sample 3.

In Figure 4.21 apparent viscosity (μ) changes were given at 300 s^{-1} during storage and it was expressed as mPa.s. Highest viscosity was observed in sample 2, on the other hand lowest viscosity was determined on sample 3. Sample 2 and sample 3 apparent viscosity changes exposed no significantly differences during storage ($P>0.05$). However, in sample 1 and among the all sample there was a significantly differences was observed during storage ($P<0.05$).

Oat milk and aroma concentrations induced to changes in viscosity. Increase of these concentrations evoked to lowering effect on viscosity.

L. Garrote reported that different kefir samples prepared with different kefir grains apparent viscosity at 629 s^{-1} were determined between 7.5 and 15.4 mPa.s. (Garrote, et al. 2000).

Viscosity of samples also affected sensory properties, sample 3 had a low consistency and it caused disliking in sensory profile. However, for sample 1 and sample 2 viscosities affected the sensory profile positively. It was studied that in fermented milk products, high consistency index and high pseudoplasticity increased the acceptability of samples in lactic acid beverages (Penna, et al. 2001).

Viscosity increased after the first day analyses until the 6^{th} day of storage in sample 1 and 3, and then decreased to the 21^{th} day of analyses. In sample 2 viscosities showed similar results; however, viscosity was increased in the 21^{th} day of storage. It was reported that viscosity of cow milk kefir decreased during the 1^{st}, 5^{th}, and 10^{th} day of storage at 158 s^{-1} (Tratnik, et al. 2006).

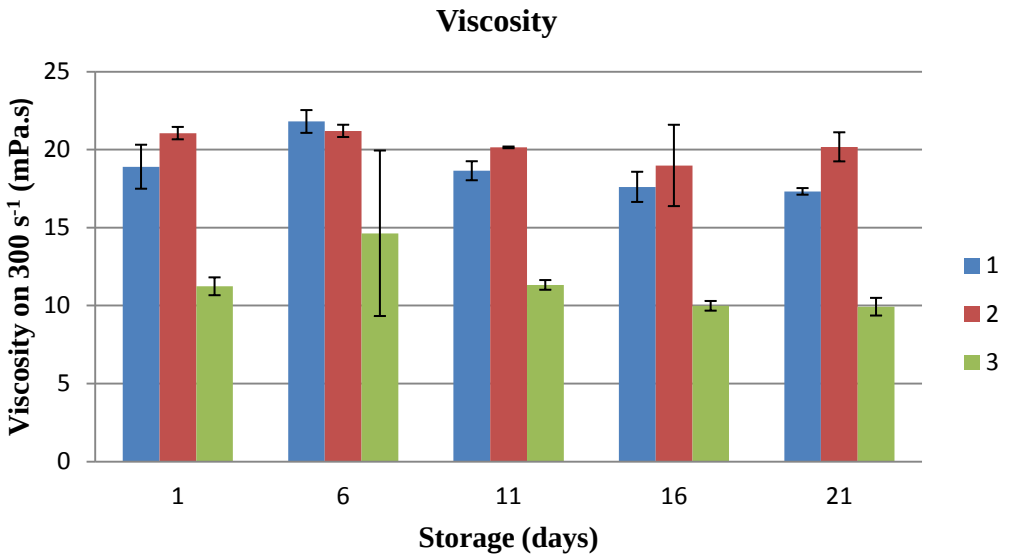


Figure 4.21. Viscosity changes in kefir samples during storage

4.3.7. Color Results

Color results were given by color features of *L** (lightness), *a** (red–green component), and *b** (yellow–blue component) in Table C.7 (Appendix C).

*L** (lightness) changes was determined between 69.45 and 76.29. There was significant differences were observed in all samples and among the all samples during storage ($P<0.05$).

*L** changes of kefir samples during storage was given in Figure 4.22. Highest *L** was performed in sample 2, lowest one was determined in sample 3. Oat milk concentration might be affected the *L** value. Increase of oat milk concentration caused decrease in *L**

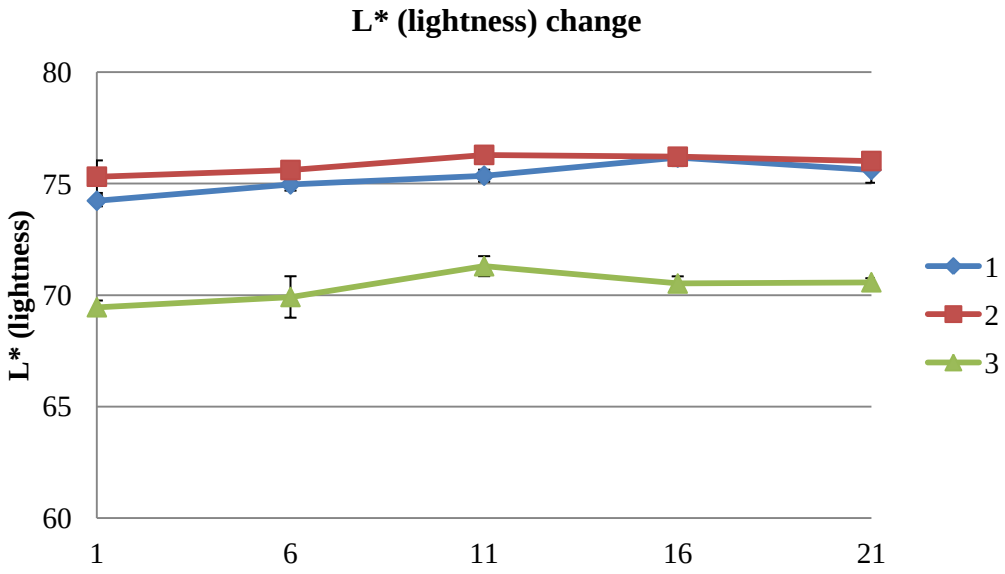


Figure 4.22. L* (lightness) change in kefir samples during storage

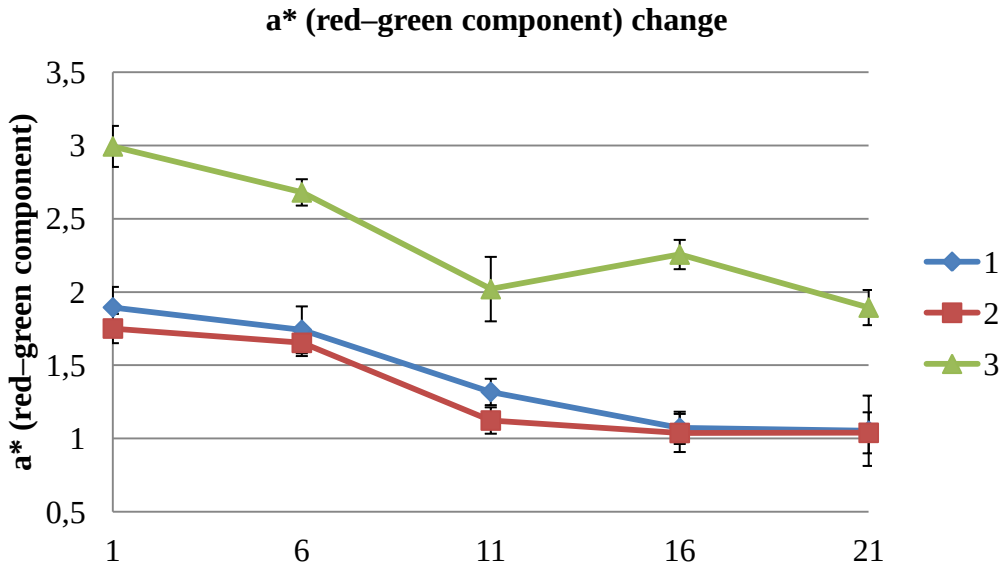


Figure 4.23. a* (red-green component) change in kefir samples during storage.

*a** (red-green component) changes was determined between; 1.04 to 3.00. Significant changes were observed in all samples and among the all samples during storage ($P<0.05$). *a** changes of kefir samples during storage was given in Figure 4.23. The highest *a** was obtained in sample 3, lowest one was determined in sample 2. Blueberry aroma had a red color so *a** value might be affected by aroma concentration. An increase of aroma concentration caused increase in *a** values.

During storage *a** values showed decreases in all sample. Only in sample 3, *a** value increased after the 11th day of storage then decreased after the 16th day of storage. In sample 1 and sample 2 *a** changes in values were determined nearly same. Color effect of aroma was lost after the 11th day of storage in these samples.

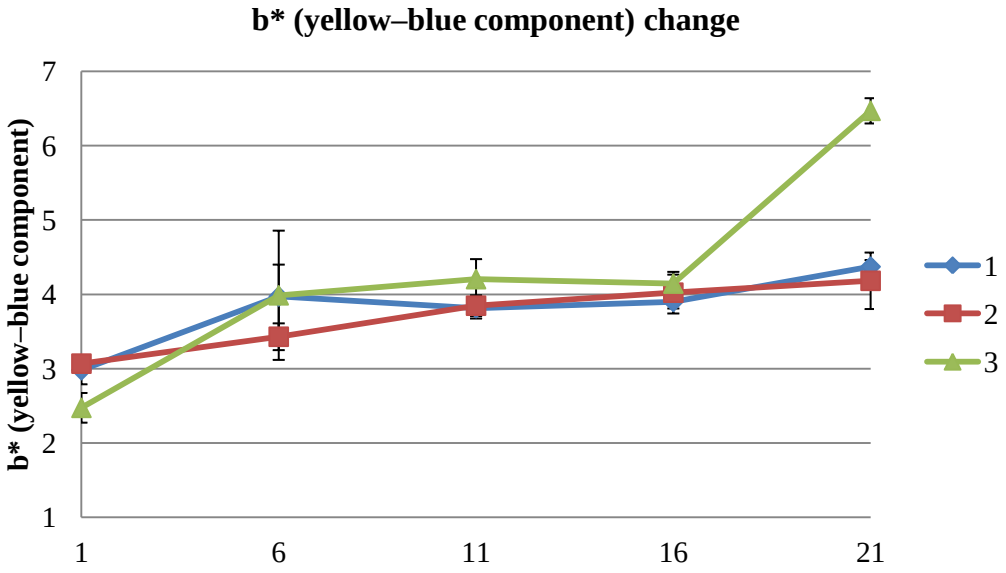


Figure 4.24. b* (yellow-blue component) change in kefir samples during storage

*b** (yellow-blue component) changes was observed between 2.47 and 6.47. Significant differences were observed among each sample within the storage time ($P<0.05$). However, no significant differences were observed among the all samples within each storage time ($p>0.05$).

Changes in *b** of kefir samples during storage was given in Figure 4.24. The highest and the lowest *b** was obtained in sample 3 during storage. Red color of aroma and yellow-brown color of oat milk affect the samples *b** value.

During storage *b** values showed increases in all samples. In the first day of analyses low *b** values were determined in all samples. This might be caused by red color of aroma. The *b** values got equilibrium after the 6th day of analyses to the 16th day of analyses and values increased slowly. After the 16th day of storage, effect of aroma was lost in all samples so yellow-brown color of oat milk affected the *b** values greatly. In sample 3 high percentage of oat milk concentration increased *b** values greatly.

4.3.8. Volatile Profile Results

Aroma is an important parameter for quality of product and also important for selection and consumption by consumer. Volatile compounds and organic acids are not only efficient on formation of aroma, but also preservation of food. Acetaldehyde, acetone, acetoin diacetly, and ethanol are important compounds for fermented dairy products.

Ethanol (2.00 min.), ethyl acetate (2.54 min.), diacetly (3.17 min.), toluene (4.73 min.), acetoin (9.47 min.), D-limonene (11.22 min.), 2-heptanone (11.67 min.), 1-hexanol (12.45 min.), eucalyptol (12.91 min.), dimethylamine (15.45 min.) benzaldehyde (17.54 min.), 2-nonanone (17.94 min.), 2-octanol (18.30 min.) limonene oxide (18.64 min.), propanedioic acid (20.71 min.), octanoic acid (25.45 min.), phenol (29.65 min.), unknown-1 (5.40 min.), unknown-2 (23.60 min.), unknown-3 (28.22 min.) were determined during storage. Chromatogram of volatile compounds was given in Figure 4.25.

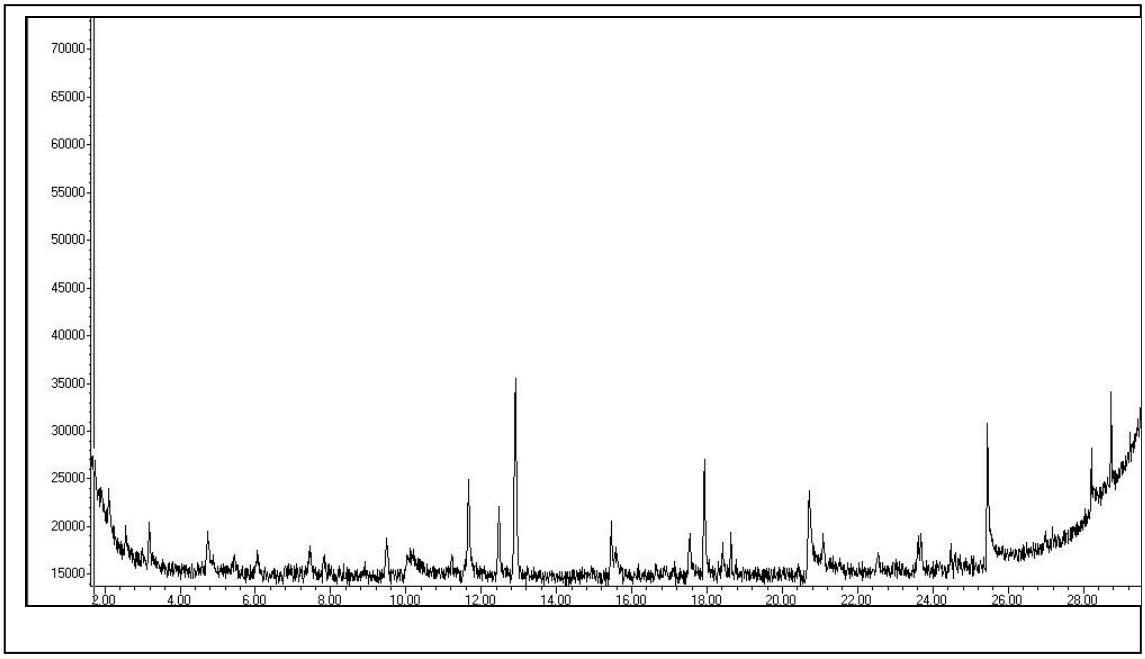


Figure 4.25. A Representative of volatile compounds in kefir

Chromatogram of oat milk and aroma of blueberry was given in Appendix E. Hexanal (7.81 min.) was determined in oat milk with a great percentage. Aroma of blue

berry contained lots of volatile compounds. Toluene, D-limonene, eucalyptol, phenol was determined in aroma greatly. Also other alcohol types and compounds of blueberry determined.

%area volatile compounds by for sample 1 during storage was given in Table 4.8. All compounds were determined during storage

Table 4.8. Percent area of volatile compounds for sample 1 during storage

	Storage				
Compounds	**1**	**6**	**11**	**16**	**21**
Ethanol	1.22±0.06	0.98±0.05	0.97±0.11	1.79±0.17	1.78±0.22
Ethyl acetate	0.75±0.05	1.31±0.22	4.13±0.36	1.50±0.26	5.32±1.02
Diacetyl	3.94±0.31	4.01±0.02	2.11±0.32	0.79±0.09	0.00±0.00
Toluene	4.29±1.22	5.51±0.50	5.16±0.61	5.92±1.17	1.56±0.46
Acetoin	23.17±0.19	10.55±1.42	2.03±0.67	3.46±1.05	0.00±0.00
D-limonene	1.59±0.25	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2-Heptanone	7.34±1.49	8.28±0.75	6.42±0.73	7.83±1.02	17.95±1.53
1-Hexanol	3.15±0.63	6.03±1.74	14.67±1.21	8.45±1.19	23.72±1.82
Eucalyptol	34.04±5.79	39.73±4.43	45.96±2.45	25.09±3.37	17.24±3.62
Dimethylamine	0.86±0.11	0.00±0.00	1.58±0.02	4.27±0.74	1.10±0.23
Benzaldehyde	0.00±0.00	1.89±0.22	0.00±0.00	4.62±1.23	0.00±0.00
2-Nonanone	4.29±1.01	6.91±1.41	5.53±2.11	4.63±0.98	7.65±1.80
2-Octanol	0.00±0.00	0.00±0.00	0.00±0.00	2.49±0.76	4.47±0.63
Limonen oxide	2.64±0.30	2.02±0.12	2.17±0.74	2.68±0.94	1.27±0.16
Propanedioic acid	4.10±1.01	5.26±1.55	6.10±0.43	10.83±1.24	6.05±0.85
Octanoic acid	2.94±0.77	6.19±1.24	3.16±1.11	7.81±0.96	9.47±2.08
Phenol	0.00±0.00	0.00±0.00	0.00±0.00	0.53±0.02	1.79±0.22
Unknown 1	2.66±1.21	1.32±1.65	0.00±0.00	0.00±0.00	0.00±0.00
Unknown 2	3.01±0.75	0.00±0.00	0.00±0.00	3.70±0.26	0.00±0.00
Unknown 3	0.00±0.00	0.00±0.00	0.00±0.00	3.60±0.96	0.62±0.74

Mean ± S.D. (n=2)

Ethanol content of %area decreased till to 11th day of storage than increased. Ethyl acetate generally increased during storage. However, diacetly content decreased during storage and no diacetly was determined on 21th day of storage. Toluene content was found similar during 16th day of storage. Only 21th day of storage decreases was observed. Acetoin showed closely to diacetly results with decreasing during storage and

acetoin not observed on 21th day of storage. D-limonene was observed only in first day analyses. 2-heptanone indicated close results till to 16th day of storage, on the other hand increase was observed on 21th day analyses. 1-hexanol increased during storage. Eucalyptol was the main observed volatile compounds during storage. It was decreased after 11th day of storage. Benzaldehyde and dimethylamine showed similar results during storage. Highest areas of them were determined on 16th day of storage. However, other days of analyses their level determined low. 2-nonanone showed similar results during storage. 2-octanol determined after 11th day analyses and increased up to 21th day of storage. Close results were investigated in limonene oxide only 21th day of analyses decrease was observed. Level of Propanedioic acid were increased till to 16th day of storage, and then decreased on 21th day analyses. Octanoic acid showed increase during storage, only 11th day of storage a decrease was seen. Phenol content of sample 1 was observed on 16th and 21th day of analyses. Unknown volatile compounds also observed during storage in sample 1.

For sample 2 %area of volatile compounds during storage, was given in Table 4.9. Except unknown-2 all volatile compounds were investigated during storage. Ethanol level decreased after first day analyses to 6th day, then decreases was observed up to 21th day of analyses. Ethyl acetate level was determined between; 2.89% to 0.46%. During storage rise and fall was observed ethyl acetate levels. Level of diacetyl was observed closely during 11th day of analyses. On 16th and 21th day of results were investigated lower. No toluene was determined on first day analyses. But after first day toluene was determined and level of toluene was decreased up to 16th day, and then increased on 21th day of analyses. Acetoin was determined great percent with 32.49% and 21.11% on 1st and 6th day of analyses then decreased to 0.44% on 16th day of storage and increased to 1.20% on 21th day of storage. D-limonene observed only first day of storage. Decrease was observed in 2-heptonone during storage 3.61% to 18.47%. 1-Hexanol levels was determined in common with 2-heptonone. But on first day of storage no 1-hexanol was investigated. Eucalyptol determined with a high level during storage. Decrease was observed 1th to 16th day of storage. Increase was seen on 21th day of analyses on eucalyptol level. Dimethylamine was determined between; 0.00 % to 5.63% during storage. Benzaldehyde was investigated on 16th and 21th day of storage. 2-nonanone was observed between; 5.04% to 13.15% during storage. 2-Octanol was determined after 11th day of storage. Low level of limonene oxide was determined during storage between; 1.63% to 3.67%. Propanedioic acid level was decreased till to

16th day of storage and then decrease was observed on 21th day analyses. Highest level of octanoic acid was determined with 11.19% on 16th day of analyses. However lowest level was observed with 4.47% on 6th day of storage. Phenol was observed on 1st, 16th and 21th days of storage. Unknown-2 and unknown-3 also determined during storage in sample 2.

Table 4.9. Percent area of volatile compounds for sample 2 during storage

	Storage				
Compounds	**1**	**6**	**11**	**16**	**21**
Ethanol	0.26±0.06	0.00±0.00	0.21±0.02	0.94±0.33	3.24±0.37
Ethyl acetate	1.77±0.39	0.95±0.32	2.89±0.92	0.46±0.12	1.89±1.09
Diacetyl	3.59±1.07	2.31±0.74	3.33±1.12	0.65±0.08	1.82±0.86
Toluene	0.00±0.00	4.63±0.09	3.60±1.02	1.90±0.25	4.24±1.26
Acetoin	31.49±0.94	21.11±3.86	11.94±2.17	0.44±0.17	1.20±0.52
D-limonene	17.80±2.17	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2-Heptanone	3.61±0.17	6.53±0.48	10.94±1.15	13.19±1.54	18.47±3.93
1-Hexanol	0.00±0.00	4.77±1.37	8.18±2.28	8.63±0.64	15.21±0.77
Eucalyptol	20.02±1.39	33.92±5.20	24.26±2.20	15.70±1.28	20.81±0.75
Dimethylamine	1.47±0.18	0.00±0.00	5.63±1.12	3.45±1.11	2.26±0.88
Benzaldehyde	0.00±0.00	0.00±0.00	0.00±0.00	4.99±1.02	4.05±1.23
2-Nonanone	5.04±0.39	10.58±2.11	8.95±1.30	13.15±2.14	9.69±1.31
2-Octanol	0.00±0.00	0.00±0.00	0.00±0.00	3.56±0.15	1.88±0.85
Limonen oxide	1.97±0.25	3.67±0.56	1.63±0.22	2.75±0.75	1.91±0.46
Propanedioic acid	2.62±0.29	7.06±1.57	7.84±1.64	9.33±0.35	5.71±0.62
Octanoic acid	9.00±2.37	4.47±1.18	9.18±0.28	11.19±1.49	5.26±3.17
Phenol	1.37±0.33	0.00±0.00	0.00±0.00	4.60±0.81	0.93±0.06
Unknown 1	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Unknown 2	0.00±0.00	0.00±0.00	1.42±0.75	1.53±0.22	1.44±0.41
Unknown 3	0.00±0.00	0.00±0.00	0.00±0.00	3.52±0.74	0.00±0.00

Mean ± S.D. (n=2)

Volatile compounds were given by %area for sample 3 during storage in Table 4.10. Expect unknown-1, all compounds was observed during storage.

Table 4.10. Percent area of volatile compounds for sample 3 during storage

	Storage				
Compounds	**1**	**6**	**11**	**16**	**21**
Ethanol	1.28±0.29	1.32±0.42	0.45±0.19	7.70±0.55	8.41±0.63
Ethyl acetate	0.54±0.15	3.10±0.22	5.13±1.02	1.79±0.05	4.81±0.85
Diacetyl	3.03±0.22	3.40±0.84	1.73±0.14	0.95±0.19	2.97±1.01
Toluene	0.58±0.02	5.44±0.28	5.79±0.99	20.37±1.47	2.87±0.73
Acetoin	22.08±1.87	8.42±1.75	4.04±0.80	3.92±0.56	0.51±0.07
D-limonene	1.87±0.05	1.89±0.17	0.00±0.00	0.00±0.00	2.07±0.12
2-Heptanone	5.57±0.43	4.98±0.11	8.83±0.14	4.20±0.19	4.57±1.21
1-Hexanol	1.11±0.04	5.92±1.10	9.24±0.27	7.53±0.75	12.97±1.91
Eucalyptol	41.51±5.27	46.66±7.12	24.49±0.25	17.65±1.88	21.91±2.16
Dimethylamine	0.00±0.00	0.83±0.01	2.17±1.17	1.62±0.15	1.28±0.26
Benzaldehyde	3.99±0.75	1.89±0.12	10.18±2.66	3.17±0.09	1.63±0.72
2-Nonanone	3.18±0.05	4.00±0.79	6.05±0.79	5.42±1.23	5.43±0.96
2-Octanol	0.00±0.00	0.00±0.00	1.20±0.29	2.30±0.27	6.26±1.69
Limoneneoxide	2.76±0.14	3.58±0.35	2.88±0.15	3.45±0.09	5.89±0.03
Propanedioic acid	3.93±0.90	3.99±0.11	7.05±0.52	6.08±1.12	6.21±0.40
Octanoic acid	4.34±0.70	2.41±0.15	8.16±0.42	6.91±1.27	9.08±0.85
Phenol	2.73±0.02	0.00±0.00	0.92±0.03	2.06±0.32	0.00±0.00
Unknown 1	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Unknown 2	1.50±0.29	2.16±0.48	1.69±0.11	2.37±0.23	2.32±0.20
Unknown 3	0.00±0.00	0.00±0.00	0.00±0.00	2.51±0.25	0.80±0.02

Mean ± S.D. (n=2)

After first day analyses ethanol content was decreased to minimum level with 0.45% on 11th day of storage and then increased to 8.41% on 21th day of storage. Level of ethyl acetate was decreased after first day analyses to 11th day of storage and then decrease was observed on 16th day of storage and increased on 21th day again. Diacetly level was decreased up to 16th day and increased on last day. Increases were monitored on level of toluene till to 16th day then toluene level was decreased on 21th day of storage. Regularly decrease was observed on acetoin level during storage. D-limonene was investigated on 1th, 6th and 21th day of storage. On the other hand no level of D-limonene was observed on 11th and 16th day of storage. 2-heptanone was calculated between; 4.20% to 8.83% during storage. 1-hexanol increased during storage. Only a bit decrease was observed on 16th day. Level of eucalyptol was decreased during storage

but it was determined greatly all of days. Dimethylamine was increased up to 11th day then increases were determined to 21th day of storage. Rise and fall was observed on level of benzaldehyde between; 1.63% to10.18% during storage. 2-nonanone level was increased till to 16th day then it balanced. Level of 2-octanol was determined after 6th day and increases were observed up to 21th day. Limonene oxide was determined between; 2.76% to 5.89% during storage. Also Propanedioic acid was calculated between; 3.93% to 7.05%. Higher results were obtained after 6th day on Propanedioic acid level. Octanoic acid was detected between; 2.41% to 9.08%. Phenol was determined on 1st, 11th and 16th day of storage. Unknown-2 determined in all days. However unknown was detected after 11th day of storage.

In comparison of all samples, ethanol level firstly decreased in all samples after first day, than increases was detected after 11th day. During storage in all days ethanol was observed expect 6th day of sample 2. Highest ethanol level was calculated on sample 3 than sample 1 and lowest one was observed on sample 2. Yeast count affected the level of ethanol. Yeast results supported these results highest yeast and ethanol levels were calculated on 16th and 21th day. It was reported that for production of ethanol in kefir yeasts are responsible (Wouters, et al. 2002). Seydim, et al. (2000), informed that level of ethanol increased during 21 day of cold storage. In Norwegian and Brazilian kefir, ethanol content was increased parallel to yeast count during storage (Gronnevik, et al. 2011; Magalhaes, et al. 2011).

Similar results were observed in ethyl acetate, rise and fall observed in all samples. Level of diacetyl balanced up to 11th day. Then great decreases were detected in all samples.

Highest toluene level was observed on sample 3 than sample 1 lowest one was calculated on sample 2. In aroma toluene was investigated clearly. Aroma of blueberry caused detection of toluene in samples. Increase of aroma concentration increased the level of toluene

Acetoin was decreased in all samples during storage. Highest acetoin level was investigated in sample 2, than sample 1. However, lowest acetoin level was calculated in sample 3. Decreases were observed greatly on 6th and 11th days. It was reported that Aceotin content increases during fermentation. However, decrease was observed on acetoin level during cold storage (Seydim, et al. 2000).

D-limonene was detected in sample 1 and 2 only on first day analyses. However it was detected on 1st, 6th and 21th day of storage in sample 3. Increases was observed in

level of 2-heptonen during storage on sample 1 and 2. On the other hand rise and fall was determined in sample 3. Level of 1-hexanol increased in all samples during storage. Highest increase was calculated between; 16^{th} and 21^{th} day results.

Eucalyptol was determined in all samples during storage with clear peak. It was detected in blueberry aroma and it come to samples from aroma. Highest %area of eucalyptol was calculated in sample 3 than sample 1 and lowest was observed in sample 2. Aroma concentration affected the level of eucalyptol positively.

Dimethylamine and benzaldehyde content changed during storage in all sample. Increases and decreases were observed. 2-nonanone was detected in all samples in all storage days. Rise and fall was determined in all samples.

2-octanol was determined in sample 1 and sample 2 after 11^{th} day of storage. In sample 3 it was detected after 6^{th} day of analyses. Increases were determined in sample 1 and sample 3. On the other hand decrease was detected in sample 2. Yeast population might affect the 2-octanol content. Increase of yeast caused to increase in 2-octanol.

Increases and decreases were calculated on level of limonene oxide, Propanedioic acid and octanoic acid. Limonene oxide was more balanced than others. Phenol content did not determine in all days. On 16^{th} day of storage it was detected in all samples.

Acetaldehyde did not detect in kefir samples. However, ethanol, acetoin and diacetly were determined. These are main aroma compounds of fermented dairy products. Other volatile compounds might be formed from composition of aroma and oat milk. It was reported that ethanol, ethyl acetate, 2-heptanone, benzaldehyde, 3-octanone, benzyl alcohol, limonene, 2-nonanone, methyl benzoate, methyl 3-methylbutanoate etc. was determined volatiles of wild blueberry (Lugemwa, et al. 1989). Also Benzaldehyde, limonene, nonanal, (Z)-3-hexenol was determined in oat leaves (Buttery, et al. 1982).

4.3.9. Organic Acid Profile Results

Organic acids are important compounds for the flavor in fermented dairy products. Organic acids affect sensory profiles so affect the acceptability of product by consumers. More over organic acids are natural preservatives. It was reported that lactic

acid inhibited pathogenic bacteria and used as a biopreservatives (Magnusson, et al. 2004).

Lactic acid, acetic acid, orotic acid and uric acid amounts were determined during storage in all samples by HPLC. Chromatogram of organic acid for a sample was given in Figure 4.26. Retention times were determined for orotic acid on 10.098 minutes, for lactic acid on 12.968 minutes, for uric acid 14.169 minutes and for acetic acid 15.231 minutes. Results were given in Appendix C, Table C.8. Chromatograms of blueberry aroma and oat milk also were given in Appendix E.

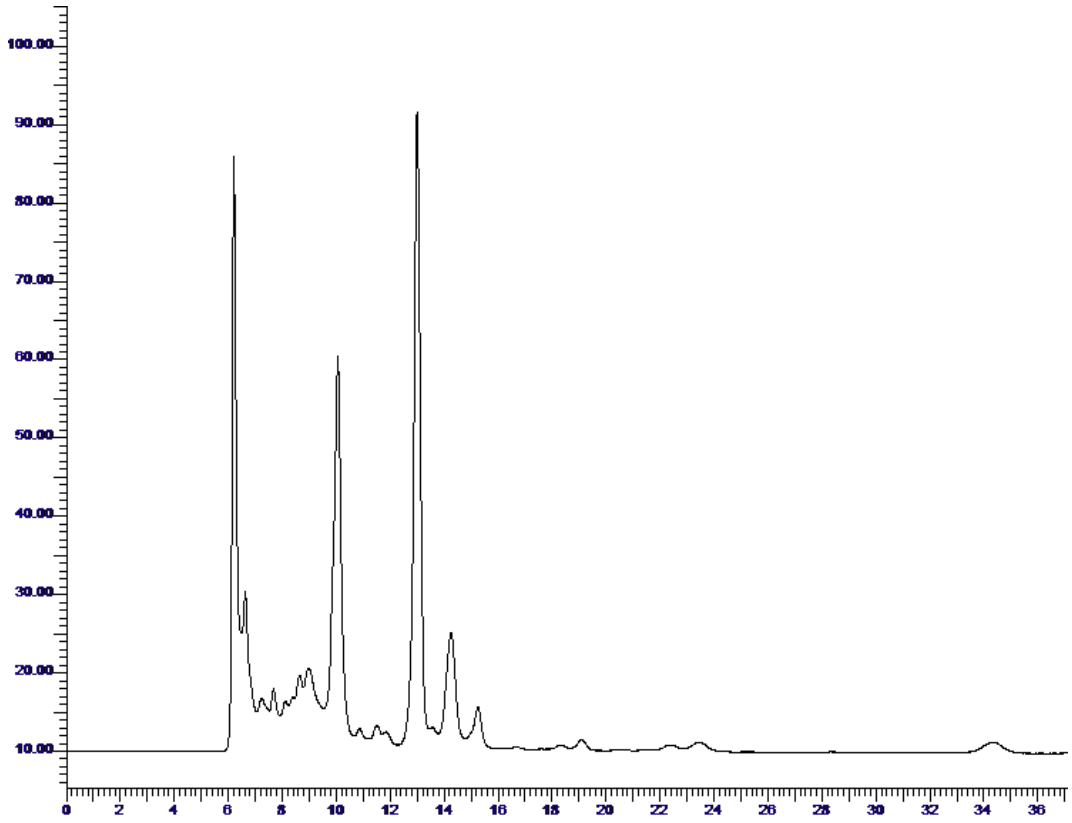


Figure 4.26. A representative organic acid chromatogram of kefir

Lactic acid and acetic acid changes during storage for sample 1 were given in Figure 4.27. No significant differences were observed during storage ($P>0.05$). Average lactic acid amount was determined 9404.66 ppm. Minimum was determined 8147.47 ppm. on 6^{th} day of storage and maximum was obtained 10175.67 ppm. on 21^{th} day of storage. Lactic acid amount decreased after first day of analyses then increased up to 21^{th} day of storage.

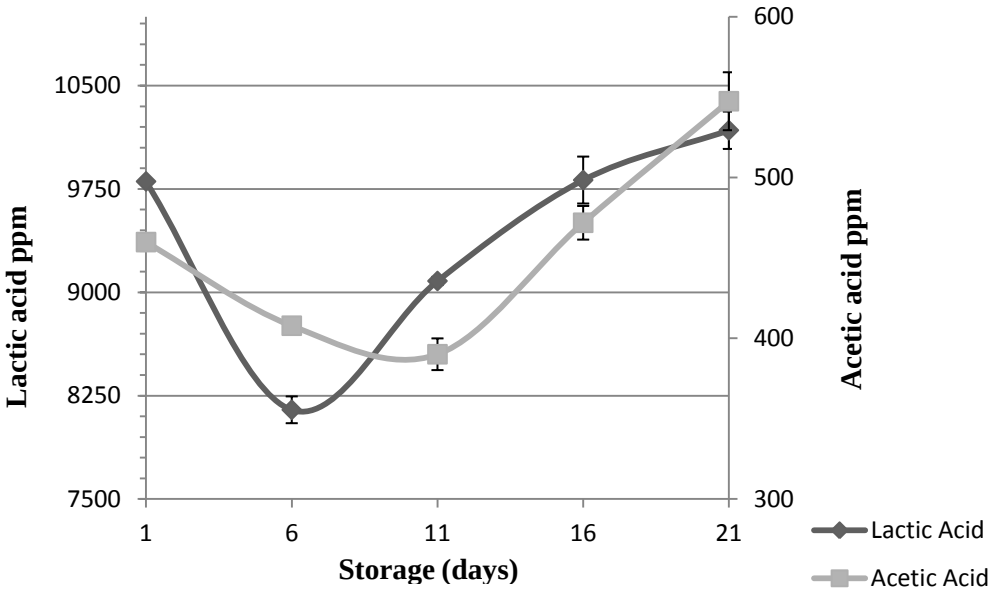


Figure 4.27. Lactic acid and acetic acid changes in sample 1 during storage

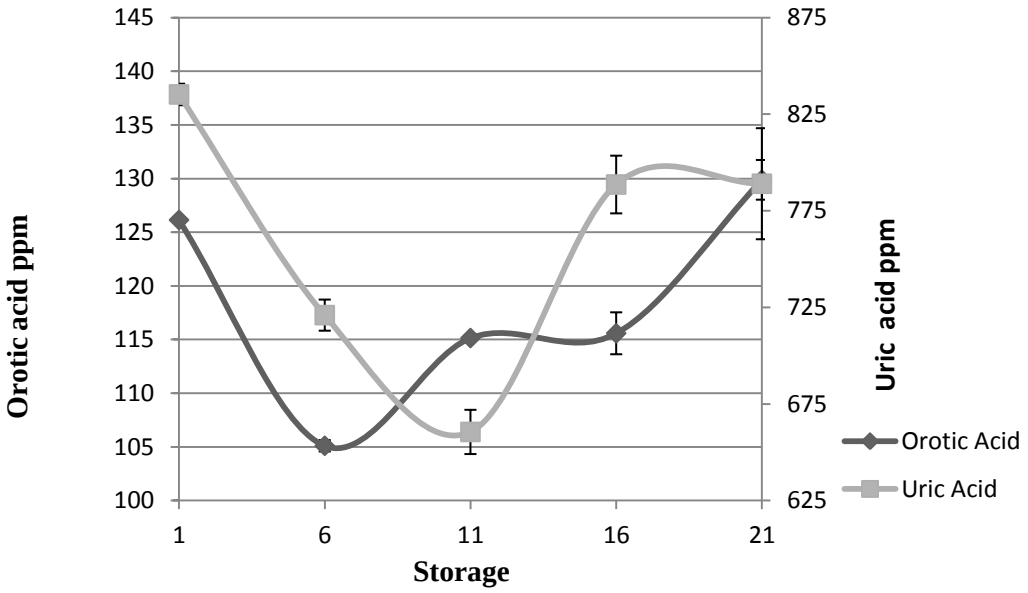


Figure 4.28. Orotic acid and uric acid changes in sample 1 during storage

Acetic acid amount was determined between; 390.02 ppm to 547.40 ppm. Average amount of acetic acid was calculated 455.35 ppm. Acetic acid decreased till to 11th day of storage then increased to 21th day of storage.

Orotic acid and uric acid changes during storage for sample 1 were given in Figure 4.28. No significant differences were observed during storage ($P>0.05$). Average

orotic acid amount was determined 118.37 ppm. Minimum was determined 105.13 ppm. on 6th day of storage and maximum was obtained 129.88 ppm. on 21th day of storage. Orotic acid amount decreased after first day of analyses to 6th day of storage. Then it was increased up to 21th day of storage.

Uric acid amount was determined between; 660.47 ppm to 835.19 ppm. Average amount of uric acid was calculated 758.83 ppm. Uric acid decreased till to 11th day of storage then increased to 21th day of storage.

Lactic acid and acetic acid changes during storage for sample 2 were given in Figure 4.29. No significant differences were observed during storage (P>0.05). Average lactic acid amount was determined 9422.25 ppm. Minimum was determined 7826.25 ppm. on 6th day of storage and maximum was obtained 10203.69 ppm. on 21th day of storage. Lactic acid amount decreased after first day of analyses then increased up to 21th day of storage.

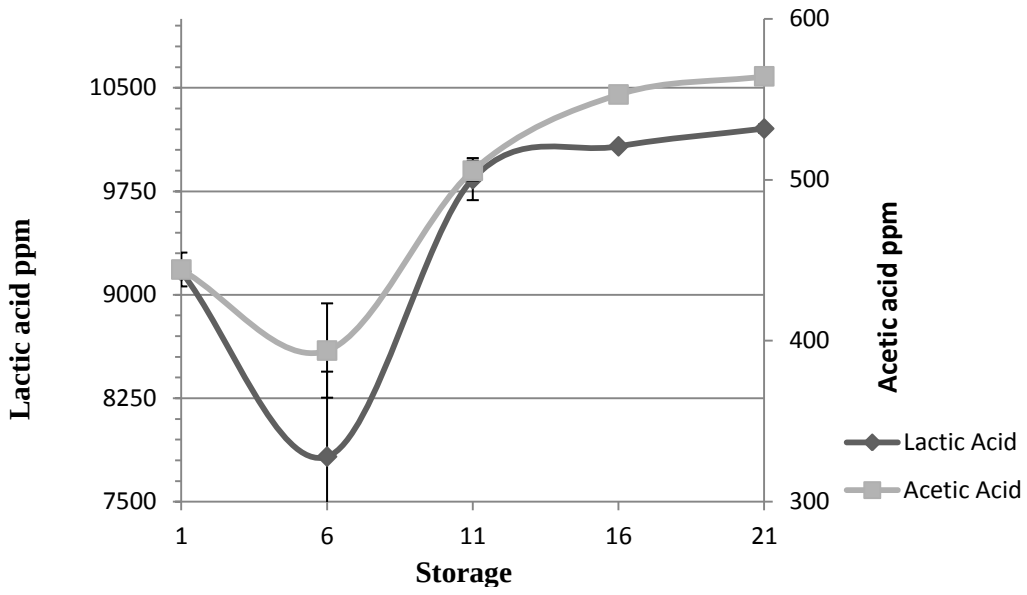


Figure 4.29. Lactic acid and acetic acid changes in sample 2 during storage

Acetic acid amount was determined between; 393.86 ppm to 564.23 ppm. Average amount of acetic acid was calculated 492.16 ppm. Acetic acid decreased after first day to 6th day of storage then increased to 21th day of storage.

Orotic acid and uric acid changes for sample 2 were given in Figure 4.30. during storage. No significant differences were observed during storage (P>0.05). Average orotic acid amount was determined 124.19 ppm. Minimum was determined 94.81 ppm.

on 6th day of storage and maximum was obtained 140.53 ppm. on 21th day of storage. Orotic acid amount decreased after first day of analyses to 6th day of storage. Then it was increased up to 21th day of storage.

Uric acid amount was determined between; 699.80 ppm to 895.15 ppm. Average amount of uric acid was calculated 818.47 ppm. Uric acid decreased till to 6th day of storage then increased to 21th day of storage.

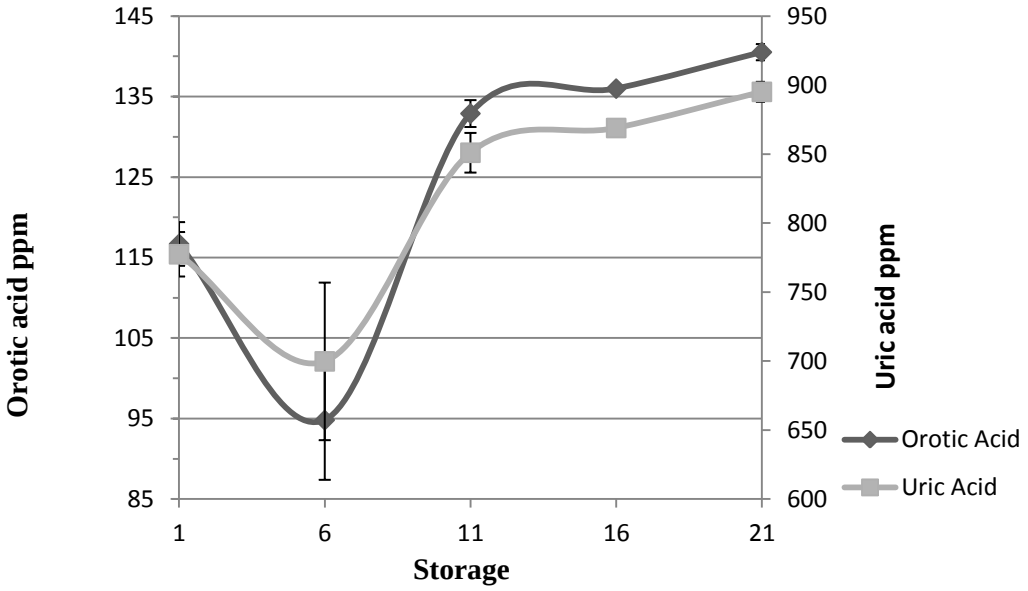


Figure 4.30. Orotic acid and uric acid changes in sample 2 during storage

In Figure 4.31. Lactic acid and acetic acid changes for sample 3 were given during storage. No significant differences were observed during storage (P>0.05). Average lactic acid amount was determined 8524.93 ppm. Minimum was determined 8049.92 ppm. on 6th day of storage and maximum was obtained 8817.99 ppm. on 6th day of storage. Lactic acid amount decreased after first day of analyses then increased up to 11th day of storage and got equilibrium to 21th day of storage.

Acetic acid amount was determined between; 357.11 ppm to 559.41 ppm. Average amount of acetic acid was calculated 488.05 ppm. Acetic acid increased till to 11th day of storage then decreased on 16th day of storage. Further it got maximum value on 21th day of storage.

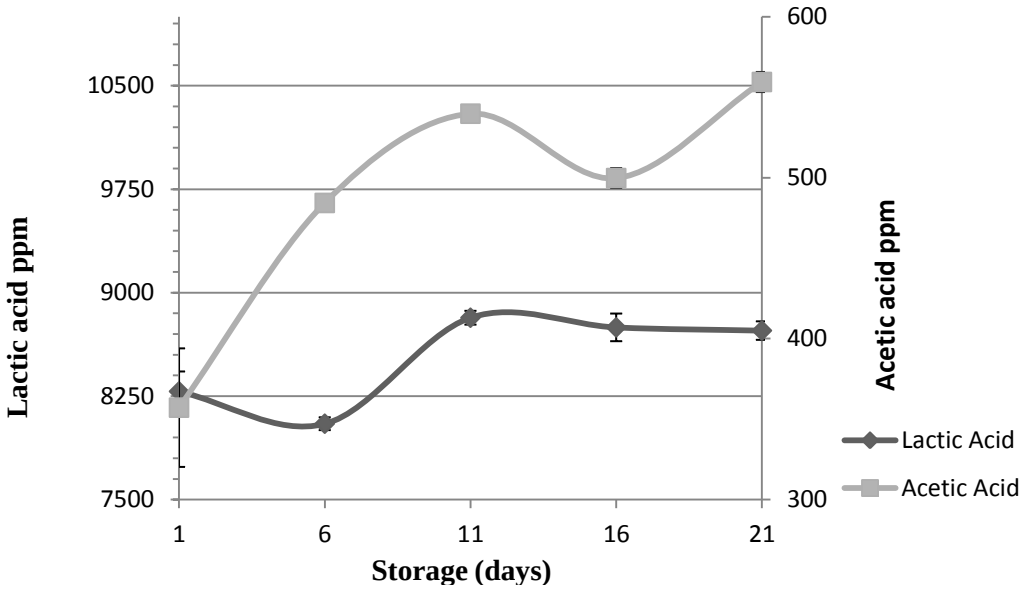


Figure 4.31. Lactic acid and acetic acid changes in sample 3 during storage

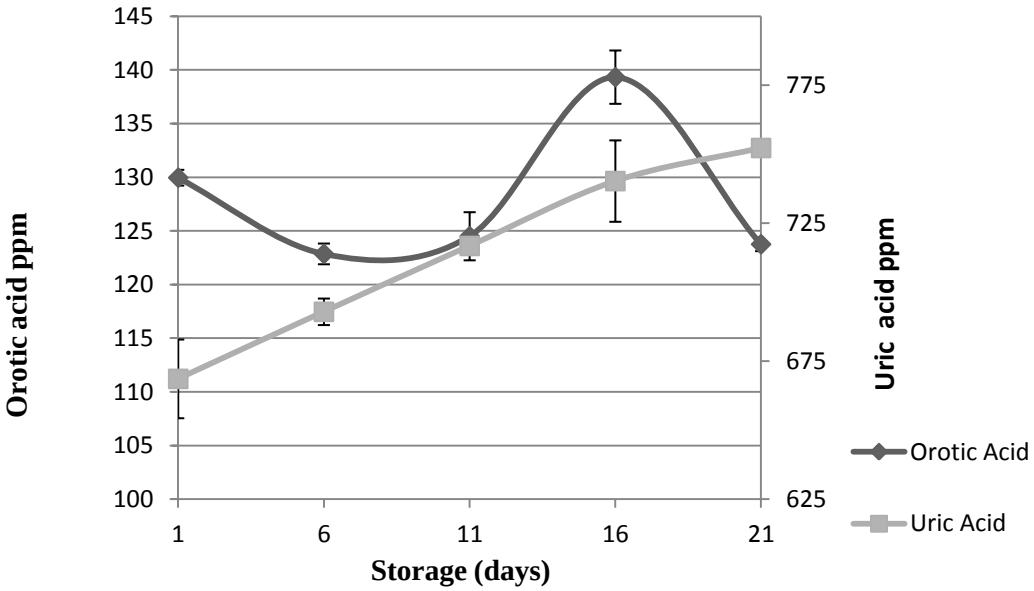


Figure 4.32. Orotic acid and uric acid changes in sample 3 during storage

Orotic acid and uric acid changes during storage for sample 3 were given in Figure 4.32. No significant differences were observed during storage (P>0.05). Average orotic acid amount was determined 128.08 ppm. Minimum was determined 122.86 ppm. on 6th day of storage and maximum was obtained 139.33 ppm. on 16th day of storage.

Uric acid amount was determined between; 668.59 ppm to 752.26 ppm. Average amount of uric acid was calculated 714.16 ppm. Uric acid increased to 21th day of storage.

Comparison of all samples, during storage sample 2 had the highest average lactic acid amounts; average of sample 1 was very close to sample 2. Besides, sample 3 had the lowest average lactic acid amount. Oat milk concentration might be affected the lactic acid amount. In all samples lactic acid amount firstly decreased than increases was detected after 6th day of storage.

Acetic acid amounts were determined highest in sample2, then sample 3. Lowest amount of acetic acid was observed in sample 1 during storage. Among the 1st day to 21th day results increase was observed in all samples.

Uric acid changes showed similar results with lactic acid results. Highest amount of uric acid was calculated in sample 2. However, lowest was determined in sample 3. Level of uric acid increased in sample 2 and sample 3 during storage. However decrease was observed in sample 1 during storage.

Orotic acid results had shown differences with other organic acids. Sample 3 had the highest orotic acid amount. On the other hand orotic acid amount of sample 1 was determined lowest. In all samples orotic acid level decreased on 6th day of storage and then increased during storage. Only in sample 3 a decrease was determined after 16th day of storage.

It was reported that slightly increase in lactate, orotate, urate and citrate production was observed during storage. Pyruvate did not determine and it converted to other substances (Seydim, et al. 2000).

4.3.10 Total Phenol Content

Total phenol content of kefir samples were given in Appendix C. Table C.9. Samples total phenol contents determined between; 188.65 to 262.98 µg/ml gallic acid. An average highest total phenol content calculated in sample 3 with 229.37 gallic acid (µg/ml) amount during storage. However, lowest one was established in sample 1 with 199.10 gallic acid (µg/ml).

Total phenol content change during storage was given in Figure 4.33. No significant differences were observed within all samples and among all samples during

storage (P>0.05).Total phenol content of blueberry aroma was determined 933.87 µg/ml gallic acid. Aroma concentration was the effective factor on total phenol content but results showed that oat milk concentration also affected the total phenol content. Even the sample 2 had less aroma concentration, total phenol content of sample 2 was not the least. This might be caused by making complex blueberry aroma with oat milk

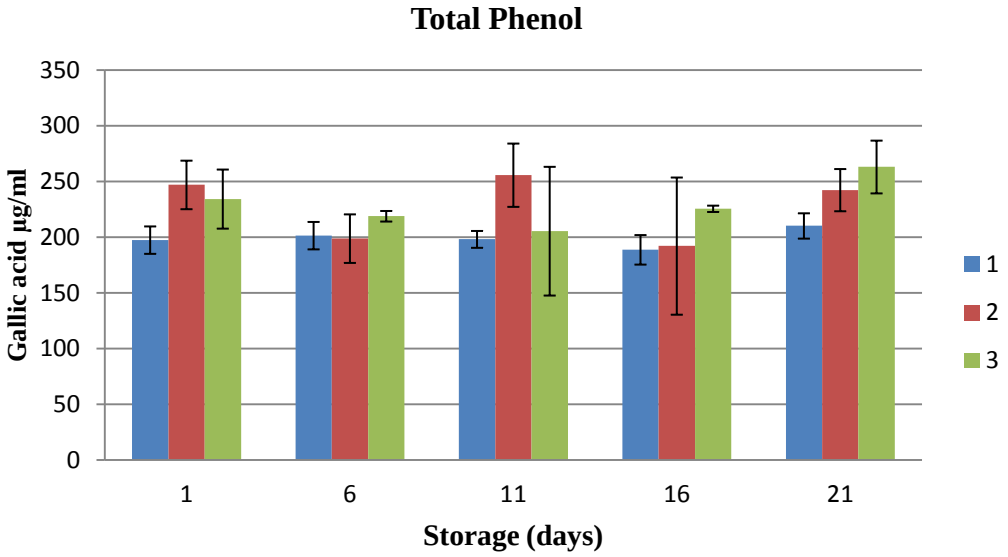


Figure 4.33. Total phenol content of samples during storage

4.3.11. Total Beta Glucan Results

Total beta glucan content of kefir samples were given in Appendix C, Table C.10. Total beta glucan content for samples were determined between; 0.011 to 0.096. Total beta glucan (w\w) changes were given in Figure 4.34. Averages results were calculated for sample 1; 0.049, for sample 2; 0.029 and for sample 3; 0.057. Sample 3 had the highest beta glucan content than sample 1 and lowest one determined on sample 2. All samples and all among the samples was significantly different (P<0.05). Oat milk concentration was the main factor for total beta glucan content. Increase of oat milk concentration increased the beta glucan content. Beta glucan content was oat milk also analyzed and it was determined 0.17 (w\w).

Increases were seen in total beta glucan content during storage in all samples. Only in sample 2 a bit decrease was detected on 11th day of storage.

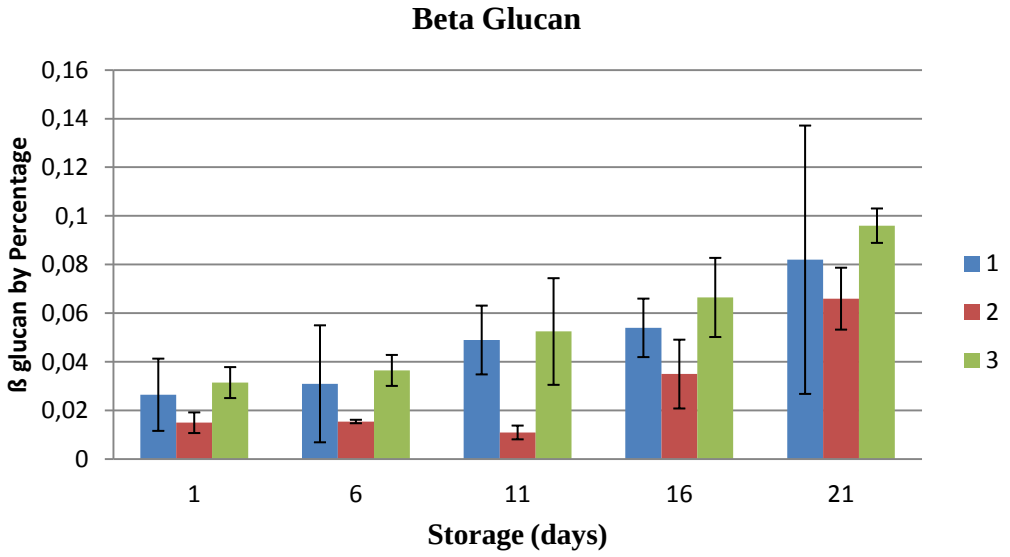


Figure 4.34. Total beta-glucan content of samples during storage

4.4. Microbiological Resulsts

4.4.1. *Lactococcus* spp.

Lactocccoccus ssp. enumeration of kefir samples were given in appendix C, Table C.11. Results were observed between $10^{7,78}$cfu/ml to $10^{9,18}$cfu/ml during storage. The average *Lactocccoccus* ssp. enumeration values of sample 1; $10^{8,37}$cfu/ml, for sample2; $10^{8,34}$cfu/ml and for sample $10^{8,47}$cfu/ml were observed.

In Figure 4.35. *Lactococcus* ssp. enumeration changes in kefir samples during storage, was given. According to figure *Lactobacillus* ssp. counts decreased during storage till 16th day of storage. 21th days of storage *Lactobacillus* ssp. count increased. For sample 1 and sample 2 *Lactococcus* ssp. showed similarity. In sample 3 *Lactococcus* ssp. enumeration was higher. Results were expectable according to optimization results. Oat milk concentration affected *lactococcus* ssp. count positively. It was studied that use of oat support the growth of lactic acid bacteria such as *Lactobacillus plantarum* to probiotic levels has been reported (Kedia et al. 2008).

It was reported that *Lactococcus* ssp. decreased till to 14th day of storage and increased on 21th day of storage (Irigoyen, et al. 2005).

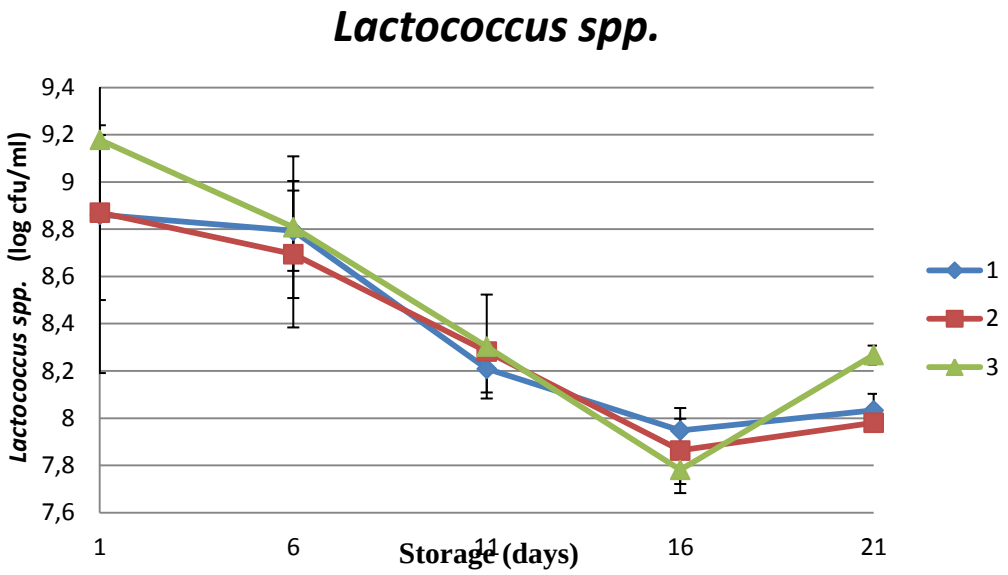


Figure 4.35. *Lactococcus* ssp. enumeration changes in kefir samples during storage

According to Turkish Food Codex, Lactococcus ssp. count was found in standards (Turkish Food Codex; Fermente Sütler Tebliği 2001)

4.4.2. *Lactobacillus* ssp.

Lactobacillus ssp. enumeration of kefir samples were given in appendix C, Table C.12. Results were observed between $10^{4,17}$cfu/ml to $10^{7,18}$cfu/ml during storage. The average *Lactoccoccus* ssp. enumeration values of sample 1; $10^{6,18}$cfu/ml, for sample2; $10^{6,32}$cfu/ml and for sample $10^{6,53}$cfu/ml were analyzed. Significant differences observed in all sample respectively and together during storage ($P<0.05$). However no significant differences were observed among the all samples ($p>0.05$).

In Figure 4.36. *Lactobacillus* ssp. enumeration changes in kefir samples during storage, was given. According to figure *Lactobacillus* ssp. counts decreased during storage. Decrease was slowly till 11th day of storage. After 16th and 21th days of storage decrease of *Lactobacillus* ssp. count became sharply.

It was reported that *lactobacillus* ssp. of kefir had reached 10^8 cfu/ml during 2nd day of storage, and then decreased slowly till to 14th day storage (Irigoyen, et al. 2005).

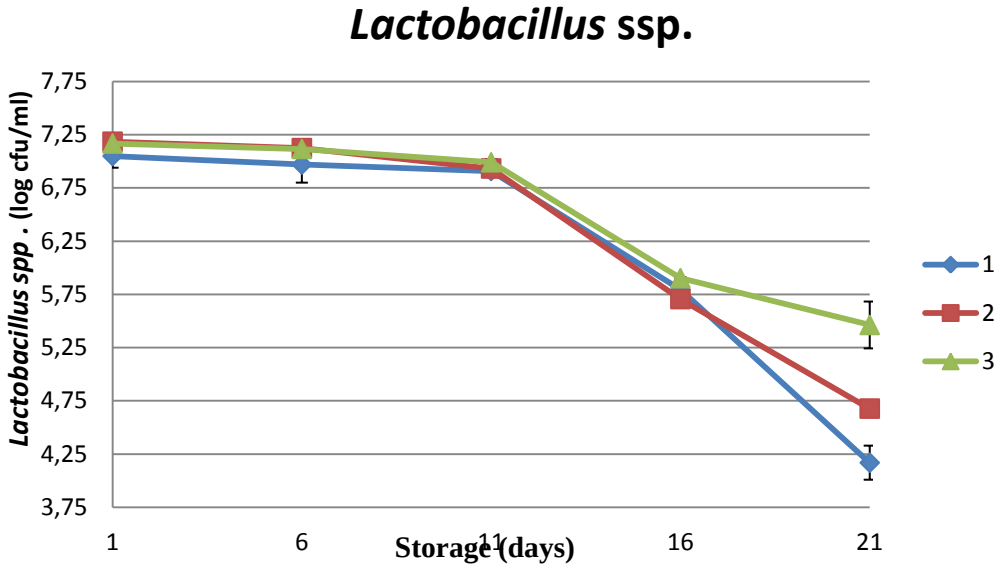


Figure 4.36. *Lactobacillus* ssp. enumeration changes in kefir samples during storage

4.4.3. Yeast

Yeast enumeration of kefir samples were given in appendix C, Table C.13. Results were observed between $10^{3,43}$ to $10^{5,44}$ during storage. The average yeast enumeration values of sample 1; $10^{4,07}$, for sample2; $10^{3,93}$ and for sample $10^{4,49}$ were analyzed.

Yeast enumeration changes during storage were given in Figure 4.37. Sample 1 and sample 2 showed similar results. All samples first day of enumerations were close each other then decreased all of them in 6th day of analyses. On 11th day of storage sample 1 and sample 2 continued to decrease. However sample 3 increased sharply. 16th day of storage yeast enumeration of sample 1 and sample 2 started to increase, On the other hand sample 3 decreased a bit. 21th day of storage all samples increased and all of them got the highest yeast count.

It was reported that yeast enumeration of kefir was determined 6.28; 5.77; 6.52 and 6.56 log cfu/ml during 1st, 7th, 14th, 21th day of storage (Seydim, et al. 2001).

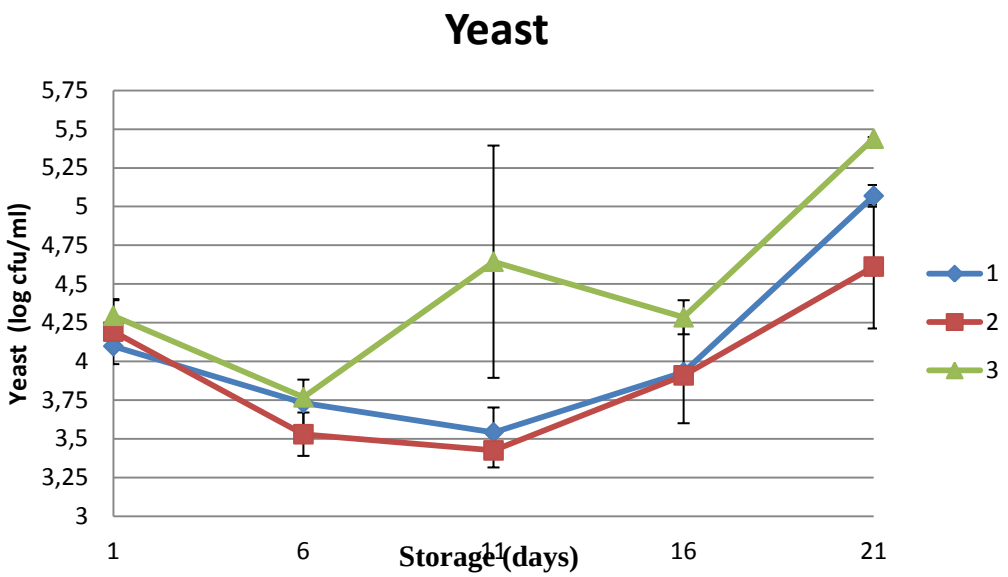


Figure 4.37. Yeast enumeration changes in kefir samples during storage

According to Turkish Food Codex, yeast counts should be higher than 10^4 cfu (Turkish Food Codex; Fermente Sütler Tebliği2001).

4.5. Sensory Profile Analyses

Panelists scored sample 1 to 10 according to personal liking in sensory profile analyses. Sensory analyses results given in Appendix C Table C.14., C.15., and C.16. According the results, samples sensory profile points were determined between; 7.40 to 4.31. Sensory profile of Sample 1 and sample 2 were closed each other. On the other hand sensory profile of sample 3 point was lower than other samples. Storage time affected sensory characteristic. For all samples sensory characteristics were given one by one and all together during storage in Figure 4.38., 4.39., 4.40., 4.41., 4.42., 4.43., 4.44. and 4.45

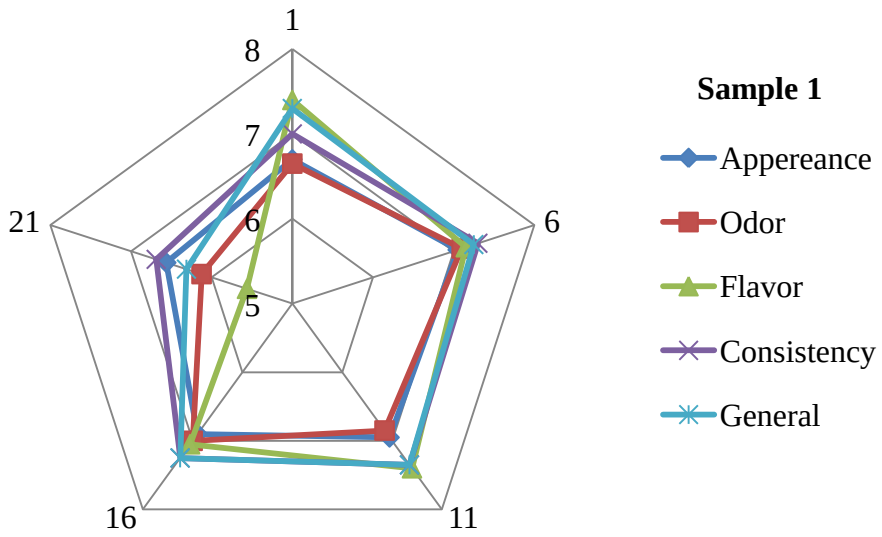


Figure 4.38. Sensory profile analyses during storage for sample 1

According to Figure 4.38 appearance of sample 1 determined between; 6.56 to 7.05. 6th day analyses showed highest point with 7.05. Besides, 21th day was the lowest pointed with 6.56. No significant differences were determined in appearance during storage (P>0.05).

Panelists scored odor profile of sample 1 between; 6.13 to 7.05. Highest scored was given on 6th day of odor analyses. However panelist gave lowest score on 21th day. Significant differences were determined in odor score during storage (P<0.05). Odor profile scores were closed each other till the 16th day. A decrease to be seen was observed in odor on 21th day scores. Souring in samples might be caused this. Also Fruit smell of aroma might be lost.

For flavor characteristics of sample 1, it was determined between; 7.40 to 5.56. Statistically differences were observed in flavor scores (P<0.05). Scores were very close each other during storage but great decrease was seen on 21th day of storage. Souring and decrease on aroma effect might be caused this.

Consistency scores indicated that between; 7.35 to 6.69 during storage. There was no differences determined in consistency during storage (P>0.05). No differences seen in 1st to 16th day analyses. On 21th analyses a bit decrease was observed.

In overall acceptability panelists scored sample 1 between; 7.35 to 6.31. Best liked sample was chosen 11th day of analysis. Sample was scored close points in 1st to 16th day. However, on 21th analyses a bit decrease was observed like other sensory

characteristic. Statistically differences were determined in overall acceptability during storage (P<0.05).

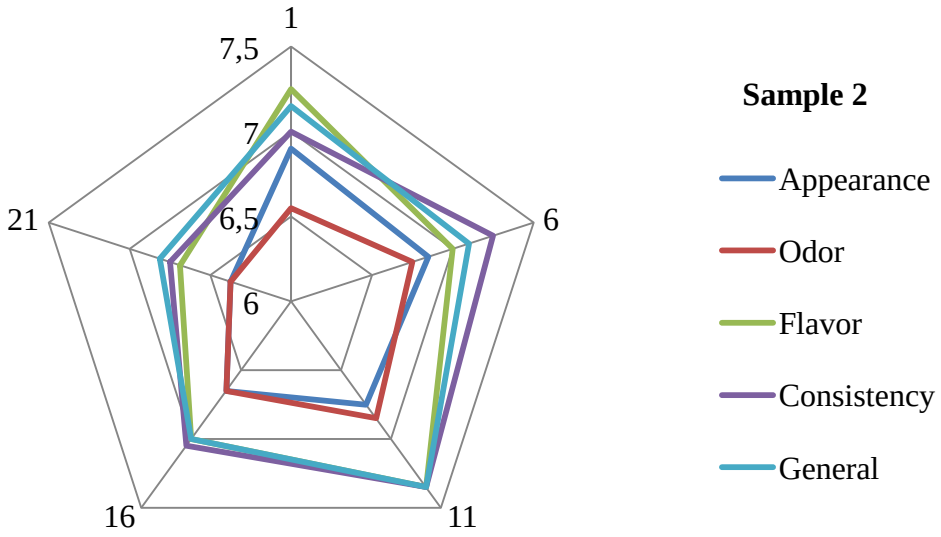


Figure 4.39. Sensory profile analyses during storage for sample 2

Sensory profile of sample 2 during storage was given in Figure 4.39. Appearance results were determined between; 6.38 to 6.90. Panelists scored highest point with 6.90 on 1st day. Besides, 21th day was the lowest pointed with 6.38. No significant differences were investigated in appearance for sample 2 during storage (P>0.05).

Odor profile of sample 2 were determined between; 6.38 to 6.85. Highest scored was given on 11th day of odor analyses. However panelist gave lowest score on 21th day. No significant differences were determined in odor score during storage (P>0.05). Souring and lost on aroma effect in sample 2 during storage caused a decrease in odor on 21th day scores.

Flavor characteristics of sample 2 were determined between; 6.69 to 7.35. Statistically no differences were observed in flavor scores (P>0.05). Scores were very close each other during storage.

Consistency were scored between; 6.75 to 7.35 during storage. There was no differences determined in consistency during storage (P>0.05). Consistency scores increased till to 11th day with a highest point 7.35. Then, scores decreased to 21th day.

Overall acceptability scores in sample 2 were determined between; 6.81 to 7.35. Best liked sample was chosen 11th day of analysis. Samples were scored close points in 1st to 11th day and highest pointed sample was determined on this day. Then decrease was seen in overall acceptability till to 21th day of analyses. No significant differences were observed (P>0.05).

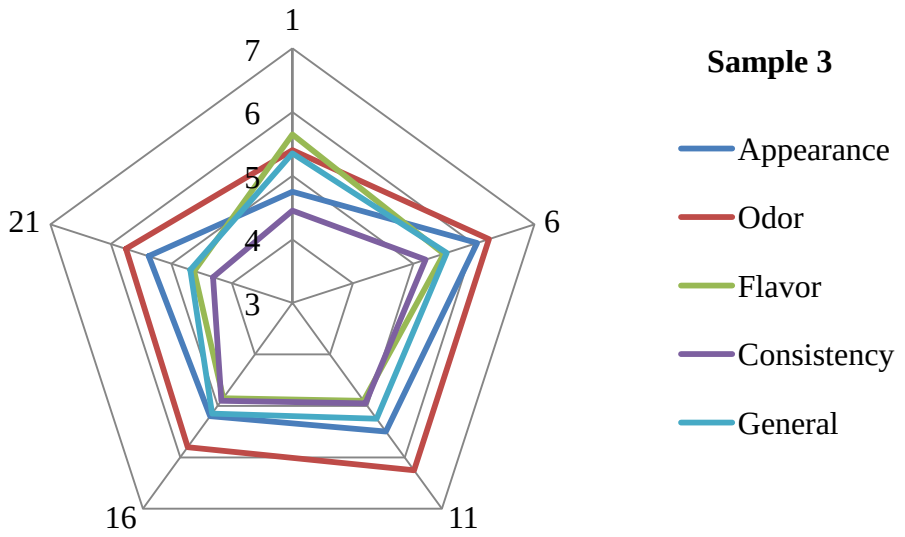


Figure 4.40. Sensory profile analyses during storage for sample 3

Sensory characteristics of sample 3 during storage were given in Figure 4.40. Appearance scores were determined between; 4.75 to 6.05. Panelists scored highest point with 6.05 on 6th day. Besides, 1st day was the lowest scored with 4.75. Significant differences were determined in appearance for sample 3 during storage (P<0.05).

Odor characteristics of sample 3 were scored between; 5.40 to 6.25. Highest scored was given on 6th and 11th day. Besides, panelist gave lowest score on 1st day. Significant differences were determined in odor score during storage (P>0.05). Souring, smell of oat milk and lost on aroma effect in sample 3 affected odor characteristics.

Flavor scores of sample 3 were obtained between; 4.63 to 5.65. Statistically significant differences were observed in flavor scores (P>0.05). Panelists scored highest point to 1st day sample with 5.65. Then during storage flavor scores decreased till to 21th day of analyses. Lose on aroma and souring affected flavor negatively during storage.

Consistency were scored between; 4.31 to 5.20 during storage. Differences were determined in consistency during storage ($P<0.05$). Rise and fall were seen in consistency scores during storage.

Overall acceptability scores were determined between; 4.69 to 5.55 in sample 3. Best liked sample was chosen 6th day of analysis. Samples were scored close points in 1st to 16th day. Then great decrease was seen in overall acceptability till on 21th day of analyses. No significant differences were observed during storage ($P>0.05$).

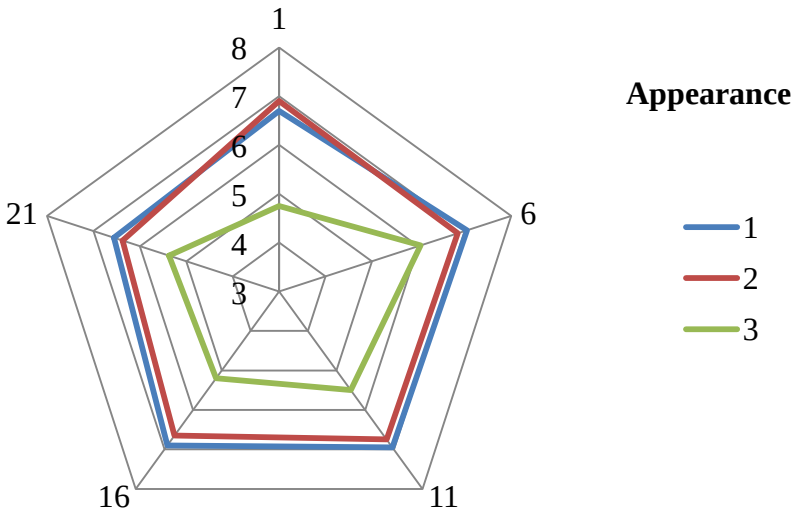


Figure 4.41. Appearance profile analyses during storage for all samples

In Figure 4.41. comparison of appearance during storage for all samples was given. Average scores in appearance for sample 1; 6.83, for sample 2; 6.71 and for sample 3; 5.37 were observed. No significant differences were determined among the all samples ($P>0.05$).However significant differences were observed during storage ($p<0.05$). According to figure, sample 1 and sample 2 had nearly same points during storage. However sample 3 had lower point. Oat milk concentration caused phase separation in sample 3 because of that, panelists gave low point. Yeast produced CO_2 in kefir. CO_2 induced foam in some sample, especially on sample 3 and that affected the appearance negatively.

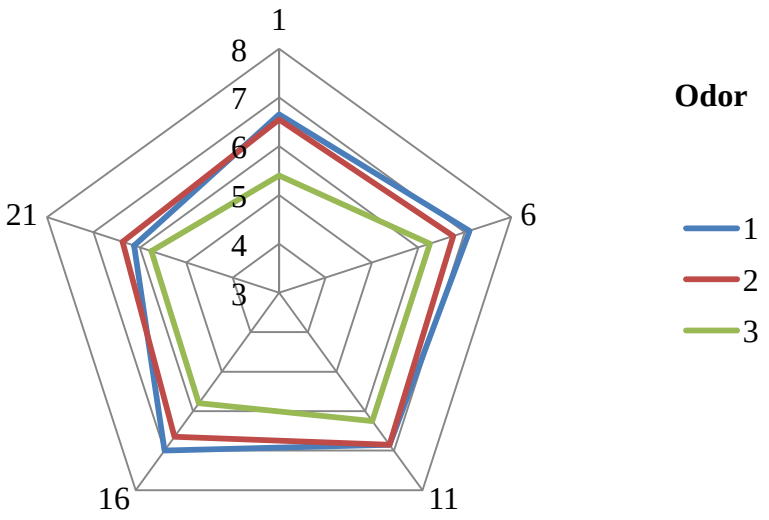


Figure 4.42. Odor profile analyses during storage for all samples

Comparison of odor during storage for all samples was given in Figure 4.42. Ssignificant differences were obtained among the all samples during storage ($P<0.05$). Average scores in odor were determined for sample 1; 6.74, for sample 2; 6.63 and for sample 3; 5.89. Sample 1 and sample 2 had scored close each other during storage. However sample 3 scored lower. Oat milk induced cereal smell in sample 3 and this caused to disliking in odor. However in sample 1 and sample 2 cereal smell of oat milk not realized by panelists. After 16th day of storage, souring and losing of aroma effect bonded to disliking.

Flavor comparison during storage for all samples was given in Figure 4.43. Significant differences were obtained among the all samples during storage ($P<0.05$). Average scores of flavor were determined for sample 1; 6.91, for sample 2; 7.06 and for sample 3; 5.11. Sample 1 and sample 2 had scored close each till to 16th day. However sample 1 decreased greatly on 21th day of storage. Sample 3 scored less during storage. Oat milk concentration was the most affective factor in flavor. Increase of oat milk concentration caused to disliking. These results showed similarity with optimization results. Souring and aroma also affected the flavor of samples.

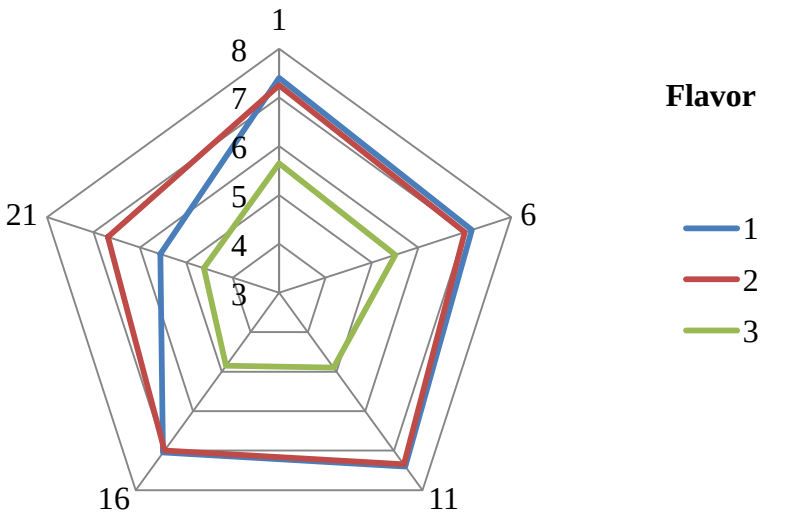


Figure 4.43. Flavor profile analyses during storage for all samples

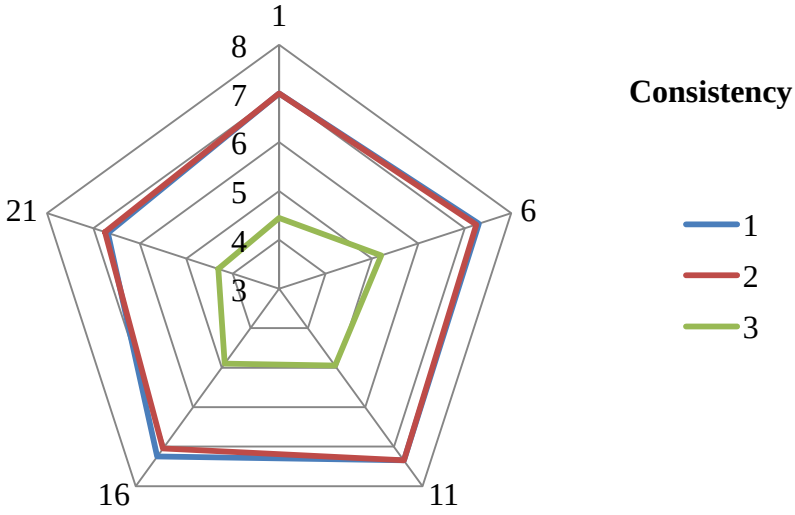


Figure 4.44. Consistency profile analyses during storage for all samples

Consistency comparison during storage for all samples was given in Figure 4.44. Significant differences were obtained among the all samples during storage ($P<0.05$). Average scores of flavor were determined for sample 1; 7.12, for sample 2; 7.08 and for sample 3; 4.76. Sample 1 and sample 2 had scored close each during storage. However sample 3 got low point during storage. Oat milk and aroma concentration affected

consistency greatly. Especially oat milk concentration affected negatively. Viscosity results bear a resemblance to sensory results. More viscous samples acceptability was higher than less viscous samples. High consistency index increased the acceptability of samples in lactic acid beverages (Penna et. al. 2001).

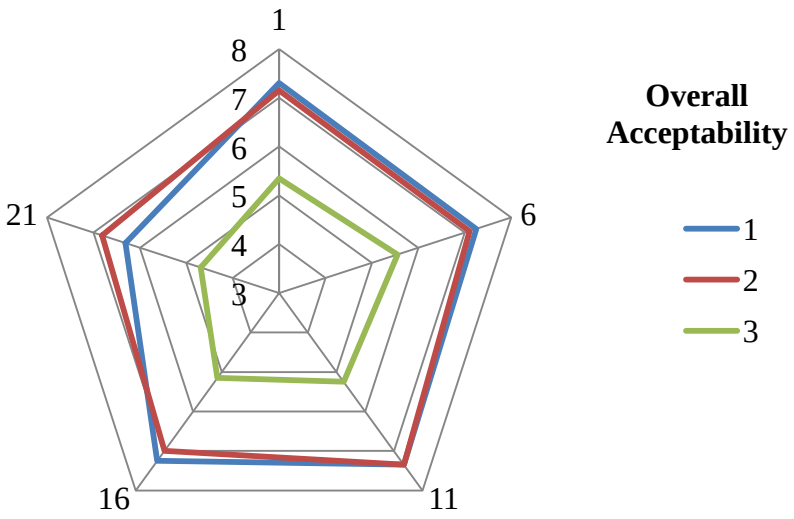


Figure 4.45. Overall acceptability profile analyses during storage for all samples

Comparison of overall acceptability during storage for all samples was given in Figure 4.45. Significant differences were obtained among the all samples during storage ($P<0.05$). Average scores in overall acceptability were determined for sample 1; 7.09, for sample 2; 7.08 and for sample 3; 5.20. Overall acceptability scores showed similarity with flavor scores. Sample 1 and sample 2 had scored close each other during storage. Only sample 1 more decreased on 21th day of storage. Sample 3 scored lower during storage. These results showed that flavor was the most important parameter in overall acceptability. Consistency also affected the panelists.

Also all average sensory points calculated in total and it was found as 34.70 for sample 1, 34.56 for sample 2 and 25.88 for sample 3. Sensory characteristics of sample 1 and sample 2 were close each other. However, sample 3 was worst.

Kilic, et al. (1999) reported that the scores of all the sensory attributes decreased significantly with time in kefir.

Also it was studied that sensory characteristics of kefir analyzed and best scores were detected in the first day of storage (Irigoyen, et al. 2005).

Raw material and starter culture of kefir were greatly affecting the sensory characteristics of kefir. It was reported that sensory characteristic of kefir was mainly influenced by type of milk used and storage period. Starter culture type affected the viscosity and flavor cream. Bovine milk kefir was more accepted than ovine and caprine milk kefir (Wszolek, et al. 2001).

CHAPTER 5

CONCLUSION

Development of a new cereal-based drink and determination of physical, chemical, microbiological, organoleptic, and aroma characteristics of bovine-oat milk mixture based kefir were the objectives of this study.

Results obtained during optimization in this study were summarized below:

Oat milk concentration strongly affected the samples' sensory, pH and microbiological characteristics.

1) Oat milk concentration affected sensory characteristics of kefir samples distastefully when concentration was higher than %30.

2) Oat milk concentration affected pH strongly and caused a pH decrease in kefir samples nearly to pH 4.

3) Thirty percent oat milk concentration had a positive effect on microbiological count.

Culture concentration also affected the kefir samples' properties. In sensory analysis panelists detected the culture difference, and 4% culture concentration was preferred by the panelists, but culture concentration did not influence the pH and microbiological counts strongly.

Aroma concentration strongly affected the kefir samples' pH and caused a decrease in pH due to the acidic characteristics of blueberry. It was also affected the odor characteristics of samples affirmatively.

Storage affected pH, microbiological counts and sensory profile negatively. The pH, sensory scores and microbial counts decreased during storage.

Three kefir samples were produced based on the results obtained from optimization. Determination of physical, chemical, microbiological, organoleptic, and aroma characteristics of theses samples were other objectives of this study.

Average physicochemical characteristic for sample 1 were found as 4.22±0.03 pH, 0.80% ±0.05 titratable acidity, 13.64% ±1.07 dry matter content, 2.24% ±0.13 total protein content, 1.85%±0.07 fat content, 11.20%±5.03 whey off, 199.10±7.73 μg/ml gallic acid total phenol content, 0.049±0.02 g total beta-glucan content, 18.86 ±1.78

mPa.s apparent viscosity on 300 s^{-1} and average color changes in L^* value 75.27±0.65, in a^* value 1.42±0.34, in b^* value 3.81±0.46 were detected.

Average lactococci, lactobacili bacteria and yeast counts were determined as 8.37±0.43, 6.18±1.23 and 4.07±0.59 log cfu/g, respectively.

Average sensory analyses determined as 6.83±0.20 appearance, 6.75±0.39 odor, 6.91±0.77 flavor, 7.12±0.27 consistency, and 7.09±0.44 overall acceptability. During storage, rise and fall was observed in sensory characteristics. However, a decrease was seen after the 16th day of storage in sample 1.

Average physicochemical characteristic for sample 2 were found as 4.23±0.04 pH, 0.73% ±0.08 titratable acidity, 14.58% ±0.47 dry matter content, 2.37% ±0.10 total protein content, 1.95%±0.07 fat content, 5.70%±3.44 whey off, 227.09±29.47 µg/ml gallic acid total phenol content, 0.029±0.02 g total beta-glucan content, 20.31±1.02 mPa.s apparent viscosity on 300 s^{-1} and average color changes in L^* value 75.89±0.37, in a^* value 1.32±0.31, in b^* value 3.71±0.41 were detected.

Average lactococci, lactobacili bacteria and yeast counts were determined as 8.34±0.44, 6.32±1.10 and 3.93±0.49 log cfu/g, respectively.

Average sensory analyses investigated as 6.38±0.21 appearance, 6.64±0.18 odor, 7.06±0.26 flavor, 7.08±0.23 consistency and 7.08±0.20 overall acceptability. During storage rise and fall was observed in sensory characteristics except appearance. However, a decrease was seen after the 16th day of storage in sample 2. Appearance scores decreased during storage regularly.

Average physicochemical characteristic for sample 3 were found as 4.08±0.03 pH, 0.89% ±0.15 titratable acidity, 14.77% ±0.59 dry matter content, 1.97% ±0.05 total protein content, 1.35%±0.07 fat content, 19.80%±7.60 whey off, 229.37±21.53 µg/ml gallic acid total phenol content, 0.057±0.02 g total beta-glucan content, 18.86 ±1.78 mPa.s apparent viscosity on 300 s^{-1} and average color changes in L^* value 70.35±0.63, in a^* value 2.37±0.41, in b^* value 4.25±1.28 were observed.

Average lactococci, lactobacili bacteria and yeast counts were determined as 8.47±0.54, 6.53±0.79 and 4.49±0.62 log cfu/g, respectively.

Average sensory analyses detected as 5.37±0.47 appearance, 5.89±0.36 odor, 5.11±0.44 flavor, 4.76±0.37 consistency and 5.20±0.32 overall acceptability. During storage a rise was observed on the 6th day and then regularly decrease was seen in sensory characteristics for sample 3.

Volatile compounds of kefir samples were analyzed with %area. Ethanol, ethyl acetate, diacetly, toluene, acetoin, D-limonene, 2-heptanone, 1-hexanol, eucalyptol, dimethylamine, benzaldehyde, 2-nonanone, 2-octanol, limonene oxide, propanedioic acid, octanoic acid and phenol were determined during storage. All volatile compounds data were analyzed by %area.

Ethanol was decreased then increased during storage. Fall and rise was observed in ethyl acetate. Diacetly was decreased during storage. Level of acetoin regularly decreased during storage in all samples. No acetaldehyde was determined in kefir samples.

2-octanol determined after the 11^{th} day of storage and level of 2-octanol increased during storage.

Toluene, Eucalyptol was clearly determined in samples which were come from aroma of blueberry.

Acetic acid, lactic acid, orotic acid and uric acid were detected in organic acid profile analyses. The highest lactic acid amount determined in sample 1 and the lowest one was found in sample 3. During storage lactic acid amount increased in all samples. Acetic acid was investigated in all samples. Highest one observed in sample 2. However, lowest level of acetic acid was determined in sample 3. In all samples level of acetic acid was increased during storage. Similar results were observed in uric acid with lactic acid. Sample 2 had the highest uric acid level. On the other hand, sample 3 had the lowest level. During storage, uric acid level increased in sample 2 and sample 3. But a decrease was observed in sample 1. In orotic acid results, it was analyzed that sample 3 had the highest orotic acid amount; sample 1 had the lowest orotic acid amount. During storage a decrease was observed in orotic acid levels.

A new cereal based fermented milk product was produced according to optimization results and chemical, physical, microbiological and organoleptic characteristics were determined in the developed product during storage. According to sensory results 15%-20% oat milk concentration is suitable for consumer preference. Oat milk caused an increase on total beta-glucan content, whey off and microbiological flora. However, oat milk caused a decrease on pH, total protein content, total fat content and viscosity.

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APPENDIX A

CHEMICAL AND MEDIA

Table A.1. Chemical Used

No	Chemical	Code
1	M17 agar	Merck 1.15108.0500
2	MRS agar	Fluka 69964
3	Yeast extract glucose chloromphenical agar	Difco 219001
4	Peptone water	Merck 1.07228.0500
5	NaOH	Riedel-de Haen 06203
6	Phenol ftalein	Merck 1.07233.0100
7	Kjeltabs-catalysts	Delta
8	Silicon antifoaming agent	Merck 1.07743.0100
9	Sulfuric acid	Merck 1.00729.2500
10	Filter paper(Whatman No: 42)	ISOLab
11	Boric acid	Sigma B6768
12	HCl	Reidel-de Haen 07102
13	n-Amyl Alcohol	Merck 8.07500.1000
14	Acetic acid	Merck 100063
15	Lactic acid	Sigma L1750
16	Orotic acid	Sigma O2750
17	Uric acid	Sigma U2625
18	Gallic acid	Sigma SIG7384
19	Ethanol	Merck 100986
20	Diacetyl	Merck 8035280100
21	D-limonen	Merck 814546
22	Octonaic acid	Merck 8.00192.0100
23	Sodium phosphate monobasic dihydrate	Sigma 71505
24	sodium hydroxide	Panreac 141687

(cont. on next page)

Table A.1. (cont.)

No	Chemical	Code
25	Acetaldehyde	Merck 8450010100
26	2-Nonanone	Merck 8187900025
27	Butyric acid	Merck 800457.0100
28	Heptanoic acid	Merck 8075820100
29	Ethyl butyrate	Merck 8005000100
30	Acetoin	Merck 8206640100
31	Kefir DC 1	Danisco
32	Hexanal	Merck 8026720005
33	Folin-ciocalteu’s phenol reagent	Fluka 47641

APPENDIX B

REAGENT AND SOLUTION

B.1. Phenolphthalein (0.01%)

0.5 g phenolphthalein was completed to 50 ml with 95% ethanol and mixed thoroughly.

B.2. Peptone Water

1 g peptone was dissolved in 1 L of deionized water and autoclaved 121°C for 15 min.

B.3. MRS Agar

68.2 g MRS agar dissolved in 1 L of deionized water and autoclaved 121°C for 15 min.

B.4. M17 Agar

55 g M17 dissolved in 1 L of deionized water and autoclaved 121°C for 15 min.

B.5. Yeast Glucose Chloromophenical Agar

38.1 g yeast glucose chloromophenical agar dissolved in 1 L of deionized water and autoclaved 121°C for 15 min.

B.6. Sodium Phosphate Buffer (20mM, pH 6.5)

3.12 g of sodium phospohate monobasic dihydrate ($NAH_2PO_42H_2O$) was dissolved in 900 ml of distilled water and pH was adjusted 6.50 with 100mM sodium hydroxide (4g/L). Volume adjusted 1 L and 0.2 g sodium azide was added.

B.7. Sodium Acetate Buffer (50mM, pH4.0)

2.9 ml glacial acetic acid was added to 900 ml distilled water. pH was adjusted to 4.0 with 1M sodium hydroxide solution. Final volume adjusted to 1L and 0.2 g sodium azide was added.

B.8. Sodium Acetate Buffer (200mM, pH4.0)

11.6 ml glacial acetic acid was added to 900 ml distilled water. pH was adjusted to 4.0 with 1M sodium hydroxide solution. Final volume adjusted to 1L and 0.2 g sodium azide was added.

B.9. 50% Ethanol

50 ml of ethanol and 50 ml ultra pure water were mixed.

APPENDIX C

RESULTS TABLE

Table C.1. pH Changes During Storage

Storage	Sample 1	Sample 2	Sample 3
1	4.25±0.03 ^{Aab}	4.28±0.02 ^{Aa}	4.09±0.02 ^{Aa}
6	4.20±0.01 ^{Aab}	4.25±0.04 ^{Aa}	4.12±0.05 ^{Aa}
11	4.22±0.03 ^{Aab}	4.25±0.01 ^{Aa}	4.08±0.03 ^{Aa}
16	4.18±0.00 ^{Aa}	4.18±0.01 ^{Ab}	4.03±0.01 ^{Aa}
21	4.25±0.01 ^{Ab}	4.20±0.01 ^{Ab}	4.07±0.01 ^{Aa}
Minimum	4.18	4.18	4.03
Maximum	4.25	4.28	4.12
Average	4.22	4.23	4.08

* a–b Means in the same column with different superscript letters differ significantly (P<0.05)

* Means in the same row with different superscript letters differ significantly (P<0.05)

Table C.2. Titratable Acidity Change During Storage

Storage	Sample 1	Sample 2	Sample 3
1	0.75±0.01 ^{Aa}	0.62±0.02 ^{Aa}	0.82±0.00 ^{Aa}
6	0.79±0.02 ^{Aa}	0.74±0.01 ^{Abd}	0.76±0.02 ^{Ab}
11	0.81±0.01 ^{ABa}	0.76±0.02 ^{ABb}	1.04±0.03 ^{ABc}
16	0.88±0.00 ^{Bb}	0.85±0.01 ^{Bc}	1.07±0.01 ^{Bc}
21	0.76±0.01 ^{Aa}	0.70±0.00 ^{Ad}	0.77±0.01 ^{Ab}
Minimum	0.75	0.62	0.76
Maximum	0.88	0.85	1.07
Average	0.80	0.73	0.89

* a–d Means in the same column with different superscript letters differ significantly (P<0.05)

* A-B Means in the same row with different superscript letters differ significantly (P<0.05)

Table C.3. Total Dry Matter Change During Storage

Sample 1		Sample 2		Sample 3	
Storage	% DM	Storage	%DM	Storage	%DM
1	14.73±0.56^{Aa}	1	14.22±0.09^{Aa}	1	14.36±0.30^{Aa}
6	12.56±0.14^{Ab}	6	14.26±0.90^{Aa}	6	14.12±1.76^{Aa}
11	14.75±0.82^{Aa}	11	14.23±0.97^{Aa}	11	14.71±0.47^{Aa}
16	12.64±0.31^{Ab}	16	15.00±0.47^{Aa}	16	15.08±1.45^{Aa}
21	13.51±0.50^{Aab}	21	15.19±1.00^{Aa}	21	15.59±2.57^{Aa}
Minimum	12.56	Minimum	14.22	Minimum	14.12
Maximum	14.75	Maximum	15.19	Maximum	15.59
Average	13.64	Average	14.58	Average	14.77

* a–b Means in the same column with different superscript letters differ significantly (P<0.05)

* Means in the same row with different superscript letters differ significantly (P<0.05)

Table C.4. Total Protein Change During Storage

Storage	Sample 1	Sample 2	Sample 3
1	2.44±0.05^{Aa}	2.42±0.04^{Aa}	1.91±0.06^{Aa}
6	2.10±0.04^{Ab}	2.30±0.02^{Aa}	1.95±0.00^{Aa}
11	2.18±0.01^{Ab}	2.34±0.03^{Aa}	1.95±0.01^{Aa}
16	2.17±0.04^{Ab}	2.28±0.13^{Aa}	2.00±0.09^{Aa}
21	2.31±0.09^{Aab}	2.52±0.11^{Aa}	2.04±0.12^{Aa}
Minimum	2.10	2.28	1.91
Maximum	2.44	2.52	2.04
Average	2.24	2.37	1.97

* a–b Means in the same column with different superscript letters differ significantly (P<0.05)

* Means in the same row with different superscript letters differ significantly (P<0.05)

Table C.5. Whey off Change During Storage

Storage	Sample 1	Sample 2	Sample 3
1	4±0.00 ^{Aa}	2±0.00 ^{Aa}	9±0.00 ^{Aa}
6	10±0.00 ^{ABb}	3±0.00 ^{ABa}	15±0.00 ^{ABb}
11	10.5±0.71 ^{ABb}	5.5±0.71 ^{ABb}	22.5±0.71 ^{ABc}
16	14±0.00 ^{ABc}	7.5±0.71 ^{ABb}	25.5±0.71 ^{ABd}
21	17.5±0.71 ^{Bd}	10.5±0.71 ^{Bc}	27±0.00 ^{Bd}
Minimum	4	2	9
Maximum	17.5	10.5	27
Average	11.2	5.7	19.8

* a–d Means in the same column with different superscript letters differ significantly (P<0.05)

* A-B Means in the same row with different superscript letters differ significantly (P<0.05)

Table C.6. Viscosity Change During Storage (mPA.s)

Storage	Sample 1	Sample 2	Sample 3
1	18.90±1.41 ^{Aab}	21.05±0.40 ^{Aa}	11.23±0.57 ^{Aa}
6	21.80±0.73 ^{Aa}	21.20±0.39 ^{Aa}	14.63±5.31 ^{Aa}
11	18.64±0.61 ^{Aab}	20.14±0.05 ^{Aa}	11.32±0.31 ^{Aa}
16	17.61±0.97 ^{Ab}	18.98±2.61 ^{Aa}	9.98±0.57 ^{Aa}
21	17.32±0.21 ^{Ab}	20.17±0.93 ^{Aa}	9.93±0.93 ^{Aa}
Minimum	17.32	18.98	9.93
Maximum	21.80	21.20	14.63
Average	18.85	20.31	11.42

* a–b Means in the same column with different superscript letters differ significantly (P<0.05)

* Means in the same row with different superscript letters differ significantly (P<0.05)

Table C.7. Color Results

	Storage	L	a	b
Sample 1	1	74.23±0.25 ^{Aa}	1.89±0.14 ^{Aa}	2.98±0.19 ^{Aa}
	6	75.00±0.28 ^{Ab}	1.74±0.16 ^{Aa}	3.97±0.43 ^{Bb}
	11	75.35±0.27 ^{Ab}	1.32±0.09 ^{Bb}	3.81±0.14 ^{Bb}
	16	76.17±0.38 ^{Ac}	1.07±0.11 ^{Bc}	3.90±0.09 ^{Bb}
	21	75.62±0.58 ^{Bd}	1.05±0.24 ^{Bc}	4.37±0.09 ^{Cc}
	Minimum	74.23	1.05	2.98
	Maximum	76.17	1.89	4.37
	Average	75.27	1.42	3.81
Sample 2	1	75.31±0.73 ^{Aa}	1.75±0.10 ^{Aa}	3.07±0.11 ^{Aa}
	6	75.61±0.36 ^{Aac}	1.65±0.09 ^{Aa}	3.42±0.18 ^{Bb}
	11	76.29±0.33 ^{Abc}	1.12±0.09 ^{Bb}	3.85±0.14 ^{Bc}
	16	76.21±0.32 ^{Abc}	1.04±0.13 ^{Bb}	4.02±0.28 ^{Bcd}
	21	76.01±0.34 ^{Bc}	1.04±0.14 ^{Bb}	4.18±0.38 ^{Cd}
	Minimum	75.31	1.04	3.07
	Maximum	76.29	1.75	4.18
	Average	75.89	1.32	3.71
Sample 3	1	69.45±0.30 ^{Aa}	3.00±0.14 ^{Aa}	2.47±0.20 ^{Aa}
	6	69.91±0.93 ^{Aac}	2.68±0.09 ^{Ab}	3.99±0.87 ^{Bb}
	11	71.29±0.45 ^{Ab}	2.02±0.22 ^{Bcd}	4.20±0.27 ^{Bb}
	16	70.52±0.32 ^{Acd}	2.26±0.10 ^{Bc}	4.15±0.12 ^{Bb}
	21	70.57±0.18 ^{Bd}	1.89±0.12 ^{Bd}	6.47±0.17 ^{Bc}
	Minimum	69.45	1.89	2.47
	Maximum	71.29	3.00	6.47
	Average	70.35	2.37	4.25

* a–d Means in the same column with different superscript letters differ significantly (P<0.05)

* A-C Means in the same row with different superscript letters differ significantly (P<0.05)

Table C.8. Organic Acid Profile Results

	Storage	Orotic acid	Lactic acid	Uric acid	Acetic acid
Sample 1	1	126.14±0.11^{ABab}	9804.30±7.28^{ABa}	835.19±5.57^{Aa}	459.78±3.31^{Aa}
	6	105.10±0.53^{Aa}	8147.15±97.29^{Aa}	720.94±8.06^{Aa}	407.76±3.68^{Aa}
	11	115.13±0.20^{ABab}	9081.92±29.75^{ABa}	660.47±11.44^{Aa}	390.02±9.86^{ABa}
	16	115.58±1.95^{Bab}	9184.25±170.22^{ABa}	788.56±14.94^{Aa}	471.79±10.45^{ABa}
	21	129.88±1.85^{Bb}	10175.67±135.53^{Ba}	788.97±28.75^{Aa}	547.40±18.02^{Ba}
	Minimum	105.13	8147.47	660.47	390.02
	Maximum	129.88	10175.67	835.19	547.40
	Average	118.37	9404.66	758.83	455.35
Sample 2	1	116.70±2.72^{ABa}	9168,85±18.79^{ABa}	777.43±16.12^{Aa}	444.19±10.50^{Aa}
	6	94.81±7.42^{Aa}	7826,25±615.63^{Aa}	699.80±57.10^{Aa}	393.86±29.30^{Aa}
	11	132.90±1.67^{ABa}	9837,40±152.53^{ABa}	851.02±14.37^{Aa}	505.61±6.17^{ABa}
	16	136.00±0.05^{Ba}	10075,06±14.49^{ABa}	868.95±3.60^{Aa}	552.94±4.22^{ABa}
	21	140.53±1.01^{Ba}	10203,69±30.42^{Ba}	895.15±7.26^{Aa}	564.22±3.10^{Ba}
	Minimum	94.81	7826.25	699.80	393.86
	Maximum	140.53	10203.69	895.15	564.22
	Average	124.19	9422.25	818.47	492.16
Sample 3	1	129.96±0.73^{ABa}	8283.95±144.45^{ABa}	668.59±14.27^{Aa}	357.11±36.83^{Aa}
	6	122.86±0.96^{Aa}	8049.92±46.42^{Aa}	692.90±4.81^{Aa}	484.28±2.13^{Aa}
	11	124.50±2.24^{ABa}	8817.99±50.02^{ABa}	716.76±2.13^{Aa}	539.73±1.71^{ABa}
	16	139.33±2.49^{Ba}	8748.00±100.19^{ABa}	740.27±14.75^{Aa}	499.73±6.14^{ABa}
	21	123.75±0.64^{Ba}	8724.77±67.03^{Ba}	752.26±0.44^{Aa}	559.41±6.24^{Ba}
	Minimum	122.86	8049.92	668.59	357.11
	Maximum	139.33	8817.99	752.26	559.41
	Average	128.08	8524.93	714.16	488.05

* a–b Means in the same column with different superscript letters differ significantly (P<0.05)

* A-B Means in the same row with different superscript letters differ significantly (P<0.05)

Table C.9. Total Phenol Content Results

Storage	Sample 1	Sample 2	Sample 3
1	197.36±12.31 ^{Aa}	246.91±21.78 ^{Aa}	234.19±26.52 ^{Aa}
6	201.38±12.31 ^{Aa}	198.70±21.78 ^{Aa}	218.79±4.73 ^{Aa}
11	198.03±7.58 ^{Aa}	255.62±28.41 ^{Aa}	205.40±57.77 ^{Aa}
16	188.65±13.26 ^{Aa}	192.00±61.56 ^{Aa}	225.49±2.84 ^{Aa}
21	210.08±11.36 ^{Aa}	242.22±18.94 ^{Aa}	262.98±23.67 ^{Aa}
Minimum	188.65	192.00	205.40
Maximum	210.08	255.62	262.98
Average	199.10	227.10	229.37

* Means in the same column with different superscript letters differ significantly (P<0.05)

* Means in the same row with different superscript letters differ significantly (P<0.05)

Table C.10. Total Beta Glucan Content Results

Storage	Sample 1	Sample 2	Sample 3
1	0.027±0.014 ^{ABa}	0.015±0.004 ^{ABa}	0.031±0.006 ^{ABa}
6	0.031±0.024 ^{Aa}	0.015±0.001 ^{Aa}	0.037±0.006 ^{Aa}
11	0.049±0.014 ^{Aab}	0.011±0.003 ^{Aa}	0.052±0.022 ^{Aa}
16	0.054±0.012 ^{Aab}	0.035±0.014 ^{Aab}	0.067±0.016 ^{Aa}
21	0.082±0.005 ^{Bb}	0.066±0.013 ^{Bb}	0.096±0.016 ^{Ba}
Minimum	0.027	0.011	0.031
Maximum	0.082	0.066	0.096
Average	0.049	0.029	0.057

* a–b Means in the same column with different superscript letters differ significantly (P<0.05)

* A-B Means in the same row with different superscript letters differ significantly (P<0.05)

Table C.11. *Lactococcus spp.*Results

Storage	Sample 1	Sample 2	Sample 3
1	8.86±0.67 ^{Aa}	8.87±0.37 ^{Aa}	9.18±0.02 ^{Aa}
6	8.79±0.17 ^{Aa}	8.69±0.31 ^{Aa}	8.81±0.30 ^{Aab}
11	8.21±0.10 ^{Ba}	8.28±0.03 ^{Ba}	8.30±0.22 ^{Bbc}
16	7.95±0.05 ^{Ba}	7.86±0.18 ^{Bb}	7.78±0.06 ^{Bc}
21	8.03±0.07 ^{Ba}	7.98±0.02 ^{Bab}	8.27±0.04 ^{Bbc}
Minimum	7.95	7.86	7.78
Maximum	8.86	8.87	9.18
Average	8.37	8.34	8.47

* a–c Means in the same column with different superscript letters differ significantly (P<0.05)

* A-B Means in the same row with different superscript letters differ significantly (P<0.05)

Table C.12. *Lactobacillus ssp.*Results

Storage	Sample 1	Sample 2	Sample 3
1	7.05±0.11 ^{Aa}	7.18±0.05 ^{Aa}	7.17±0.03 ^{Aa}
6	6.97±0.17 ^{Aa}	7.12±0.01 ^{Aa}	7.12±0.03 ^{Aa}
11	6.91±0.03 ^{Aa}	6.93±0.05 ^{Ab}	6.99±0.03 ^{Aa}
16	5.80±0.05 ^{Bb}	5.71±0.02 ^{Bc}	5.90±0.01 ^{Bb}
21	4.17±0.16 ^{Cc}	4.68±0.03 ^{Cd}	5.46±0.22 ^{Cc}
Minimum	4.17	4.68	5.46
Maximum	7.05	7.18	7.17
Average	6.18	6.32	6.53

* a–d Means in the same column with different superscript letters differ significantly (P<0.05)

* A-C Means in the same row with different superscript letters differ significantly (P<0.05)

Table C.13. Yeast Enumeration Results

Storage	Sample 1	Sample 2	Sample 3
1	4.10±0.02 ^{Aa}	4.19±0.21 ^{Aab}	4.30±0.10 ^{Aab}
6	3.73±0.15 ^{Aa}	3.53±0.14 ^{Aa}	3.77±0.02 ^{Aa}
11	3.54±0.16 ^{Aa}	3.43±0.11 ^{Aa}	4.64±0.75 ^{Aa}
16	3.93±0.33 ^{Aa}	3.91±0.04 ^{Aab}	4.28±0.11 ^{Aab}
21	5.07±0.07 ^{Bb}	4.61±0.40 ^{Bb}	5.44±0.01 ^{Bb}
Minimum	3.54	3.43	3.77
Maximum	5.07	4.61	5.44
Average	4.07	3.93	4.49

* a–b Means in the same column with different superscript letters differ significantly (P<0.05)

* A-B Means in the same row with different superscript letters differ significantly (P<0.05)

Table C.14. Sensory Characteristics of Sample 1

Storage	Appearance	Odor	Flavor	Consistency	Overall A.
1	6.70 ^{Aa}	6.65 ^{Aab}	7.40 ^{Aa}	7.00 ^{Aa}	7.30 ^{Aab}
6	7.05 ^{Aa}	7.10 ^{Aa}	7.15 ^{Aa}	7.30 ^{Aa}	7.25 ^{Aab}
11	6.95 ^{Aa}	6.85 ^{Aa}	7.40 ^{Aa}	7.35 ^{Aa}	7.35 ^{Aa}
16	6.90 ^{Aa}	7.00 ^{Aa}	7.05 ^{Aab}	7.25 ^{Aa}	7.25 ^{Aab}
21	6.56 ^{Aa}	6.13 ^{Ab}	5.56 ^{Ab}	6.69 ^{Aa}	6.31 ^{Ab}
Minumum	6.56	6.13	5.56	6.69	6.31
Maximum	7.05	7.10	7.40	7.35	7.35
Average	6.83	6.75	6.91	7.12	7.09

* a–b Means in the same column with different superscript letters differ significantly (P<0.05)

* Means in the same row with different superscript letters differ significantly (P<0.05)

Table C.15. Sensory Characteristics of Sample 2

Storage	Appearance	Odor	Flavor	Consistency	Overall A.
1	6.90 ^{Aa}	6.55 ^{Aa}	7.25 ^{Aa}	7.00 ^{Aa}	7.15 ^{Aa}
6	6.85 ^{Aa}	6.75 ^{Aa}	7.00 ^{Aa}	7.25 ^{Aa}	7.10 ^{Aa}
11	6.75 ^{Aa}	6.85 ^{Aa}	7.35 ^{Aa}	7.35 ^{Aa}	7.35 ^{Aa}
16	6.65 ^{Aa}	6.65 ^{Aa}	7.00 ^{Aa}	7.05 ^{Aa}	7.00 ^{Aa}
21	6.37 ^{Aa}	6.37 ^{Aa}	6.69 ^{Aa}	6.75 ^{Aa}	6.81 ^{Aa}
Minumum	6.37	6.37	6.69	6.75	6.81
Maximum	6.90	6.85	7.35	7.35	7.35
Average	6.71	6.63	7.06	7.08	7.08

* Means in the same column with different superscript letters differ significantly (P<0.05)

* Means in the same row with different superscript letters differ significantly (P<0.05)

Table C.16. Sensory Characteristics of Sample 3

Storage	Appearance	Odor	Flavor	Consistency	Overall A.
1	4.75 ^{Aa}	5.40 ^{Aa}	5.65 ^{Aa}	4.45 ^{Aab}	5.35 ^{Aa}
6	6.05 ^{Ab}	6.25 ^{Aa}	5.50 ^{Aa}	5.20 ^{Aa}	5.55 ^{Aa}
11	5.50 ^{Aab}	6.25 ^{Aa}	4.90 ^{Ab}	4.95 ^{Aab}	5.25 ^{Aa}
16	5.20 ^{Aab}	5.80 ^{Aa}	4.85 ^{Ab}	4.90 ^{Aab}	5.15 ^{Aa}
21	5.37 ^{Aab}	5.75 ^{Aa}	4.63 ^{Ab}	4.31 ^{Ab}	4.69 ^{Aa}
Minumum	5.20	5.75	4.63	4.31	4.69
Maximum	6.05	6.25	5.65	5.20	5.55
Average	5.37	5.89	5.11	4.31	5.20

* a–b Means in the same column with different superscript letters differ significantly (P<0.05)

* Means in the same row with different superscript letters differ significantly (P<0.05)

APPENDIX D

STANDARD CALIBRATION CURVE FOR TOTAL PHENOL CONTENT

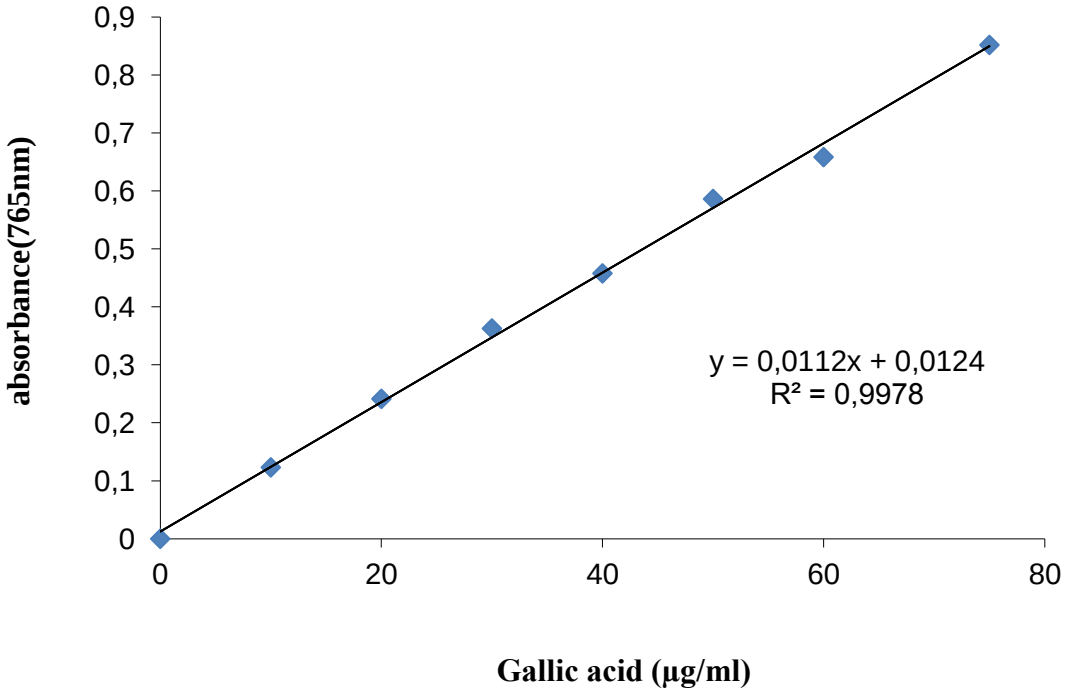

Figure D.1. Standard calibration curve for total phenol content analysis

APPENDIX E

CHROMATOGRAMS OF ORGANIC ACID AND VOLATILE COMPOUNDS IN AROMA AND OAT MILK

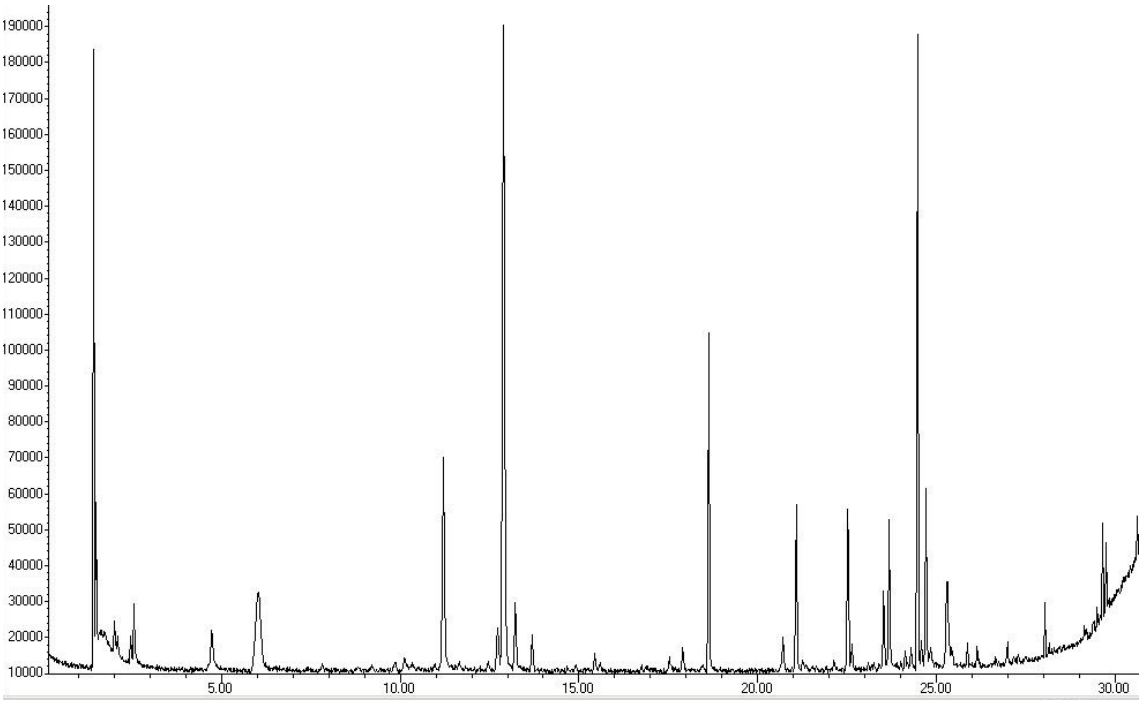

Figure E.1. A representative of blueberry aroma in GC-MS

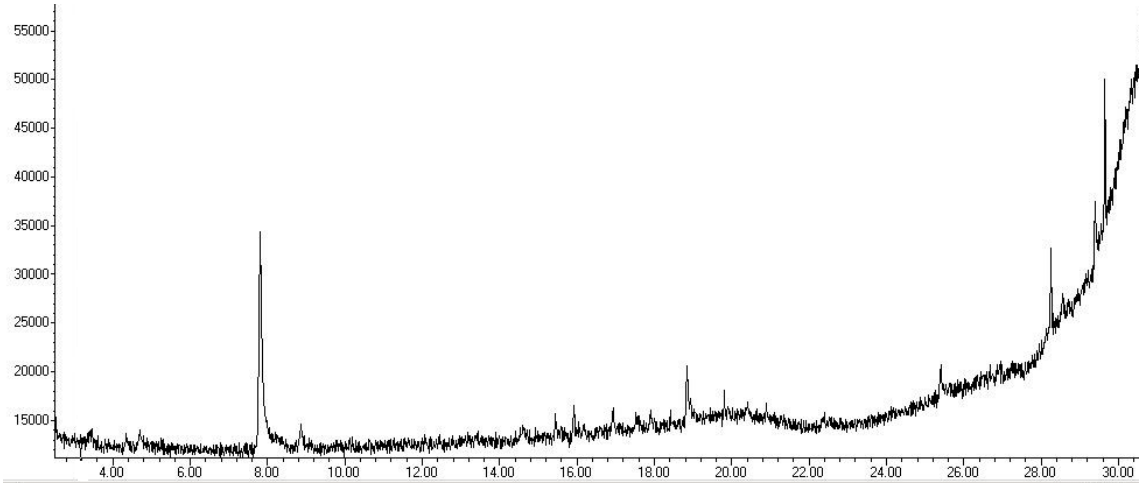

Figure E.2. A representative of oat milk in GC-MS

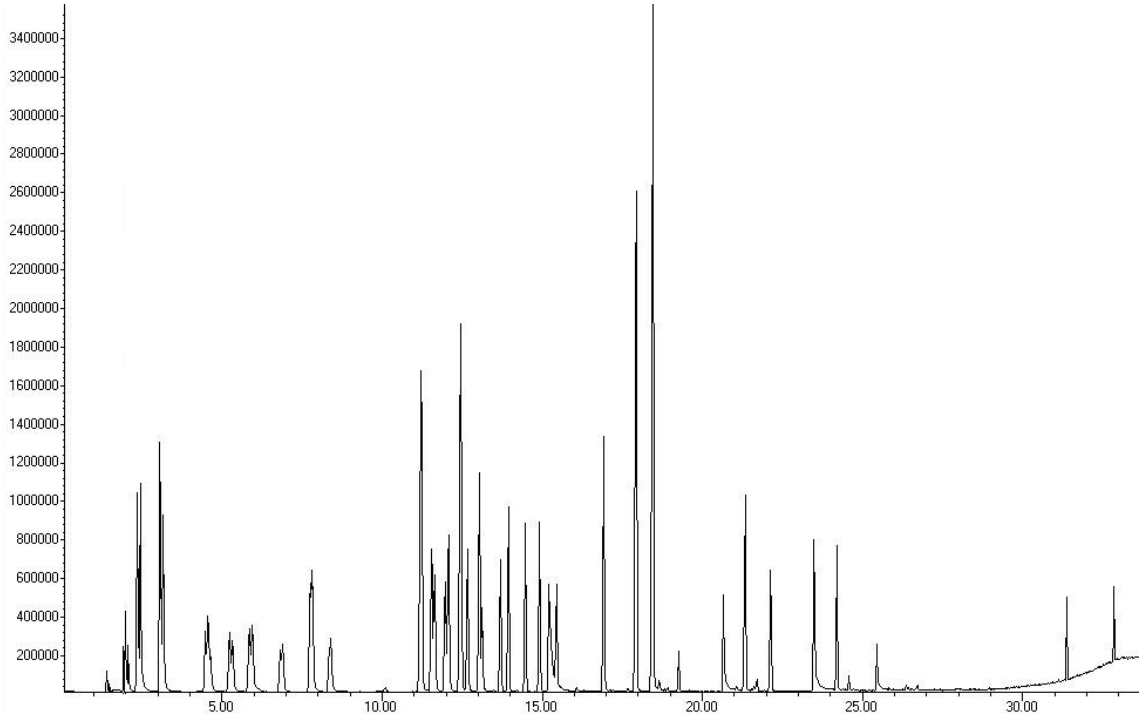

Figure E.3. A representative of standards in GC-MS

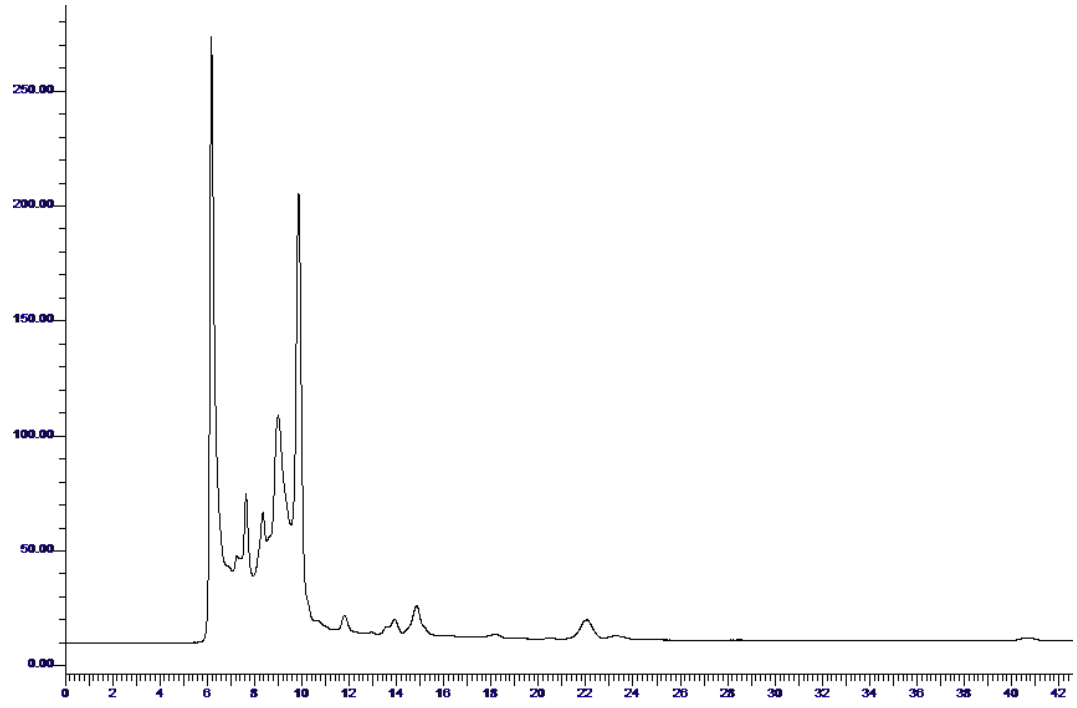

Figure E.4. A representative organic acid profile for blueberry aroma

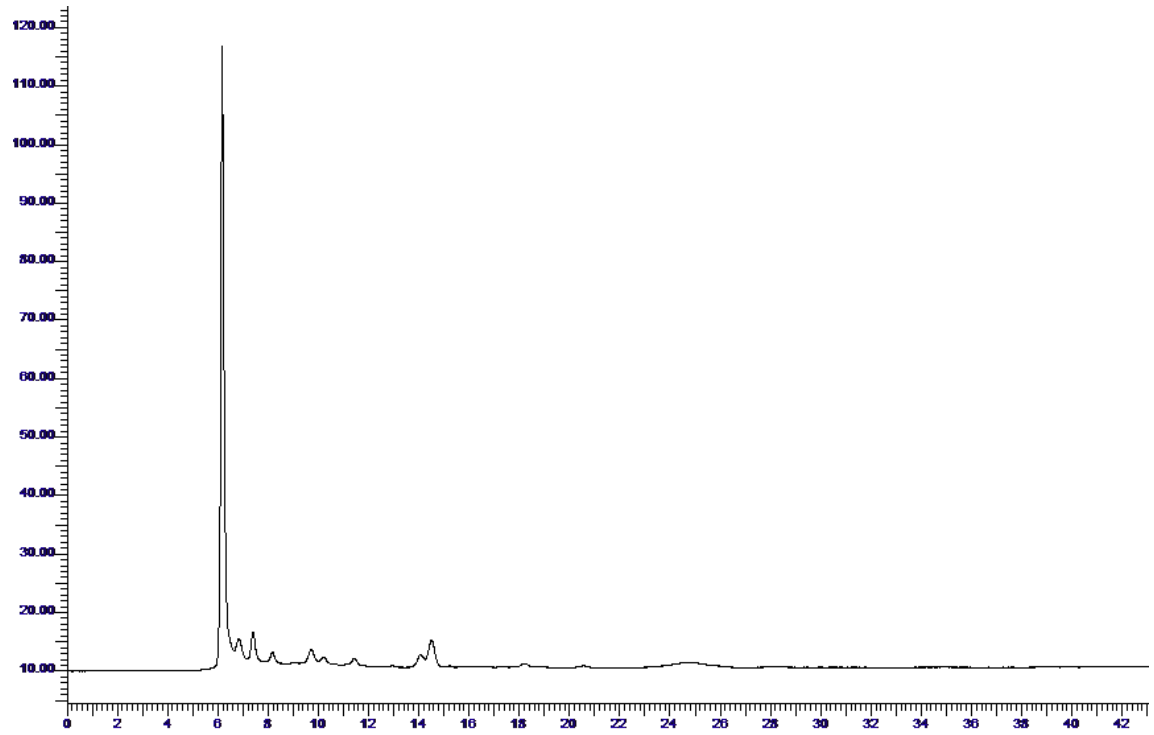

Figure E.5. A representative organic acid profile for oat milk

APPENDIX F

SENSORY EVALUATION SHEET

Name; Date;
/ / 2011

Age;

Give score to kefir samples according to personal liking
(1= worst ☹ ……. 10= is best ☺)

Kefir sample	Appearance	Odor	Flavor	Consistency	Overall acceptability
320					
274					
986					
671					
576					
735					
127					
404					
813					
689					
311					
515					

Figure F.1. Sensory evaluation sheet of kefir